MPharm Maciej Korczak

Strategies of utilizing bioactive potential of natural products-derived postbiotic metabolites

Dissertation for the degree of doctor of medicine and health sciences in the discipline of pharmaceutical sciences

Supervisor: prof. Jakub Piwowarski

Microbiota Lab, Department of Pharmaceutical Biology,

Medical University of Warsaw

Defense of the doctoral dissertation to the Pharmaceutical Sciences Council,

Medical University of Warsaw

Warsaw, 2024

Mgr farm. Maciej Korczak

Strategie wykorzystania potencjału biologicznego metabolitów postbiotycznych powstałych ze związków pochodzenia naturalnego

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki farmaceutyczne

Promotor: prof. dr hab. n. med. i n. o zdr. Jakub Piwowarski

Microbiota Lab, Katedra i Zakład Biologii Farmaceutycznej,

Warszawski Uniwersytet Medyczny

Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Farmaceutycznych

Warszawskiego Uniwersytetu Medycznego

Warszawa, 2024

I dedicate this work to my beloved Father.

Acknowledgments

Writing these words, I am painfully aware that I will not be able to thank everyone who contributed to this work and who helped me navigate through the four years of my doctoral studies. Nonetheless, it would be disingenuous of me not to mention at least a few People to whom I am most grateful.

Initially, I am much obligated to Dr. Przemysław Kurowski for being the first to believe in me during my studies and encouraging me to pursue further scientific adventure.

I thank wonderful and inspiring scientists from Braga and Vienna, who made me feel at home during my internships in each of these cities.

I would like to thank all the staff of the Department of Pharmaceutical Biology at Medical University of Warsaw, for creating a workplace that so often was more than just another job.

I wish to express my gratitude to Professor Sebastian Granica for his calm, warmth, patience in sharing his vast knowledge, and being the best boss, I could have dreamed of.

I am grateful to all past and present members of the Microbiota Lab for collectively creating the best laboratory in the entire university. I thank Dr. Karolina Pawłowska and Dr. Aleksandra Kruk for always being an invaluable source of substantive support for me. Dr. Marcin Równicki for his contribution to broadening my horizons. Dr. Dominik Popowski, I thank for every thought-provoking hour spent in conversation and every coffee shared together. I thank the younger Ph.D. students: Natalia Melnyk, Inna Vlasova, Yuliia Kostenko, Diana Dolzhko, for making me a better scientist through our collaborative work.

I would like to offer my special thanks to my closest family, grandparents, aunt Marta, parents, and brother, for believing in the significance of what I do, even when I had doubts. I deeply regret that I did not manage to thank all of you from this place and time.

I am beyond grateful to Professor Jakub Piwowarski, for calling me in June 2020 and changing my life, for being everything a supervisor should be. For the scientific atmosphere he created in the Microbiota Lab and the model of a scientist he provides to us all.

My sincerest thanks go to Dr. Weronika Skowrońska, who was my greatest support during the most challenging moments in my professional and personal life. This work is just a small part of all that I owe to that support and understanding.

Funding

Studies on the stability and biological activity of synthetic urolithin A derivatives were conducted within the project "Influence of chemical modifications of urolithin A molecule on its metabolism and biological activities *in vitro*." (grant no. 2019/35/O/NZ7/00619) funded by the National Science Center under the PRELUDIUM BIS 1 funding scheme.

NATIONAL SCIENCE CENTRE
POLAND

Implementation-oriented research on the development of a stable formulation containing the urolithin A molecule was conducted within the project entitled "Synthesis of API, development of formulation and *in vivo* studies of a cream containing human gut microbiota postbiotic metabolite - U228 for topical treatment of atopic dermatitis." (grant no. 0118/L-11/2019) by the National Center for Research and Development under the Leader XI program.

NCBR
National Centre for Research and Development

Two research internships held during the doctoral program in which the candidate developed his skills in molecular biology were funded by the Polish National Agency For Academic Exchange within the NAWA STER and NAWA PRELUDIUM BIS 1 programs.

NAWA
POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE

1. List of publications constituting the doctoral dissertation

The basis for applying for the degree of doctor of medical and health sciences in the discipline of pharmaceutical sciences is a series of 3 scientific publications published in 2022-2023 with a total impact factor (IF) of 20.

Scientific papers included in the dissertation:

1. Maciej Korczak, Maciej Pilecki, Sebastian Granica, Aleksandra Gorczyńska, Karolina Aleksandra Pawłowska, Jakub Patryk Piwowarski. Phytotherapy of mood disorders in the light of microbiota-gut-brain axis. Phytomedicine. 2023 Mar;111:154642. doi: 10.1016/j.phymed.2023.154642. Epub 2023 Jan 4. PMID: 36641978.
   Impact Factor: 7,9; MEiN: 140
2. Maciej Korczak, Piotr Roszkowski, Sebastian Granica, Jakub Patryk Piwowarski. Conjugates of urolithin A with NSAIDs, their stability, cytotoxicity, and anti-inflammatory potential. Scientific Reports 2022 Jul 8;12(1):11676. doi: 10.1038/s41598-022-15870-8. Erratum in: Scientific Reports. 2022 Nov 2;12(1):18503. PMID: 35804000; PMCID: PMC9270351.
   Impact Factor: 4,6; MEiN: 140
3. Maciej Korczak, Piotr Roszkowski, Weronika Skowrońska, Klaudia Małgorzata Żołdak, Dominik Popowski, Sebastian Granica, Jakub Patryk Piwowarski. Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function. Biomedicine & Pharmacotherapy. 2023 Dec 31;169:115932. doi: 10.1016/j.biopha.2023.115932. Epub 2023 Nov 24. PMID: 38000358.
   Impact Factor: 7,5; MEiN: 140

2. Table of contents

3. List of figures

4. List of abbreviations

AhR - aryl hydrocarbon receptor

API - Active Pharmaceutical Ingredients

AspUA – mixture of esters of urolithin A with acetylsalicylic acid (aspirin)

cGMP - current Good Manufacturing Practice

COX-2 - cyclooxygenase-2

DicloUA – mixture of esters of urolithin A with diclofenac

EA – ellagic acid

EMA - European Medicines Agency

GLP – Good Laboratory Practice

HPLS-MS/MS - high-performance liquid chromatography coupled with mass spectrometry

IBD - inflammatory bowel disease

IbuUA – mixture of esters of urolithin A with ibuprofen

ISAPP - The International Scientific Association for Probiotics and Prebiotics

IsoUA -isourolithin A

LPS - lipopolysaccharide

MefUA – mixture of esters of urolithin A with mefenamic acid

NF-κB - nuclear factor kappa-light-chain-enhancer of activated B cells

NSAIDs – nonsteroidal anti-inflammatory drugs

Ph. Eur - European Pharmacopoeia

RO5 – Lipinski's rule of five

SCFAs - short-chain fatty acids

SULTs - sulfotransferases

TEER - transepithelial electrical resistance

TJPs- tight junction proteins

TMA – trimethylamine

TMAO - trimethylamine-N-oxide

UA – urolithin A

UB- urolithin B

UADs – urolithin A derivatives

UGTs - UDP-glucuronosyltransferases

5. Abstracts

Tytuł: Różne strategie wykorzystania potencjału biologicznego metabolitów postbiotycznych powstałych ze związków pochodzenia naturalnego

Ostatnie lata przyniosły gwałtowny wzrost wiedzy na temat roli ludzkiej mikrobioty jelitowej oraz produkowanych przez nią związków w utrzymaniu homeostazy organizmu. Bakterie jelitowe wykazują ekspresję olbrzymiej liczby enzymów przekształcających cząsteczki, które dostają się do przewodu pokarmowego, często znacząco zmieniając ich strukturę i właściwości biologiczne. Metabolity postbiotyczne powstałe z produktów naturalnych stanowią niszę interesujących substancji aktywnych o obiecujących parametrach, które mogą służyć do rozwoju nowych substancji czynnych.

W swojej pracy podjąłem się badania metabolitów postbiotycznych poprzez zaproponowanie 3 różnych strategii badawczych dążących do wykorzystania ich potencjału terapeutycznego. Pierwsza strategia, przedstawiona na przykładzie substancji roślinnych wykorzystywanych w terapii łagodnych zaburzeń nastroju, dotyczy nowego spojrzenia na mechanizmy aktywności leków roślinnych z perspektywy osi mikrobiota-jelita-mózg i podkreślenia roli powstających metabolitów postbiotycznych w działaniu tradycyjnie stosowanych produktów pochodzenia naturalnego. Pozostałe dwie strategie wdrożyłem wybierając urolitynę A, znany metabolit postbiotyczny, jako cząsteczkę wiodącą do dalszych prac. Zaproponowałem dwa nowatorskie podejścia umożliwiające wykorzystanie jej potencjału terapeutycznego związanego z silną aktywność przeciwzapalną, która *in vivo* ograniczana jest przez metabolizm II fazy: pierwsze, poprzez dokonanie chemicznej modyfikacji jej struktury, tak by powstałe pochodne zwiększyły biodostępność aktywnej formy oraz drugie, poprzez podanie na skórę niezmienionej urolityny A, unikając w ten sposób działania enzymów detoksykacyjnych.

Wyniki z badań *in vitro* nad pochodnymi urolityny A opublikowałem w formie dwóch prac oryginalnych, które wraz z pracą przeglądową poświęconą aktywności metabolitów postbiotycznych powstałych z substancji roślinnych stosowanych w zaburzeniach nastroju wchodzą skład cyklu publikacji stanowiących przedstawioną rozprawę doktorskiej. Ze względu na wdrożeniowy charakter prac nad syntezą urolityny A na skalę półtechniczną oraz wytworzeniem formulacji do podania na skórę szczegółowe wyniki badań są zawarte w raportach projektowych i zostały zwięźle opisane w przedstawionej pracy. Wykonane przeze mnie badania nakreślają obiecujące podejścia do wykorzystania metabolitów postbiotycznych, i stanowią innowacyjne strategie, będąc podstawą do dalszych badań i prac wdrożeniowych nad tym bogatym światem produktów metabolizmu jelitowego.

Słowa kluczowe:

mikrobiota jelitowa, metabolity postbiotyczne, stan zapalny, urolityna A, związki pochodzenia naturalnego

Title: Different strategies of utilizing bioactive potential of natural products-derived postbiotic metabolites.

In recent years we have witnessed a significant increase in our understanding of the role of human gut microbiota and its metabolites in maintaining body homeostasis. Gut bacteria express a vast number of enzymes that transform compounds entering the gastrointestinal tract, often significantly changing their structure and biological properties. Postbiotic metabolites derived from natural products represent a niche of active substances and a rich reservoir of promising compounds for the development of new active pharmaceutical ingredients.

In my work, I have presented 3 research strategies on utilizing therapeutic potential of postbiotic metabolites. The first strategy, using as examples plant substances traditionally employed in the therapy of mild mood disorders, offers a new perspective on their mechanisms of action from the microbiota-gut-brain axis viewpoint, highlighting the role of emerging postbiotic metabolites in the efficacy of traditionally used natural products. The remaining two strategies involved urolithin A, a known postbiotic metabolite, as a lead molecule for further research. I proposed two innovative approaches to exploit its therapeutic potential related to its strong anti-inflammatory activity, which is limited *in vivo* by phase II metabolism: firstly, through chemical modification of the original compound's structure to enhance the bioavailability of its active form; and secondly, through topical application of the unchanged urolithin A, thus avoiding the action of detoxifying enzymes.

The results from *in vitro* studies on urolithin A derivatives have been published in the form of two original papers, and along with a review article focused on the activity of postbiotic metabolites derived from plant substances used in mood disorders, form part of a series of publications constituting the presented doctoral dissertation. Due to the implementation-oriented nature of the work on the semi-technical scale synthesis of urolithin A and development of formulations for topical application, the research findings are contained in the project reports and are briefly described in this work. The conducted studies outline promising approaches for utilizing postbiotic metabolites and represent innovative strategies, laying the groundwork for further research and implementation work on this rich world of gut metabolism products.

Keywords:

gut microbiota, inflammation, natural products, postbiotic metabolites, urolithin A

6. Scientific profile of the doctoral candidate

Master of Pharmacy Maciej Korczak

I commenced my Master's studies in Pharmacy at the Medical University of Warsaw in 2014 and completed them in 2020. During my studies, I was actively engaged in the scientific activities within the Cerebrum students' scientific circle, which was affiliated with the Department of Human Physiology and Pathophysiology at the Faculty of Pharmacy. My involvement in Cerebrum enabled me to obtain a student mini-grant, present the results of my research at two international student conferences (14th Warsaw International Medical Congress and 11th Leiden International (Bio)Medical Student Conference), and ultimately led to the publication of a review paper, for which I was the first author, in the European Journal of Pharmacology in 2020. In my undergraduate research, I explored the phenomenon of prolonged depolarization in the neurons of the prefrontal cortex and searched for cellular effectors responsible for its onset in rats. I utilized patch-clamp electrophysiology techniques in my work, examining changes in voltage and current measurements in prefrontal cortex neurons sourced post-decapitation of rats and tissue isolation. This research culminated in the defense of my Master's thesis titled "Mechanism of prolonged depolarization resulting from hyperpolarization of the cell membrane controlled by $GABA_B$ receptors," supervised by dr. Przemysław Kurowski, in 2020.

Within my doctoral research, which began in October 2020, I was working on the project "Influence of chemical modifications of urolithin A molecule on its metabolism and biological activities *in vitro*," funded by the National Science Centre under the Preludium Bis 1 program, with prof. Jakub Piwowarski serving as the Principal Investigator and supervisor of my doctoral thesis. From November 2022 to May 2024, I was also employed as a Specialist – Researcher within the Lider XII project funded by the National Centre for Research and Development, titled "Synthesis of API, development of formulation and *in vivo* studies of a cream containing human gut microbiota postbiotic metabolite – U228 for topical treatment of atopic dermatitis," with prof. Jakub Piwowarski also serving as the Principal Investigator. The results obtained from these two research projects on the molecule of urolithin A and its derivatives form the basis of my doctoral dissertation. In 2023, as the principal investigator, I received funding for two projects focused on the interaction between compounds found in hop cones and gut bacteria, which allowed me to open an independent and autonomous chapter of research on postbiotic metabolites not derived from ellagitannins.

The research I have conducted has contributed to the development of knowledge on the role of gut microbiota for human health, primarily focusing on compounds arising from its metabolic activity and potential strategies for utilizing these substances in the development of new medicinal substances. Apart from research focused strictly on gut microbiota and postbiotic metabolites,

between 2021-2022, I also participated as a contractor in the project "Birch extract with an exceptional content of active ingredients" within the Innovation Incubator 4.0 program funded by the Ministry of Science and Higher Education, studying the anti-inflammatory and diuretic activities of a modified birch leaf extract, which is covered by the national patent P.441246, and for which I am a co-author.

During my doctoral studies, I completed two internships significant for my scientific career: a one-month stay in May 2023 at the Department of Biology, University of Minho under the supervision of Prof. Alberto Dias, and a six-month internship at the Molecular Targets group, University of Vienna under the supervision of Prof. Elke Heiß. These internships primarily served to develop my skills in molecular biology and introduce me to new, advanced techniques that could potentially be implemented in domestic units in the future. In addition, another 6-month internship in the Molecular Targets group under the supervision of Professor Elke Heiß received funding from the NAWA Walczak program (project number BPN/WAL/2023/1/00034). Scheduled to begin in November 2024, this project will study the anti-inflammatory properties of hop-derived postbiotic metabolites using advanced molecular biology techniques. Throughout my doctoral studies, I have developed an interdisciplinary skill set, encompassing techniques such as semisynthetic methods for modifying compounds of natural origin, and various molecular biology and microbiology methods. Additionally, I have conducted studies involving human gut microbiota sourced *ex vivo*, and, after completing training in the use of animals in experimental research, I engaged in *in vivo* research utilizing rodent models

As part of the teaching activities between 2020-2023, I led laboratory courses in phytochemistry as part of the Pharmacognosy for the third year of the Pharmacy program. I supervised two Master theses and actively participated in the work of Herbarium students' scientific circle at the Department of Pharmaceutical Biology, gaining funding for a project involving club members. In my mentorship work with students, I was also engaged in the international SCORE program organized by The International Federation of Medical Students Associations (IFMSA), and in 2023 I was a tutor for two international students during their stay in Poland, during which they conducted research in the Microbiota Lab. I also participated in the peer review process for scientific articles in 4 Web of Science indexed journals with an impact factor: Journal of Ethnopharmacology, Biochemical and Biophysical Research Communications, Scientific Reports, and Prospects in Pharmaceutical Sciences. During the course of my PhD, I managed to establish a documented network of scientific collaborations with national centers in Warsaw and Lublin, as well as international centers in Egypt, Portugal, and Austria.

6.1 Detailed information on the candidate's scientific activity

I am co-author of 6 publications, being the first author in 4 of them.

- The cumulative Impact Factor of these publications at the time of their release stands at 41,3

- The total number of citations my publications, excluding self-citations, is 18 (according to the Scopus database).
- My Hirsch index, excluding self-citations, is 2 (according to the Scopus database).

6.1.1 Research projects

- **Principal Investigator**

  Project title: Impact of xanthohumol on the structure and diversity of the human gut microbiota

  Grant for Young Scientists, Medical University of Warsaw, Poland 06.2023-06.2024

- **Principal Investigator**

  Project title: Isolation of hops-derived postbiotic metabolites using human intestinal microbiota

  Grant Students scientific associations create innovations, Ministry of Science and Higher Education, Poland, 05.2023-05-2024

- **Specialist - Researcher**

  Project title: Synthesis of API, development of formulation and *in vivo* studies of a cream containing human gut microbiota postbiotic metabolite – U228 for topical treatment of atopic dermatitis

  Lider 12, The National Centre for Research and Development, Poland, 11.2022-04.2024

- **Contractor**

  Project title: Birch extract with an exceptional content of active ingredients.

  Grant Innovation Incubator 4.0, Ministry of Science and Higher Education, Poland, 12.2021-10.2022

- **Ph.D. student**

  Project title: Influence of chemical modifications of urolithin A molecule on its metabolism and biological activities *in vitro*

  Preludium Bis 1, National Science Centre, Poland, 10.2020-09.2024

- **Principal Investigator**

  Project title: Mechanisms of the modulatory effects of divalent ions on the phenomenon of prolonged membrane depolarizations in prefrontal cortex pyramidal neurons

  Student mini-grant, Medical University of Warsaw, Poland, 06.2017-03.2018

6.1.2 Scientific internships

- Molecular Targets, Division of Pharmacognosy, University of Vienna, Vienna, Austria
  Supervisor: Prof. Elke Heiß

  Accepted, planned 11.2024-04.2025

Internship funded by the National Agency for Academic NAWA Walczak program.

- Molecular Targets, Division of Pharmacognosy, University of Vienna, Vienna, Austria
  Supervisor: Prof. Elke Heiß
  10.2023-03.2024
  Internship funded by the National Agency for Academic Exchange Preludium Bis 1 NAWA program.
- Department of Biology, University of Minho, Braga, Portugal
  Supervisor: Prof. Alberto Dias
  05.2023 (*one-month internship*)
  Internship funded by the National Agency for Academic Exchange NAWA STER internationalization of Doctoral Schools 2023 program.

6.1.3 Publications outside the doctoral dissertation cycle

- Dominik Popowski , Aleksandra Kruk, Karolina A. Pawłowska, Diana Dolzkho, **Maciej Korczak**, Jakub P. Piwowarski, Marek Roszko, Sebastian Granica
  Evaluating birch leaf tea as a functional herbal beverage: Beneficial impact on the urinary tract, and metabolism in human organism
  Food Research International. *Article in press, accepted, peer reviewed, citable using DOI.* 10.1016/j.foodres.2024.114481
  Impact factor: 8,1
- Fatema R. Saber, Ahmed H. Elosaily, Engy A. Mahrous, Łukasz Pecio, Solomiia Pecio Yasser A. El‑Amier, **Maciej Korczak**, Jakub P. Piwowarski, Łukasz Świątek, Katarzyna Skalicka-Woźniak.
  Detailed metabolite profiling and *in vitro* studies of Urospermum picroides as a potential functional food.
  Food Chemistry. 2023 Nov 30;427:136677. doi: 10.1016/j.foodchem.2023.136677. Epub 2023 Jun 23. PMID: 37390739.
  Impact Factor: 8,8
- **Maciej Korczak**, Przemysław Kurowski, Anna Leśniak, Alfhild Grönbladh, Anna Filipowska, Magdalena Bujalska-Zadrożny.
  $GABA_B$ receptor intracellular signaling: novel pathways for depressive disorder treatment?
  European Journal of Pharmacology. 2020 Oct 15;885:173531. doi: 10.1016/j.ejphar.2020.173531. Epub 2020 Aug 29. PMID: 32871173
  Impact Factor: 4,4

6.1.4 Participation in Conferences[1]

- Maciej Korczak, Sheyda Bahiraii, Martina Redl, Piotr Roszkowski, Sebastian Granica, Judith M. Rollinger, Elke Heiß, Jakub Piwowarski

  Oral presentation: „Design and anti-inflammatory evaluation of ellagitannin-derived postbiotic metabolite conjugated with serotonin and dopamine"

  International Congress on Natural Products Research, Kraków, Poland, 07.2024 (*scheduled; officially accepted contribution*)
- Maciej Korczak, Dominik Popowski, Aleksandra Kubiszewska, Sebastian Granica, Jakub Piwowarski

  Poster: „Bilateral interactions between human gut microbiota and xanthohumol"

  International Congress on Natural Products Research, Kraków, Poland, 07.2024 (*scheduled; officially accepted contribution*)
- Maciej Korczak, Sheyda Bahiraii, Piotr Roszkowski, Sebastian Granica, Elke Heiß, Jakub Piwowarski

  Poster: „Synthesis and anti-inflammatory properties *in vitro* of Urolithin A derivatives conjugated with neurotransmitters"

  9th International Conference on Pharmacy and Pharmaceutical Science. Seoul, South Korea, 06.2024
- Maciej Korczak, Piotr Roszkowski, Klaudia Żołdak, Dominik Popowski, Sebastian Granica, Jakub Piwowarski

  Poster: „Urolithin A Conjugates with Non-Steroidal Anti-Inflammatory Drugs: Synthesis, Impact on Phase II Metabolism and Caco-2 Monolayer Integrity"

  American Society of Pharmacognosy Annual Meeting: Innovation Through Interaction. Rockville, Maryland, USA, 07.2023
- Maciej Korczak, Aleksandra Gorczyńska, Sebastian Granica, Jakub Piwowarski

  Poster: „Bilateral interactions between gut microbiota and natural products applied in anxiety and mood disorders"

  International Bioresource Conclave & Ethnopharmacology Congress. Imphal, Manipur, India, 02.2023
- Maciej Korczak, Piotr Roszkowski, Sebastian Granica, Jakub Piwowarski

  Oral presentation: „Monoesterified analogs of postbiotic metabolites of ellagitannins improve Caco-2 monolayer integrity and increase the bioavailability of urolithin A *in vitro*"

[1] The authors presenting the results are underlined.

Young Researchers Workshop - GA 2022; 70th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA). Thessaloniki, Greece 08.2022.

- Dominik Popowski, Weronika Skowrońska, Maciej Korczak, Aleksandra Kruk, Karolina Pawłowska, Jakub Piwowarski, Sebastian Granica

  Poster: „Main non-flavonoid constituent of birch leaves water extract – 3-hydroxy-1-(4-hydroxyphenyl)-propan-1-one 3-O-β-D-glucoside: isolation, permeability, and antiadhesive activity"

  70th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA). Thessaloniki, Greece 08.2022.

- Dominik Popowski, Maciej Korczak, Karolina Pawłowska, Aleksandra Kruk, Jakub Piwowarski, Sebastian Granica

  Oral presentation „Silver birch (Betula pendula Roth) – phytochemical characterization of the constituents and their metabolites present in the urine"

  Young Researchers Workshop - GA 2021; 69th International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA). Virtual conference, 12.2021.

- Maciej Korczak, Piotr Roszkowski, Sebastian Granica, Jakub Piwowarski

  Oral presentation: „Synthetic derivatives of urolithin A increase Caco-2 monolayer integrity, impede intestinal glucuronidation, and suppress TNF alpha production in THP-1 derived macrophages"

  Targeting Microbiota 2021, International Society of Microbiota. Paris, France, 10.2021.

- Maciej Korczak, Przemysław Kurowski

  Oral presentation: „Rebound depolarization – the hidden secret of our brains"

  11th Leiden International (Bio)Medical Student Conference. Leiden, Netherlands, 03.2019

- Przemysław Kurowoski, Maciej Korczak, Paweł Szulczyk

  Poster: „Characterization of rebound depolarization in medial prefrontal cortex pyramidal neurons *in vitro*"

  14th International symposium molecular basis of pathology and therapy in neurological disorders, Warsaw, Poland, 10.2018

- Maciej Korczak, Przemysław Kurowski

  Oral presentation: „Rebound depolarization – the hidden secret of our brains"

  14th Warsaw International Medical Congress, Warsaw, Poland, 05.2018

6.1.5. Other significant information

- Tutorship in the International Federation of Medical Students Associations – SCORE exchange program – 08.2023

Project title: Gut microbiota assisted isolation of xanthohumol-derived postbiotic metabolites

- Patent application in the territory of the Republic of Poland (P.441246)

  Title: A method for producing a lyophilizate from birch leaves with a standardized content of 3,4'-DHPPG, a lyophilizate obtained by the present method, and a composition containing such a lyophilizate.

  Authors: Dominik Popowski, Weronika Skowrońska, **Maciej Korczak**, Sebastian Granica, Jakub Piwowarski
- Medical University of Warsaw Rector Scholarship for best PhD students. 2022 – 2023
- Completed course for individuals responsible for participating in, planning, and conducting procedures and experiments on animals, as well as euthanizing animals used in procedures – Medical University of Warsaw, 2023
- Completed course “Good Laboratory Practice - implementing the system and ensuring the highest quality of GLP-compliant research” Bioszkolenia, 2023
- Completed course “Introduction to metagenomics” – Data2biology, 2022

7. Introduction and justification of research themes

7.1. Molecules of natural origin as a source of new active substances

The history of utilizing compounds of natural origin in contemporary evidence-based medicine did not conclude in the last century with the discovery of penicillin or the introduction of paclitaxel for cancer therapy. Furthermore, significant technological advancements in bioinformatics and metagenomics have led some researchers to believe that the "golden era" of using natural products in drug development is still ahead of us [1]. A complementary approach to designing medicinal substances, merging pharmacognostic expertise with knowledge from the field of medicinal chemistry, enables to bypass some limitations of compounds isolated from the natural environment. A well-known, albeit historically dust-covered example is acetylsalicylic acid. This compound was first synthesized in 1887 by Felix Hoffmann to minimize side effects associated with the gastrointestinal irritation caused by salicylic acid, found in, for example, the bark and leaves of plants of the *Salix* genus, used in traditional medicine even 3500 years ago [2]. Another historical example of synthetic derivatives of compounds isolated from medicinal plants used in therapy is quinine, isolated from the bark of Cinchona species, which through chemical modifications enabled the synthesis and introduction of antimalarial drugs such as chloroquine and mefloquine in the first half of the 20th century [3]. The concept initiated by Hoffmann and his colleagues at Bayer in the late 19th century has never ceased to inspire scientists, and the desire to "improve" nature, though it may seem a boastful challenge, often leads researchers to new, surprising discoveries, with suffering patients as the ultimate beneficiaries.

The widespread use of synthetic drugs and the increasingly robust development and market introduction of new biological macromolecules, such as proteins or polymer conjugates with active substances, may cast doubt on the validity of searching for new promising "leading molecules" among plant substances. The traditional and well-documented effects of many herbal substances over centuries may suggest that their use in modern medicine has been exhausted. However, a review of drugs registered and approved for sale by the U.S. Food and Drug Administration and related agencies between 1981-2019 showed that among all 1881 new drugs, 18.9% were derivatives of compounds, another 11% contained a pharmacophore known from natural substances, and a further 3.8% and 0.8% were, respectively, unchanged plant substances and mixtures of them [4]. It is important to highlight that the use of compounds of natural origin in the design of new medicinal substances is highly varied depending on the drug group and its potential therapeutic use: nearly 65% of registered medicinal products used in cancer treatment are derived from these compounds, while for some drugs, e.g., diuretics or antihistamines, the use of compounds found in nature is of marginal importance.

Adapting and up-scaling of synthesis of compounds originally derived from natural sources is often a considerable technological challenge. Modifications to the structure of molecules of natural origin often do not correlate with an increase in their bioactivity or a reduction in their undesirable impact on the organism, but are required to ensure the profitability of the production process. The main characteristics of naturally occurring compounds that complicate their retrosynthesis and market introduction are their high diversity along with chemical structure complexity and the presence of many chiral centers, with the possibility of creating stereoisomers with varied biological activity [5]. Therefore, the synthesis of active derivatives of natural product-derived compounds often relies on simplifying the structure of original compounds. The most known and classic example of simplifying the chemical structure of a compound of natural origin to obtain active (often even more active than the unmodified molecule) and easier-to-produce analogs is undoubtedly morphine, originally isolated from *Papaver somniferum* [6]. Thanks to a series of modifications, including the removal of additional rings from the morphine molecule, other approved drugs were obtained, which at first glance have a significantly different structure from the starting molecule (Fig. 1). Interestingly, a completely different strategy is sometimes used in the development of small molecule drugs, involving the conjugation of naturally occurring molecules with other active compounds to increase their overall biological activity [7,8].

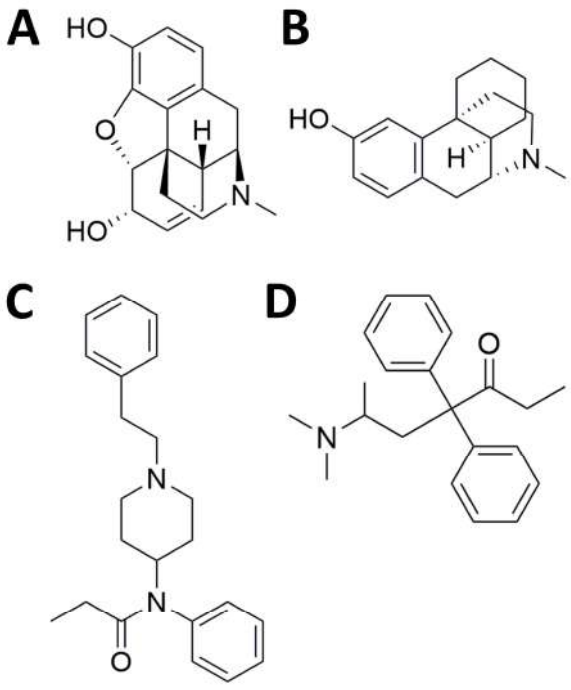


Figure 1. Chemical modifications of the morphine molecule (A) led to the synthesis of new, active derivatives. The removal of additional rings facilitated the synthesis of levorphanol (B), fentanyl (C), and ultimately resulted in the synthesis of the simplest opioid drug - methadone (D).

In the search for new, active, and bioavailable molecules, researchers must consider a range of factors originating from their chemical structure that affect their physicochemical properties and, consequently, their potential implementation as active pharmaceutical ingredients (API). To facilitate the search through increasingly large databases of small molecules, Lipinski's rule of five (RO5; Fig. 2) has been used for preliminary screening to filter out compounds that, due to their chemical structure, do not seem to be ideal candidates for further, costly research and, crucially, for this rule, are not bioavailable after oral administration.

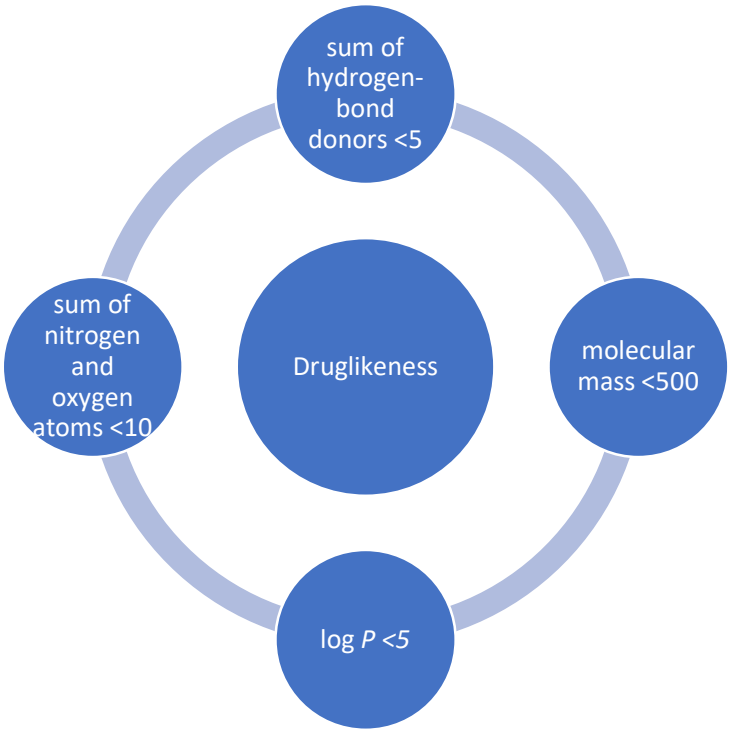


Figure 2. RO5 comprises theoretical requirements for potential active substances to be effective in the human body when administered orally.

Of course, as with any such rule, there are numerous exceptions. For example, out of 74 kinase inhibitors approved and marketed by 2023, 30 did not meet the requirements set by RO5 [9]. Like any arbitrary tool created by humans, Lipinski's rule should be treated as a guideline for prioritizing the development of small molecules, rather than a definitive indication of which compounds should be further researched and invested in time and money. This is especially significant for compounds based on molecular structures derived from natural products and molecules that are substrates for transporters found in the cell membrane, for which Lipinski's rule does not apply. Moreover, focusing exclusively on the oral bioavailability of molecules, as many researchers do, is considered too stringent and may deprive access to many important drugs; for example, penicillin V, bioavailable after oral administration, was discovered 15 years after the first life-saving penicillin G, which might not have been approved for market at all if the trend was towards the development of substances available for

oral administration [10]. Many diseases do not require orally bioavailable drugs, and intravenous or topical administration on the skin can serve as alternatives.

The already mentioned penicillin G also represents a classic example of another group of compounds of natural origin, this time not derived from medicinal plants but produced thanks to the enzymatic activity of microorganisms. The role of both fungi and bacteria producing specialized metabolites in drug design and development is not limited to the production of antibiotics (e.g., penicillins and cephalosporins) but also includes immunosuppressants (cyclosporine), cholesterol-lowering drugs (mevastatin, lovastatin), and antiparasitic drugs (ivermectin) [3]. Just as with compounds isolated from traditionally used medicinal plants, metabolites produced through the enzymatic activity of microorganisms continue to be a valuable source of leading molecules, promising in further stages of drug development. In recent years, research focused on searching for biologically active metabolites derived from microorganisms living in non-standard ecological niches, such as plant-associated bacteria, organisms developing in diverse marine ecosystems, or those living in invertebrates, has gained popularity [11]. Some of these compounds have already been approved for medical use, such as the anti-cancer drug used in soft-tissue sarcoma and ovarian cancer, ecteinascidin-743, produced by symbiotic bacteria (*Candidatus Endoecteinascidia frumentensis*) living in the sea squirt belonging to the species *Ecteinascidia turbinata* [12]. Similar to compounds obtained from medicinal plants, sometimes compounds isolated from these less studied ecosystems require significant simplification of their structure, such as eribulin (anti-cancer drug), which is a synthetic derivative of halichondrin B initially isolated from the sponge *Halichondria okadai* (Fig 3) [13].

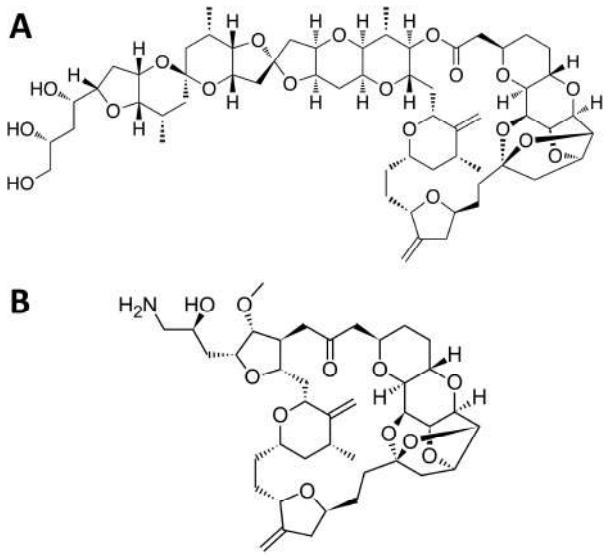


Figure 3. Halichondrin B (A), like morphine, is an example of compound of natural origin whose chemical structure has been simplified to obtain a new API, in this case eribulin (B).

The wealth of yet unexplored niches, together with the development of medical chemistry related to the derivatization of already known compounds, indicate that compounds of natural origin will remain one of the main sources of molecules for the development of new drugs for a long time. However, the next newly discovered source of promising medically and biologically active molecules is much closer than the depths of the oceans. The role of organisms living in the human gastrointestinal tract in maintaining the body's homeostasis is increasingly recognized to such an extent that some researchers propose to stop considering the human body as an independent and indivisible evolutionary entity, but instead suggest the concept of the holobiont, which emphasizes the role of bacteria not only for human health but also for its development as a species [14].

7.2. The role of postbiotic metabolites in maintaining homeostasis

The 21st century, particularly its second decade, has witnessed a huge gain in scientific interest regarding the role of gut bacteria on human health and the alterations within this complex ecosystem. This phenomenon is most evident in the number of scientific articles indexed in the PubMed database, addressing issues related to the gut microbiota (though it's worth noting that, especially in the early stages, it was often inaccurately referred to as gut microflora) (Fig 4). This intricate community within our intestines consists not only of bacteria but also of archaea, fungi, and viruses inhabiting this unique niche, collectively with their genome, referred to as the microbiome [15]. Particularly, the advancement of bioinformatic tools, next-generation sequencing, and metabolomics has contributed to a deeper understanding of the significant impact that bacteria and other microorganisms have on maintaining the body's homeostasis, though, in reality, we still do not fully comprehend the role and impact of certain elements, especially non-bacterial components of the gut microbiota. Estimating the population of bacteria living in our digestive system is challenging; it is thought to reach up to 100 trillion bacteria, and moreover, it is characterized by significant variability within the gastrointestinal tract due to changing pH levels and oxygen availability [16,17]. Furthermore, the structure of the human gut microbiota is highly susceptible to changes related to external factors such as stress, diet, and age [18].

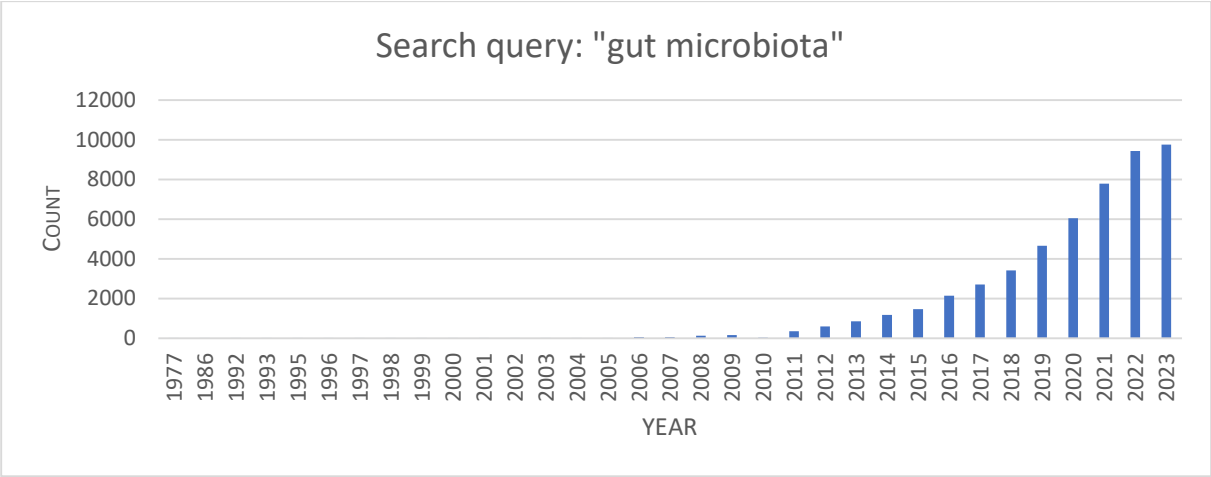


Figure 4. Number of results in PubMed database after searching „gut microbiota" term

Primarily, some changes in the gut microbiota structure are strongly correlated with the occurrence of numerous diseases, including obesity, diabetes, depression, and certain cancers [19–22]. Although defining what constitutes a "healthy" microbiome remains a challenge, undoubtedly, specific fingerprints in the alterations of this microenvironment, particularly the loss of its biodiversity associated with the so-called "Western lifestyle," can serve as prognostics in disease development [23–25]. Modifying complex structure of the gut microbiota for disease treatment appears to be an extremely complicated task, yet attempts to reverse dysbiosis are areas of research for scientists and clinicians. Currently, fecal microbiota transplantation, which involves obtaining gut microbiota from healthy donors and administering it to patients, is used in treating Crohn's disease and recurrent *Clostridium difficile* infections, with meta-analyses showing statistically significant improvements following its application [26,27]. The use of pre-, pro-, post-, and synbiotics to modify the gut microbiota structure remains a question. Like with the microbiome, defining these terms seems crucial and represents the first step towards systematizing their use in treatment, for instance, to avoid incorrectly calling postbiotics postbiotic metabolites and vice versa. To this end, The International Scientific Association for Probiotics and Prebiotics (ISAPP) has defined:

- Prebiotics as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" [28]. This definition limits prebiotics only to those substances whose beneficial properties are microbiota-mediated.
- Probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [29]. This definition emphasizes the importance of applying the correct number of microorganisms in using probiotics to induce a specific therapeutic effect.
- Synbiotics as "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host" [30]. ISAPP noted that

synbiotics are not merely a mix of pre- and probiotics but a combination of compounds and microorganisms that must act cooperatively.

- Postbiotics as "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" [31]. This definition highlights the significance of including whole or parts of inactivated microorganisms, not just the metabolites they produce.

The issue of defining metabolites produced thanks to the activity of gut bacteria remains an open question. Some researchers suggest using of the term "metabiotics," however, due to the aforementioned lack of consensus on this matter, in the presented dissertation these compounds are referred to as "postbiotic metabolites". Importantly, these compounds can be part of postbiotics and, together with cell fragments, may exert a beneficial effect on the human body [32].

While numerous studies document the positive effects of using probiotics, for instance, in preventing diarrhea in children following antibiotic therapy, the issue of whether orally administered microorganisms in the form of probiotics or synbiotics have the ability to colonize the gastrointestinal tract, and if so, how long this effect is maintained, remains a matter of debate and insufficiently explored [33,34]. The presented questions and the complexity of the colonization mechanism of the gut wall by orally administered bacteria undoubtedly represent some of the more important issues that need resolution.

A separate issue is the high metabolic activity of microorganisms residing in the human intestines and the role of the metabolites produced in maintaining the body's homeostasis and immunological balance. The vast gut microbiome is characterized by the expression of bacterial enzymes, which aid in colonizing the intestinal lumen and, for example, through the activity of β-glucosidases, enable intestinal bacteria to break down complex polysaccharides into their readily available monomers, serving as their energy material. Flagship representatives of the metabolites with proven positive health effects are short-chain fatty acids (SCFAs), primarily acetic, propionic, and butyric acids, produced mainly during the fermentation of indigestible saccharides by intestinal bacteria. SCFAs exhibit strong immunomodulating actions, serving as substrates for G-coupled receptors and inhibiting histone deacetylases, demonstrating anti-inflammatory activity, increasing intestinal wall integrity. Considering that receptors for which they are ligands are found in the central nervous system, it is speculated that they are, at least in part, responsible for communication along the microbiota-gut-brain axis [35,36]. Additionally, a reduction in the number of bacteria producing SCFAs is associated with the occurrence of inflammatory bowel disease (IBD) [37].

Other important postbiotic metabolites are compounds derived from the intestinal biotransformation of tryptophan. Gut microbiota microorganisms transform this amino acid into

indole and a range of its bioactive derivatives, which are agonists of the aryl hydrocarbon receptor (AhR), activated by diverse xenobiotics [38]. Transmission pathways associated with AhR activity modulation play a key role in maintaining immunological balance in the intestinal wall and preserving its integrity [39]. Moreover, like SCFAs, tryptophan-derived indole compounds produced by gut bacteria seem to mediate the crosstalk between the intestinal lumen ecosystem and the central nervous system by activating AhR in brain microvessels, consequently modulating the blood-brain barrier integrity [40]. Noteworthy, several gut bacteria produce neurotransmitters, including dopamine, serotonin, and gamma-aminobutyric acid. Numerous animal models suggest clear influence between the gut microbiota and neurodegenerative and affective diseases, and one of the proposed mechanisms of indirect brain influence by intestinal bacteria is precisely the synthesis of neurotransmitters by them, though this connection has not yet been sufficiently proven in clinical studies, and further research is required to fully understand this complex relationship in the human body [41].

An extremely important metabolic activity of intestinal bacteria for maintaining normal body function is their ability to synthesize de novo vitamins crucial for cellular metabolism. Particularly, intestinal bacteria belonging to the *Bifidobacterium* genus demonstrate a strong ability to produce B vitamins, reducing the risk of avitaminosis [42]. In the case of vitamin A, it has been shown that commensal bacteria convert vitamin A into its biologically active form, and in the germ-free mice model, deprived of intestinal bacteria, a statistically significant reduction in these metabolites in the body was presented compared to mice with an unchanged microbiome [43]. Another vitamin produced by the gut microbiota (according to some estimates, even up to 50% of the daily requirement; however, it is worth noting that this is difficult to verify *in vivo*) is vitamin K [44]. Moreover, vitamins ingested with food or orally supplemented significantly affect the composition of the gut microbiota, influencing its richness and diversity [45].

The metabolic activity of intestinal bacteria also affects certain basic physiological aspects related to the proper functioning of the organism. A good example can be bile acids and the associated enterohepatic circulation. Primary bile acids are synthesized in the liver from cholesterol, conjugated with amino acids, and secreted into the duodenum in conjugated form to facilitate the absorption of fats and fat-soluble vitamins through emulsification. Then, these compounds are mostly reabsorbed into the body and recirculated; however, some that do not undergo active reuptake in the intestine are deconjugated by intestinal bacteria, preventing their absorption by transporters located in intestinal epithelial cells, and these compounds undergo further metabolism [46]. The resulting so-called secondary bile acids, e.g., lithocholic acid, deoxycholic acid, or ursodeoxycholic acid. Importantly, in patients suffering from IBD, reduced levels of secondary bile acids have been observed, which, as

numerous *in vitro* and *in vivo* studies show, exhibit strong anti-inflammatory action in intestinal inflammations [47].

Considering the evolutionary history of coexistence between humans and microorganisms residing in their gastrointestinal tract and the series of benefits derived from this complex symbiosis, it is necessary to emphasize how fragile and susceptible to external factors is the balance between the host organism and gut bacteria. This is best seen in the case of lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, including a significant portion of gastrointestinal tract bacteria belonging to Bacteroidetes and Proteobacteria – the main phyla of gram-negative bacteria of the gut microbiome [48]. Considering that it is an essential structural element of the bacterial membrane, earlier definitions may define it as a postbiotic; however, at least for some bacteria, its release has been shown not to be directly related to the disruption of the continuity of the outer membranes of bacteria and their death, which in these cases prevents categorization in this way [49]. Under physiological conditions, the intestinal barrier effectively separates the intestinal lumen environment and bacterial LPS from entering the systemic circulation; however, in the case of increased permeability, associated, for example, with decreased expression of tight-junction proteins (TJPs) in intestinal epithelial cells, it can enter the body and cause a series of undesirable effects [50]. Although the pathogenesis of the so-called "leaky gut syndrome" remains unclear, undoubtedly, increased intestinal wall permeability and the associated increase in circulating LPS in the body are linked to the occurrence of numerous inflammatory diseases. LPS is an agonist of toll-like receptors 4, which stimulation results in the activation of intracellular signal transmission pathways, including the activation of the pathway associated with nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and consequently increased expression of inflammatory mediators [51]. In the case of disruption of the intestinal integration, the role of bacterial LPS seems to be one of the main pathogenic factors in the development of obesity, insulin resistance associated with type 2 diabetes, cardiovascular diseases, and even neurodegenerative and affective disorders [52–54].

The postbiotic metabolites presented so far represent only a fraction of the products of the metabolic activity of gut bacteria and their significant impact on health and disease development in humans. An interesting and relatively recently discovered postbiotic metabolite is trimethylamine (TMA), which is produced from choline through the metabolic activity of microorganisms residing in the human intestines. Once absorbed into the body, TMA is converted in the liver to trimethylamine-N-oxide (TMAO). TMAO is associated with an increased risk of cardiovascular diseases in humans, and the proposed mechanisms of its adverse effects are related to the promotion of myocardial hypertrophy and pro-inflammatory action [55]. The gut microbiota interacts with virtually every substance administered orally that was not absorbed in the earlier stages of the gastrointestinal tract.

However, it is worth emphasizing and requires special attention in the design of new studies, whether the analyzed compounds produced by the gut microbiota are actually active substances or merely biomarkers of changes induced by other factors [56]. This also applies to other xenobiotics, including drugs. On one hand, the powerful enzymatic machinery of the gut microbiota can transform some prodrugs into their active forms, such as in the case of prontosil, which active form exhibits strong antibacterial action; on the other hand, it can deactivate them and reduce their bioavailability, as in the case of amlodipine – a popular drug used in the treatment of hypertension [57]. Moreover, by expressing β-glucuronidases, gut bacteria actively increase the level of the active form of those drugs that are eliminated from the body via the biliary route after conjugation with glucuronic acid, to be deconjugated in the intestinal lumen and reabsorbed [58]. This deglucuronidation process is responsible for the severe diarrhoea induced by the chemotherapeutic drug irinotecan, as intestinal bacteria convert its inactive and excreted metabolite - SN38-glucuronide - into its highly toxic form SN38, sometimes necessitating termination of therapy [59]. Similar mechanisms consequently accompany compounds contained in medicinal plants, often of complex structure, which drastically change their pharmacokinetic and pharmacodynamic properties due to the activity of gut bacteria, and part of these interactions will be dedicated to the next subsection.

7.2.1 The role of gut microbiota in the metabolism of plant specialized metabolites

Specialized metabolites, which are active components of medicinal plants, as previously discussed, often possess a complex chemical structure and a multitude of functional groups and pharmacophores. These can undergo biotransformation by gut bacteria, and similar to the case with synthetic xenobiotics, this process can significantly alter the biological activity of the initial compounds. While some modifications of the molecules of specialized metabolites of natural origin lead to an increase in the molecular weight and complexity of the compound, such as in the case of amentoflavone, a biflavonoid found in *Ginkgo biloba* leaves (Ginkgo folium, Ph. Eur.), which can be methylated by gut bacteria, the vast majority of biologically interesting postbiotic metabolites are formed as a result of simplifying their structure, leading to compounds with potentially increased bioavailability due to reduced molecular weight [60]. One of the most common modifications is the removal of the sugar residue from glycosides by bacteria-secreted glucosidases. This biotransformation significantly changes the pharmacokinetic properties of the compounds, producing more lipophilic aglycones, which are relatively well absorbed into the body and can exert a biological effect [61]. There are many compounds of natural origin whose sugar residues are detached due to the metabolic activity of gut microorganisms, resulting in the production of biologically active metabolites. An exceptionally interesting example of the impact of gut metabolism on the biological activity of compounds is the biotransformation of crocin, a carotenoid found in *Crocus sativus*, into its aglycone, crocetin. Utilizing a pseudo germ-free rats model, researchers have demonstrated the key

role of gut biotransformation of the glycoside into crocetin in its effect on brain ischemia outcomes. In the absence of glycoside transformation by gut bacteria, the beneficial effect of crocin administration was not observed, highlighting the significant impact of bacterial transformations on the expected therapy outcomes [62]. Another well-documented example of such biotransformation is the modification of the structure of the widely occurring flavonol– hyperoside, which is transformed into quercetin by detaching the beta-D-galactosyl residue from position 3 [63]. Importantly, this type of molecular change often serves only as a prelude to further modifications of the structure; quercetin is further transformed into compounds with smaller molecular weight, including phenolic acids such as 3-(3,4)-dihydroxyphenylacetic acid, whose potential impact on the body's homeostasis has not yet been fully determined [64].

From the perspective of impact on biological activity and bioavailability, the bacterial catabolism of condensed tannins, proanthocyanidins, e.g., procyanidin B2 found in red wine or hawthorn, and the formation of (–)-epicatechin, is significant. The breakdown of the dimer such as procyanidin B2 into epicatechin molecules through gut transformation can be crucial for the beneficial action of substances containing it [65]. While a series of *in vitro* studies indicate the high biological activity of procyanidin B2, including its strong anti-inflammatory and neuroprotective effects, studies focused on its bioavailability have revealed its low, nanomolar concentrations in the human body after consumption of products containing it, well below concentrations capable of inducing a biological effect [66,67]. The metabolism of procyanidin B2 into biologically active epicatechin significantly increases its bioavailability, and it is the monomeric form that may be responsible for the systemic action of traditionally used medicinal plants containing it [68]. Notably, as in the case of hyperoside, being a precursor to quercetin and then small phenolic acids, so too procyanidins undergoing hydrolysis to epicatechins, initiate a series of further transformations resulting from the activity of gut bacterial enzymes. Epicatechins, as well as other flavan-3-ols, are metabolized by the gut microbiota into bioavailable metabolites phenyl-γ-valerolactones and phenylvaleric acids; their further biotransformation in the intestinal lumen may lead to the production of 3-(3,4)-dihydroxyphenylacetic acid, serving as a link in the gut metabolism of hyperoside and proanthocyanidins (Fig 5) [69]. Although these compounds are increasingly receiving attention due to promising results concerning their anti-inflammatory and neuroprotective actions, they are still largely regarded as mere biomarkers of consumption of flavan-3-ol-rich products, such as green tea [70]. As with many postbiotic metabolites produced after oral administration of natural compounds, the biological activity and potential medical applications of phenyl- γ-valerolactones and phenylvaleric acids and their derivatives require further research and represent a promising area for the discovery of new API for further drug design and development.

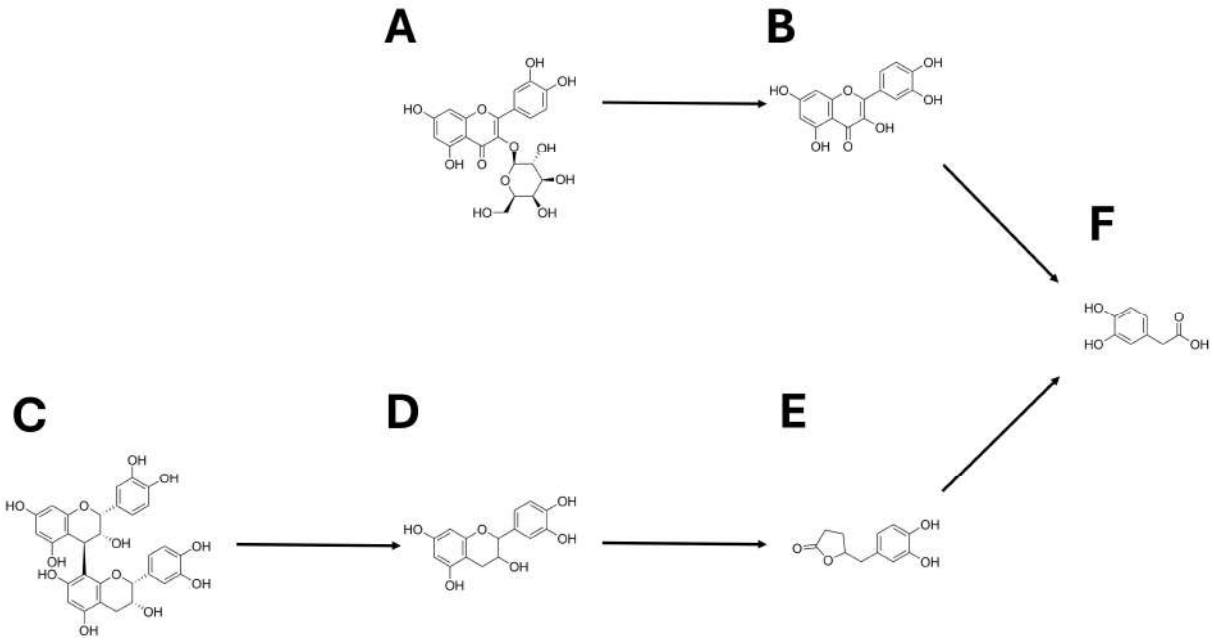


Figure 5. Gut bacteria catabolism of selected naturally occurring compounds (A) Hyperoside, (B) Quercetin, (C) Procyanidin B2, (D) (-)-Epicatechin, (E) 5-(3',4'-Dihydroxyphenyl)-γ-valerolactone, (F) 3-(3,4)-dihydroxyphenylacetic acid.

Another characteristic group of transformations of naturally occurring compounds in the intestinal lumen is the reduction of their oxidation state, particularly promoted due to the low oxygen content in the gut atmosphere. For some compounds, such transformations significantly influence bioactivity, as is the case with anthraquinones, active compounds traditionally used in laxatives such as senna leaf (Sennae folium, European Pharmacopoeia, Ph. Eur.) or Frangula bark (Frangulae cortex, Ph. Eur). The reduction of anthraquinones to anthrones by gut bacteria enhances their laxative effect, and individual variability among the gut microbiota and its ability to reduce anthraquinones in different patients may explain the varied metabolite profiles detected and potentially different responses to therapy [71]. Similarly, the reductive metabolism in the gut lumen is shared by another molecule well-known to the scientific community – resveratrol. This compound, belonging to the stilbenes and found among others in red wine, with a documented beneficial effect on the functioning of the vascular endothelium, is reduced by gut bacteria to two postbiotic metabolites – dihydroresveratrol and lunularin [72]. Importantly, it has been shown that the bioavailability of both compounds is higher than that of unmetabolized resveratrol, and at concentrations achieved in the body after oral administration, these metabolites exhibit greater anti-inflammatory activity than the unmetabolized compound [73].

A further intriguing example of the impact of gut bacteria on altering the activity of naturally occurring compounds is the intestinal metabolism of xanthohumol – the main flavonoid, more precisely, a chalcone, found exclusively in hops (*Humulus lupulus*). Hops cones are cultivated by

humans to be used in beer production as an additive providing the desired bitterness and it has been observed that women involved in harvesting hops suffer from menstrual cycle disorders and other side effects related to hormonal balance disturbance [74]. This observation has led to research into the bioactive compounds in hop cones responsible for this phenomenon. Although xanthohumol has proven strong anti-inflammatory action and is positioned as a potential anti-cancer agent, it does not affect estrogen balance [75]. However, following oral administration of xanthohumol or plant extracts containing it, it undergoes biotransformation by gut bacteria, presumably through isomerization to isoxanthohumol, to 6- and 8-prenylnaringenin, which are most likely to be responsible for the symptoms observed in hop pickers [76]. In addition to the mentioned postbiotic metabolites, xanthohumol is also a precursor to several other compounds obtained thanks to the activity of gut bacteria, although their biological activity has not been as well described as that of the parent compound [77].

When discussing plant-derived phytoestrogens, one cannot forget another postbiotic metabolite, equol, which is a product of the biotransformation of daidzein, an isoflavone found in soy. After cleavage of one of the daidzein's rings, biologically more active enantiomer - S-(-)-equol is formed in the gut [61]. This phenomenon is noteworthy as gut bacteria often produce only one optically active enantiomer of postbiotic metabolites, which may differ in biological activity from the others, often challenging to replicate using basic laboratory synthesis methods. As with xanthohumol and 8-prenylnaringenin, in this case, the intestinal biotransformation of daidzein to equol increases its anti-androgenic activity [78]. Moreover, it is speculated that increased soy consumption in the Asian population may be associated with a reduced occurrence of certain cancers, including prostate cancer, specifically due to the production of equol by the gut microbiota, although these hypotheses require further confirmation in clinical studies [79].

The final postbiotic metabolite to be discussed in this subsection – urolithin A (UA) – will be presented in more detail due to its relevance to the research themes of the scientific articles that form the foundation of this dissertation. UA is a perfect illustration of how rapidly knowledge about the metabolic activity of gut bacteria and the ability of individual microbial species to produce biologically active substances has developed in recent years. UA, along with other urolithins, is produced in the intestinal lumen following the consumption of natural substances containing hydrolysable tannins – ellagitannins. Among the products containing them are pomegranates, which are an exceptionally rich source of ellagitannins (e.g., punicalagins), accounting for up to half of all polyphenols they contain [80]. Besides pomegranates, high concentrations of ellagitannins are detected in food products such as walnuts, raspberries, and strawberries and medicinal plants, for instance *Filipendula ulmaria* herba, *Lythrum salicaria* herba and *Quercus robur* bark [81]. Ellagitannins, composed of hexahydroxydiphenic

acid units attached to a glucose molecule, despite their interesting bioactivity *in vitro*, such as demonstrated anti-inflammatory activity, are characterized by low bioavailability when administered orally [82]. These compounds undergo hydrolysis in the gastrointestinal tract, mainly in the stomach and small intestine, to ellagic acid (EA), a molecule of considerably lower molecular weight that also exhibits a range of biological activities *in vitro* similar to ellagitannins, but still characterized by poor bioavailability and occurring in the body at concentrations insufficient to evoke a biological effect [83]. However, in some individuals, thanks to the catabolic activity of gut bacteria, the lactone ring in EA is removed, resulting in the production of dibenzopyran-6-one derivatives – urolithins.

The first urolithin to form from ellagic acid is urolithin M5 (Uro-M5), which possesses five hydroxyl groups. Further biotransformations carried out by gut bacteria lead to the removal of additional hydroxyl groups, ultimately resulting in derivatives containing two hydroxyl groups (including UA and iso-urolithin A – isoUA) and one hydroxyl group – urolithin B (UB) [84]. The previously mentioned dynamic increase in knowledge about urolithins' metabolism in the gastrointestinal tract has led to the identification in recent years of several specific strains of gut bacteria, belonging, for instance to the genera *Enterocloster* or *Enterococcus*, capable of producing UA as the final product of metabolism of ellagitannins [85,86]. Considering the health-promoting properties of UA, which will be described in more detail later, this opens the path to using these bacteria as potential probiotics; however, it should be noted that the production process involving the formulation of such probiotics would require anaerobic conditions and, as mentioned earlier, evaluation of whether orally administered bacteria can colonize the human gastrointestinal tract for an extended period. UA, in comparison to ellagitannins and EA, is characterized by significantly better bioavailability, and its derivatives are present in human plasma at micromolar concentrations [87].

As mentioned above, UA as the final product of ellagitannins and EA metabolism occurs only in a portion of the population possessing a specific type and structure of gut bacteria community. Based on the ability of the gut microbiota to produce specific urolithins, three so-called metabotypes have been identified: metabotype A, found in the part of population where UA is the exclusive postbiotic metabolite after oral administration of ellagitannins; metabotype B, found in individuals where, besides UA, the presence of UB and isoUA is detected; and metabotype 0, in which none of the above urolithins are produced [88]. As mentioned earlier, the structure of the human gut microbiota is highly susceptible to changes induced by a range of external factors and naturally undergoes modifications with age. Similarly, in the case of urolithin metabotypes, although metabotype 0 remains at a relatively constant level in the population regardless of age group (about 10%), with age, a percentage increase in people with metabotype B is observed compared to metabotype A, which in the early years of life is almost ten times rarer than metabotype A, but with age, by the time of reaching the thirtieth year

of life, almost equals in frequency of occurrence in the Caucasian population [89]. Importantly, the concept of metabotypes is not limited to the production of various urolithins but similarly scientists stratify the population, for example, in terms of the ability to produce equol from daidzein or lunularin from resveratrol; moreover, recently, clustering these different metabotypes has been proposed, seeking connections between individual groups and their ability to produce biologically active postbiotic metabolites [90]. Identifying patient metabotypes appears to be an interesting and promising strategy, especially in the case of interventions based on compounds that are potential substrates for bacterial catabolism. Depending on the type and structure of the gut microbiota, the production of active or inactivated postbiotic metabolites can significantly affect the final effect of the therapy applied and may constitute an important parameter before starting treatment.

The biological activity of urolithins, especially UA, is the subject of numerous studies on diverse models, starting from in silico studies and relatively simple tests using isolated enzymes, through *in vitro* and *in vivo* models, to clinical studies evaluating the pharmacokinetic and pharmacodynamic properties of UA in humans. To date, it has been shown that UA appears to be superior in terms of biological activity compared to other urolithins, although a large part of the research also focuses on studying the activity of UB [91–93]. The therapeutic research area of UA also includes a wide spectrum of diseases, and the molecule itself shows promising antioxidant, anti-inflammatory, anticancer, neuroprotective or cardioprotective activities, muscle and skeletal system function improving properties and beneficial effects on maintaining intestinal barrier integrity [84,87,94]. Such a broad potential application and beneficial properties of UA *in vitro* should naturally raise scientific skepticism to avoid labeling this molecule as a miracle drug and panacea, especially considering the problems with translating *in vitro* research results into *in vivo* outcomes, which will be further discussed later in this work. However, despite the known limitations of UA due to its extensive phase II metabolites, several dietary supplements in the form of capsules and other formulations for oral administration of UA are available on the market.

The molecular basis for UA's positive impact on human health is being thoroughly investigated by researchers worldwide, and several intracellular mechanisms have been identified as key to its activity. UA has been shown to inhibit the phosphorylation of kinases involved in the stress response, including ERK 1/2, JNK, phosphatidylinositol 3-kinase, and it induces the inhibition of signal propagation in molecular pathways such as JAK-STAT and NF-κB signaling [84,95,96]. Particularly, the impact on signal transduction related to the latter has been meticulously studied, demonstrating that UA inhibits the nuclear translocation of NF-κB, consequently suppressing the expression of, among others, pro-inflammatory cytokines [95,97]. Moreover, UA has been shown to be a potent natural agent promoting autophagy and mitophagy, and by removing malfunctioning organelles, it improves cellular functioning,

which may be crucial especially in the context of UA's anti-aging properties and its improvement of muscle functioning [98,99]. UA is also a ligand for AhR, and, by being its agonist, UA exerts a positive effect on intestinal barrier integrity and increases the expression of TJPs [100]. As seen, *in vitro* studies indicate UA as an extremely active natural molecule, affecting numerous intracellular signal transmission pathways. However, many *in vivo* studies have shown a surprising lack of expected systemic effects after oral administration of UA. This seemingly paradoxical lack of activity has its rational evolutionary justification. Considering the ubiquity of plants containing ellagitannins and the activity of the urolithins produced from them, to maintain homeostasis and immunological balance, the human body developed numerous detoxifying enzymes that lead to the deactivation of xenobiotics. In the case of urolithins, these molecules undergo glucuronidation and sulfation already in the intestinal epithelium, thanks to the activity of specific transferases (UDP-glucuronosyltransferases (UGTs) and sulfotransferases (SULTs)), and finally, unconjugated UA is detectable in significantly lower concentrations in the body [84]. This process, part of the phase II metabolism, leads to the formation of polar metabolites, which are primarily removed from the body through kidneys. This process is also characteristic to other postbiotic metabolites derived from natural compounds, and similar conjugates are the dominant forms in the body of 6-/8-prenylnaringenin or γ-valerolactones [69,101]. Although phase II metabolism may lead to the formation of metabolites with potentially greater activity than the original compounds, such as in the case of morphine glucuronide, which has a higher affinity for the μ-opioid receptor than morphine, in most cases, it reduces the biological activity compared to the unconjugated molecule [102]. Similarly, studies focusing solely on UA glucuronides have shown that these conjugates do not exhibit anti-inflammatory or anticancer properties, unlike UA itself [91,103]. This detoxification process is responsible for the difficulties in translating *in vitro* results into *in vivo* studies and should be especially considered when designing studies evaluating the activity of urolithins after oral administration.

In summary, presented studies indicate a still vast reservoir of active substances in traditionally used medicinal plants, which can be used in the development of medicinal products. The increasing emphasis on the role of the human gut microbiota and its metabolites also opens perspectives for further, undiscovered or insufficiently studied molecules that may constitute the backbone of new APIs. Thus, the need to utilize high-throughput screening tests and further develop metabolomics and bioinformatics sciences is not surprising. Equally necessary is a critical view of newly discovered bacterial-derived molecules, avoiding the search for an unreal panacea and considering a range of mechanisms limiting their action *in vivo*. Most postbiotic metabolites, similar to unmetabolized natural compounds, require structural changes in their composition to achieve the desired biological effect after oral administration. However, the potential of other routes of administration of active substances should not be overlooked, as evidenced by the introduction of penicillin G into medicine.

8. Aim of the thesis

The goal of this dissertation was to present the potential applications and novel approaches to the utilization of biologically active postbiotic metabolites derived from plant products. The project aimed to implement various strategies for employing therapeutic potential of compounds formed as a result of the metabolic activity of gut microorganisms following oral administration of natural substances. This study began with a review that sheds new light on the therapeutic effectiveness of traditionally used plant substances. It progressed through basic research on synthetic derivatives of gut bacteria metabolites and concluded with implementation studies within the Lider XII program, leading to the commercialization of a product containing a known postbiotic metabolite active upon topical application to the skin. Consequently, the project's objectives were divided into three subcategories related to the strategy applied in studying these compounds:

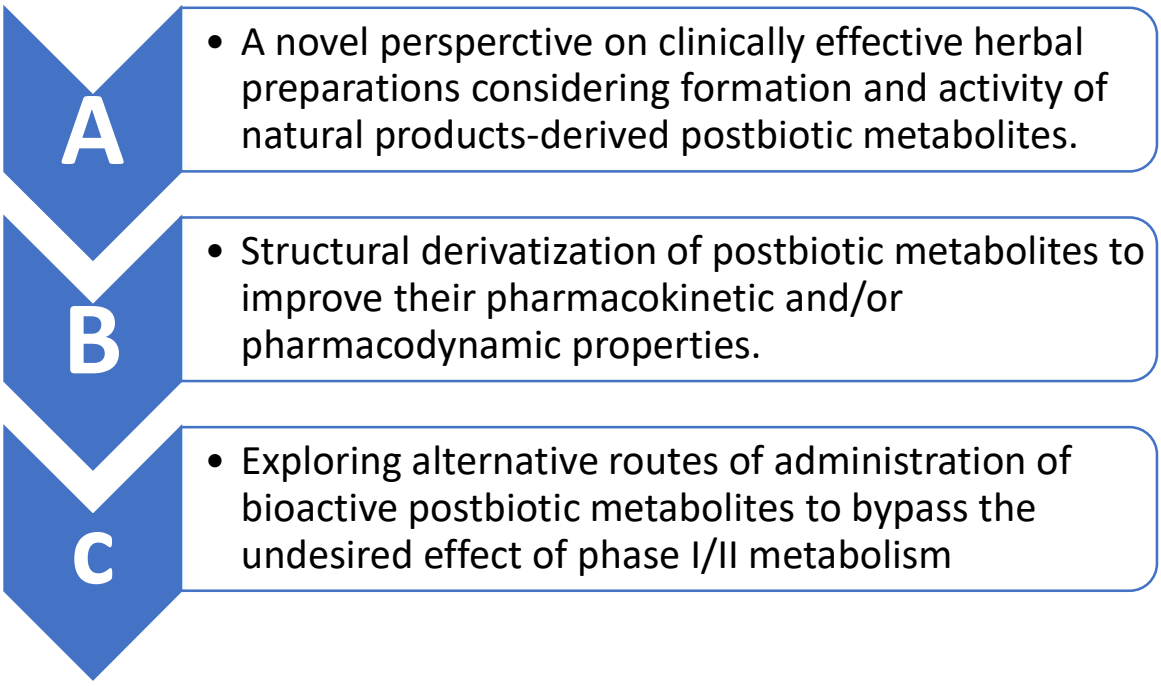


Figure 6. The proposed strategies for utilizing and investigating the properties of postbiotic metabolites derived from herbal substances.

8.1 Objectives of Strategy A

This strategy is based on a critical examination of the current knowledge concerning postbiotic metabolites formed after the consumption of natural substances, and the selection of promising compounds for further developmental research. Current scientific databases contain numerous studies on the interactions between natural substances and the gut microbiota. The abundance of articles, combined with the diversity of platforms and models for studying these relationships, may

obscure the final picture due to the overwhelming amount of data generated. Many plants used in traditional folk medicine contain compounds with demonstrated potent biological activity *in vitro*. However, *in vivo* studies, particularly those investigating their pharmacokinetic properties, reveal discrepancies between preliminary effects and actual therapeutic outcomes. This discrepancy partly arises because initial research often overlooks the influence of gut microbiota on the metabolism of natural compounds found in herbal substances. Frequently, these compounds are not sufficiently bioavailable, achieving too low concentrations in the body to produce the expected effects. It is the postbiotic metabolites derived from plant specialized metabolites, characterized by superior pharmacokinetic profiles compared to the original compounds, that may be responsible for the therapeutic effectiveness of medicinal plants, which molecular background of action often remain unexplained.

Strategy A underlines the role of postbiotic metabolites derived from natural products and postulates their formation as a key step in the light of which activity and mechanism of action of traditional herbal substances should be examined. Strategy A emphasizes the wealth of biologically active substances produced in the human gut and could serve as a starting point for further research on postbiotic metabolites. To narrow down the scope of this work and enable a critical assessment of the interactions between gut microorganisms and traditionally used natural products, plant materials applied in the phytotherapy of mild mood disorders were selected. Within Strategy A, four main objectives were demonstrated:

1. Summarizing the bilateral interactions between plant substances used in mood disorders (mainly depression and anxiety disorders), with a focus on produced postbiotic metabolites active in the context of these diseases.
2. Emphasizing the role of postbiotic metabolites in the activity of traditionally used herbal substances.
3. Identifying promising postbiotic metabolites that exhibit interesting biological activities, which could serve as potential molecules for developing active substances in the treatment of depression and anxiety disorders.
4. Highlighting current knowledge gaps and the insufficient number of preclinical studies focused on the interactions between the human gut microbiota and plant substances used in mood disorders. Especially considering the role of microbiota-gut-brain axis in mood regulation and emerging recognition for postbiotic metabolites' contribution to the activity of medicinal plants.

8.2 Objectives of Strategy B

As presented in the theoretical introduction of this dissertation, the chemical modification of the structure of naturally occurring substances is a common method for obtaining new APIs. **Similar**

modifications can be applied to postbiotic metabolites derived from compounds found in medicinal plants to enhance their beneficial properties. Strategy B involves the synthesis of derivatives of postbiotic metabolites that, while retaining the activity of the original compound, would have a more favorable pharmacokinetic profile than unmodified molecule. This process is time-consuming, often involving frequent changes in synthesis strategy and a multitude of biological tests, thus constituting the main body of the work performed within this doctorate.

Due to promising and well-documented *in vitro* biological activity and known limitations arising from *in vivo* phase II metabolism UA was chosen as the starting compound for this stage of research. The planned objectives of this strategy aimed to identify the most promising modifications of the UA molecule in terms of maintaining its biological activity while reducing susceptibility to intestinal epithelial cells' metabolism, primarily conjugation with glucuronic acid. Ultimately, these studies aimed to select those derivatives that, devoid of strong cytotoxic activity, would increase the bioavailability of UA's active form and open the door to further research, including *in vivo* studies using animal models of inflammatory diseases

1. Chemical synthesis of various synthetic UA derivatives (UADs)
2. Verification of the chemical stability of synthesized UADs under various conditions
3. Study of the cytotoxicity of UADs and their effect on cell proliferation
4. Verification of the anti-inflammatory activity of selected UADs *in vitro*
5. Investigation of intestinal transport and metabolism of UADs *in vitro*
6. Examination of the molecular background of UADs' activity

8.3 Objectives of Strategy C

Postbiotic metabolites derived from herbal substances often exhibit strong activity *in vitro*. However, as described in the chapter presenting the theoretical basis of the project, the high expression of detoxifying enzymes in the human gut wall and liver can quickly reduce the concentration of their active forms in the body and lead to the formation of biologically inactive metabolites. **In contrast to Strategy B, where derivatization of structure aimed to obtain active substances following oral administration, Strategy C proposes an approach to bypass this metabolism by applying the biologically active postbiotic metabolite, again UA, topically to the skin and utilize its anti-inflammatory potential in skin diseases like atopic dermatitis or psoriasis.** Preliminary studies, forming the basis for the patent application PCT/IB2019/060337, conducted on a rat model of topical inflammation showed that UA applied to the skin exhibits beneficial anti-inflammatory effects. Strategy C represents the initial steps towards introducing ointments containing UA as products used in treating atopic dermatitis.

In previous research, UA was obtained through chemical synthesis using a method developed by Bialonska et al [104]. According to regulations governing new medicinal products (The Rules Governing Medicinal Products in The European Community, Volume IV, EU Guidelines to Good Manufacturing Practice, Annex 13, 03 February 2010; FDA 21 CFR Part 210 Current Good Manufacturing Practice and Investigational New Drugs Intended for Use in Clinical Trials), API should be produced in compliance with cGMP requirements appropriate for each phase of preclinical and clinical studies. Moreover, it is recommended that active substances intended for toxicity studies in animals be produced in the same manufacturing facility, using the same production process, and maintaining the same contamination profile as the material to be used in phase I and II clinical trials.

According to FDA regulations "Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route", 2015, all medicinal products with a changed formula or new routes of administration must undergo acute and/or chronic toxicity studies with a full histological assessment using the clinical route of administration. The previous *in vivo* studies of UA's anti-inflammatory activity on the skin were conducted using a basic formulation containing UA emulsified in a semi-solid substrate - white petrolatum. To conduct preclinical studies on effectiveness and safety, it is necessary to develop a target formulation containing UA based on cGMP standards, which should be identical to that used in clinical studies. Considering patient preferences, the target formulation should be in the form of a cream. For these reasons, the objectives at this stage of the implementation research within Strategy C were as follows:

1. Developing the synthesis of UA on a semi-technical scale and producing the API based on standards compliant with Good Laboratory Practice (GLP) and current Good Manufacturing Practice (cGMP).
2. Developing a pharmaceutical formulation for topical application containing UA in concentrations of 0.5, 1.0, 2.0, and 5.0% based on cGMP standards.

9. Summary and Conclusions

9.1. Strategy A

9.1.1. Publication No. 1

Maciej Korczak, Maciej Pilecki, Sebastian Granica, Aleksandra Gorczyńska, Karolina Aleksandra Pawłowska, Jakub Patryk Piwowarski. Phytotherapy of mood disorders in the light of microbiota-gut-brain axis. Phytomedicine. 2023 Mar;111:154642. doi: 10.1016/j.phymed.2023.154642. Epub 2023 Jan 4. PMID: 36641978.

Publication No. 1, a review focused on the bilateral interactions between gut microbiota and plant substances traditionally used in mood disorders, presents the first summary of research as of November 2021 on this specific aspect of phytotherapy. The increasing incidence of depression and related diseases, along with the questioned efficacy of currently used medicinal products, underlines the need for new therapies and active substances [105]. On the other hand, as discussed in the previous chapters of this dissertation, scientists have identified the significant role gut bacteria play in modifying compounds found in natural products. These modifications can alter the structure and, consequently, both the pharmacodynamic and pharmacokinetic properties of the initial compounds. This biotransformation also involves compounds present in significant quantities in medicinal plants used to treat moderate depression. For this reason, the area of research on the impact of herbal medicines on mood disorders in the context of gut microbiota was chosen as the subject of this review article.

This paper is the result of a search of scientific databases such as Google Scholar, Science Direct, and Pubmed, critical analysis of the results obtained so far, and highlighting those areas that have not yet been sufficiently explored. To better understand the issues discussed in the review, it also includes an extensive introduction outlining the role of pharmacotherapy and gut microbiota in mood disorders and explaining basic concepts and parameters used to describe changes in its structure and activity. The review summarizes interactions between gut bacteria and eight species of medicinal plants used in treating depression/anxiety, chosen based on the indications of the European Medicines Agency and historically documented sources of their use in moderate mood disorders:

1. *Hypericum perforatum* L. (St. John's wort)
2. *Valeriana officinalis* L. (valerian)
3. *Piper methysticum* G.Forst. (kava kava)
4. *Passiflora incarnata* L. (maypop)
5. *Humulus lupulus* L. (hops)
6. *Melissa officinalis* L. (lemon balm)

7. *Lavandula officinalis* Chaix (lavender)
8. *Rhodiola rosea* L. (golden root)

Additionally, when discussing each of these plants, information about past preclinical and clinical studies evaluating the bioavailability of compounds contained in them and the effectiveness of these plants in treating mood disorders was added. These insights aim to highlight plants whose therapeutic effectiveness may not result directly from the absorption of contained compounds but might suggest a different mechanism, such as the formation of bioavailable postbiotic metabolites. This seems significant because many unmetabolized compounds in herbal medicines do not reach adequate concentrations in human tissues to induce a therapeutic effect [106]. The main part of the work presents how these plants and their active ingredients interact with gut microorganisms, both by changing the biodiversity parameters of the microbiota and the abundance of specific species, families, and other taxonomic levels of gut bacteria, as well as by modifying the structure of natural compounds and producing postbiotic metabolites. Numerous studies focused on the impact of plant extracts or isolated compounds on the structure of the gut microbiota, and at first glance, this area seems well-researched for many of these plants. This is undoubtedly linked to the increasing availability and reduced costs of next-generation sequencing, especially the sequencing of DNA coding bacterial 16s ribosomal RNA fragments. However, due to the variety of models used in studies, from *in vivo* research on rodent models, through *ex vivo* studies using human fecal samples incubated in anaerobic chambers, to sequencing gut microbiota samples sourced from humans participating in clinical trials where they were administered the studied substances, researchers encounter problems comparing the obtained results. Although each of these platforms has its unique advantages and disadvantages, the substantial differences in the structure of human and rodents gastrointestinal tracts and different relative amounts of specific gut bacteria compared to mice and rats, must be considered when analyzing data [106]. Moreover, despite the growing number of studies on natural compounds and their impact on the gut microbiota, it's noteworthy that these studies are not evenly distributed and while the impact of compounds such as quercetin seems well-documented in the literature, a part of less commonly occurring specialized metabolites, e.g., kavalactones, are seriously underrepresented in studies, especially those utilizing human gut bacteria.

In the context of review papers, equally if not more important than summarizing existing results is the identification of areas requiring greater attention from the scientific community, as well as outlining new directions for future research. Publication No. 1 demonstrates that one such area is undoubtedly the identification and examination of the biological activity of postbiotic metabolites derived from the plants. The bacterial catabolism of metabolites found in plants traditionally used in mood disorders has not been sufficiently explored and the antidepressant or anxiolytic activity of the

resulting postbiotic metabolites remains unknown in most cases. The results summarized in Table 2 of the review paper indicate that for some of the medical plants discussed, the production of postbiotic metabolites has not been studied at all, while for others, experiments were only conducted with isolated strains of gut bacteria. An interesting example discussed in the presented work are postbiotic metabolites derived from xanthohumol, whose biotransformation is relatively well documented, yet significantly underexplored in terms of efficacy in alleviating mood disorder symptoms. It is precisely the thorough investigation of the role of these metabolites derived from traditionally used herbal substances that should be one of the priorities in searching for new active substances for treating depression and anxiety as well as in understanding the mechanism of action of medicinal plants with confirmed effectiveness. The discussed review paper lays the groundwork for further research in these areas.

The work "Phytotherapy of mood disorders in the light of the microbiota-gut-brain axis" has opened up new areas of development for my research, unrelated to UA and its derivatives, which until now have been the main area of my scientific activity during my doctoral studies. **This article served as the basis for a grant application in the Preludium funding scheme of the National Science Center and two projects that were awarded funding, focusing on the study of interactions between human gut microbiota and active substances from hop cones.**

9.3. Strategy B

9.3.1. Publication No. 2

Korczak M, Roszkowski P, Granica S, Piwowarski JP. Conjugates of urolithin A with NSAIDs, their stability, cytotoxicity, and anti-inflammatory potential. Scientific Reports. 2022 Jul 8;12(1):11676. doi: 10.1038/s41598-022-15870-8. Erratum in: Scientific Reports. 2022 Nov 2;12(1):18503. PMID: 35804000; PMCID: PMC9270351.

As presented in the review of the current state of knowledge, phase II metabolism, occurring just after the absorption of urolithins, significantly reduces the concentration of its biologically active form in the body, resulting in the presence of inactive conjugates. Within Strategy B, structural modifications of postbiotic metabolites derived from compounds found in medicinal plants were designed, so that the newly obtained derivatives could overcome the limitations of the original compounds. In the case of the UA molecule, it is the detoxification processes occurring in the intestinal wall that limit its use as a biologically active molecule when administered orally to induce systemic anti-inflammatory action. Therefore, in the course of the research conducted by me, in collaboration with dr. Piotr Roszkowski (Faculty of Chemistry, University of Warsaw), we initiated the synthesis of UADs which were intended to overcome the limitations of UA's anti-inflammatory activity resulting from its conjugation with glucuronic acid.

To inhibit the activity of UGTs, the main enzymes responsible for the deactivation of orally administered UA, we decided to synthesize UA esters with representatives of various groups of nonsteroidal anti-inflammatory drugs (NSAIDs) - ibuprofen (IbuUA), mefenamic acid (MefUA), diclofenac (DicloUA), and acetylsalicylic acid (AspUA). NSAIDs act as UGTs' inhibitors, which was the reason for selecting them as potentially promising subunits for new UADs, intended to increase the bioavailability of the biologically active form of UA or its derivative [107,108]. It was planned that the obtained compounds could exhibit two alternative mechanisms of action:

1. UADs could cross the intestinal barrier in their unaltered form and enter the body as esters, thus acting on molecular targets involved in the site of inflammation.
2. UADs could undergo hydrolysis in the cell thanks to the activity of esterases, but the presence of free NSAIDs was to lead to increased bioavailability of the unconjugated, active form of UA in the body.

Additionally, selecting NSAIDs as potential conjugated units with UA could lead to a synergistic effect of the two UA subunits, associated with distinct anti-inflammatory mechanisms of action - UA primarily inhibiting the translocation of the NF-κB subunit to the cell nucleus and the associated expression of inflammatory mediators, and NSAIDs mainly inhibiting the activity of cyclooxygenase-2

(COX-2) and production of, among others, prostaglandins. This synergic activity could decrease the necessary dosage of NSAIDs to obtain therapeutic effects and lower the probability of, for instance, gastrointestinal tract side effects [109].

To minimize the molecular weight of the obtained UADs and to preserve one free phenolic group in the molecule, as in the case of the biologically active UB, firstly the synthesis of mono-substituted UADs was planned. Initial attempts to obtain such UADs were unsuccessful due to the low stability of the ester bond, and its disruption during demethylation stage. Therefore, we finally opted to synthesize 4 mixtures of UADs, containing isomers of molecules esterified at positions 3- or 8- with the respective NSAIDs (Fig 7). Due to the similarity in the structure of isomers in these mixtures, it was impossible to separate these compounds using preparative chromatography, so further studies were conducted on mixtures of isomers. The identity and purity of the obtained compounds were confirmed using HPLC-DAD-MS/MS and NMR techniques, which also enabled the determination of the isomer ratios in the obtained mixtures of compounds.

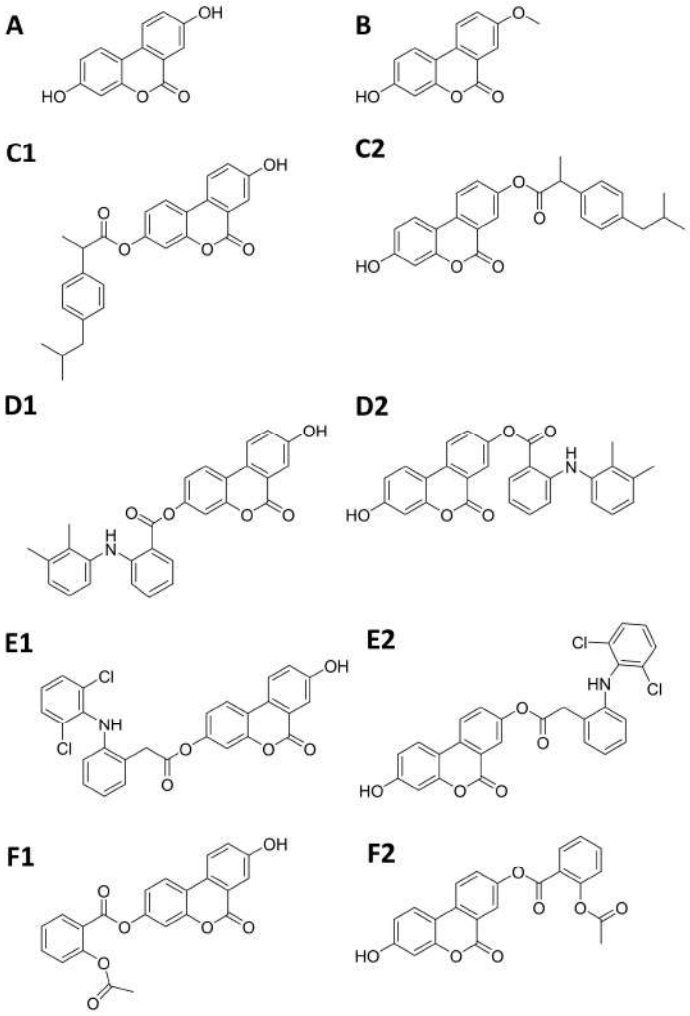


Figure 7. Structures of synthesized UADs esterified with NSAIDs. IbuUA (A1, A2), MefUA (B1, B2), DicloUA(C1, C2), and AspUA (D1, D2).

An essential step, often neglected in the investigation of newly synthesized compounds, is the evaluation of their stability under various environmental stressors. This step is of significant importance, enabling the acquisition of crucial information about new derivatives at the early stages of research, which may minimize the time and costs associated with their further investigation. It also allows for the selection of compounds that exhibit the chemical stability required for their application as new APIs and are most likely to interact within the human body. In this work, I conducted stability studies of UADs using HPLC-DAD-MS/MS under different thermal conditions, as well as under conditions of elevated and lowered pH, exposure to UV radiation, and in human plasma obtained from the Regional Blood Donation and Blood Treatment Center in Warsaw (ul. Saska 63/75, 03-948, Warsaw). The synthesized UADs displayed variable chemical stability; for instance, all underwent hydrolysis in alkaline conditions, with MefUA and DicloUA being more stable in acidic environments and after exposure to UV radiation compared to the other derivatives. However, only MefUA exhibited high stability in human plasma. For this reason, this derivative was chosen for further *in vitro* studies using human macrophages derived from the THP-1 line.

Subsequent studies aimed to preliminarily determine the biological activity of the newly synthesized derivatives and assess to what extent the esterification with mefenamic acid of one phenolic group of UA influences its anti-inflammatory properties and whether, upon reaching circulation, these derivatives could indeed produce the intended effect. The use of THP-1 macrophages, transformed from monocytes with phorbol myristate acetate, enabled the investigation of the cytotoxic properties and anti-inflammatory activity of UADs on human cells, significantly reducing the interindividual variability characteristic of studies conducted with an alternative model of peripheral blood mononuclear cells isolated from leukocyte concentrate. The use of human cells rather than mouse or rat cells at this stage of research is extremely useful, facilitating the translation of obtained results to the expected therapeutic effect. The cytotoxicity and effect on the proliferation of THP-1 monocytes of the UA ester mixture with mefenamic acid were investigated using MTT assay, neutral red uptake assay, and propidium iodide staining combined with flow cytometry techniques. The experiments showed that, unlike UA, MefUA exhibited cytotoxicity at a concentration of 50 µM, therefore, a lower concentration of the derivative was used for further investigation of anti-inflammatory action.

The effect of MefUA on modulating the inflammatory response was investigated using enzyme-linked immunosorbent assay. This made it possible to demonstrate the anti-inflammatory effect of UADs at the protein level, in this case, specific cytokines involved in the development and inhibition of inflammation released from macrophages. MefUA showed a different activity compared to unconjugated UA. MefUA nonlinearly inhibited the secretion of TNF-α after 3 hours of stimulation

of THP-1 macrophages with LPS, reducing its quantity when using 5 µM but not at 10 µM, while UA linearly inhibited its secretion. This contradictory picture may arise from the opposing actions of two MefUA subunits, where the inhibitory effect on TNF-α secretion by the UA molecule could be diminished by the mefenamic acid subunit, which increased the release of this pro-inflammatory cytokine, as demonstrated in this work. Unlike UA, MefUA did not inhibit the secretion of another pro-inflammatory cytokine - interleukin 6 but increased the release of anti-inflammatory interleukin 10.

This work has shed new light on the possibility of chemical modification of postbiotic metabolite structures. The results presented in this paper show that despite the esterification of one phenolic group of UA, the obtained derivatives still exhibit anti-inflammatory activity. However, to better understand the impact of UA molecule modification on its biological activity and to thoroughly investigate the structure-activity relationship, further studies and multi-step regioselective syntheses are required. Esterification of UA's phenolic groups appears to be a promising strategy, yet it is essential to consider the limited stability of the resulting derivatives, as demonstrated in this work. Additionally, in the course of the conducted research, I highlighted the cytotoxic effect of MefUA at a concentration of 50 µM, which may suggest a potentially limited safety and toxicity of this particular derivative, but also its significant biological activity, visible *in vitro* at a concentration of 5 µM. The results also suggest a mechanism of intracellular action of UADs different from that of UA, likely related to the parallel influence of the NSAID subunit.

9.3.2. Publication No. 3

Korczak M, Roszkowski P, Skowrońska W, Żołdak KM, Popowski D, Granica S, Piwowarski JP. Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function. Biomedicine & Pharmacotherapy. 2023 Dec 31;169:115932. doi: 10.1016/j.biopha.2023.115932. Epub 2023 Nov 24. PMID: 38000358.

The logical continuation of the research presented in publication number 2 was to assess the potential *in vitro* bioavailability of the newly obtained UADs. For this purpose, a model of human Caco-2 cells derived from colon carcinoma was utilized. These cells, when seeded on inserts and differentiated, constitute the gold standard for evaluating the intestinal absorption of compounds *in vitro*. Additionally, due to the expression of phase I and II metabolic enzymes, they allow for a preliminary assessment of compound biotransformation and metabolism. Considering the documented beneficial effect of UA on the integrity of the intestinal barrier, it was reasonable to also examine the impact of UADs on this parameter *in vitro*. To this end, a cellZscope device (nanoAnalytics, Münster, Germany) was used, enabling the continuous measurement of transepithelial electrical resistance (TEER) value, an indicator of the integrity of the cell monolayer, without the need to remove cells from the incubator. This helped to reduce TEER measurement disturbances, increasing the uniformity of results, and facilitating the study of the tested compounds' impact during prolonged incubations.

In the presented study, UA and UADs were applied to the apical sides of fully differentiated Caco-2 cell monolayers seeded on inserts and placed in a cellZscope device, followed by a 24-hour incubation. After 24 hours, the content of the medium on both the apical and basolateral sides was analyzed using HPLC-DAD-MS/MS. Due to the observed drastic decrease in TEER values during incubation of Caco-2 cell monolayers with 100 µM MefUA, indicating increased cellular barrier permeability and a decrease in cell viability, confirmed by the MTT assay and Annexin V and propidium iodide staining, a lower concentration of MefUA, which did not induce the observed cytotoxic effects, was used for *in vitro* bioavailability studies. Such toxicity was not observed for UA and other UADs. The results obtained were consistent with the findings from studies evaluating the cytotoxicity of MefUA on THP-1 cells, for which this mixture of isomers was more cytotoxic than unmodified UA.

The results from the transport studies of UADs through the Caco-2 cell monolayer confirmed earlier conclusions from publication No. 2 indicating their limited stability and did not show the presence of UADs on the basolateral side 24 hours after apical administration of the tested compounds. Glucuronides, free UA molecule, and trace amounts of sulphates were detected on the basolateral side following apical administration of UA or UADs. However, importantly, all derivatives increased the ratio of active, unconjugated UA to inactive glucuronides on the basolateral side compared to UA

administration. Notably, one of the derivatives, DicloUA, significantly increased the UA concentration on the basolateral side. The presence of intracellular esterases is utilized during the design of ester derivatives of compounds as so-called "prodrugs" that release their active form only within the cell [110]. The experiments conducted so far indicated that the second of the mechanisms of action mentioned in subsection 8.3.1. might contribute to the beneficial effect of UADs (especially DicloUA) on inhibiting systemic inflammation after oral administration.

In parallel with the transport studies, the impact of UADs on the integrity of Caco-2 cell monolayers was measured. In this case, only UA, DicloUA, and AspUA demonstrated a beneficial and statistically significant effect on enhancing the barrier function of intestinal epithelial cells *in vitro*. Utilizing Western blotting techniques to examine changes in the expression of TJPs, I confirmed the positive influence of UA and UADs on the levels of these proteins (although to varying degrees), which are responsible for maintaining the integrity of the intestinal epithelium. Notably, the most pronounced effect was observed for DicloUA and AspUA, including additionally a reduction in the expression of claudin-2, a member of the pore-forming proteins whose elevated levels increase intestinal permeability. The results obtained, both from publication No. 2, indicating the greatest stability of DicloUA in acidic environments (compared to other UADs), and its beneficial effect on inhibiting the glucuronidation of UADs and on the tightness of the intestinal barrier *in vitro* as shown in publication No. 3, allowed for the selection of this derivative for further studies aimed at precisely examining the molecular bases of the observed health-promoting effects. Caco-2 cell monolayers exposed to DicloUA underwent next-generation RNA sequencing. Transcriptome analysis after 24 hour incubation with testes compounds revealed significant changes in gene expression related to several intracellular processes following DicloUA administration, including multivesicular body organization or zinc ion homeostasis (involved in maintaining intestinal barrier integrity) [111,112]. Interestingly, no statistically significant changes were detected in the enrichment of biological processes between cells treated with DicloUA and UA alone. This suggests that the observed changes in metabolism and biological activity between these compounds were not due to changes at the mRNA level, but possibly due to direct inhibition of UGTs by diclofenac or other post-translational modifications.

Results published in publications No. 2 and 3 served to collect an extensive set of preclinical data that could be used further the development of UA derivatives conjugated with NSAIDs and other molecules as part of the proposed Strategy C. The research presented in publication No. 3 identifies DicloUA as a promising molecule for further study using animal models, to confirm the hypothesis that esterification with diclofenac may lead to increased bioavailability of free UA *in vivo*. However, animal studies would require scaling up the synthesis of the compounds under investigation and developing an appropriate drug formulation, which, similar to the development of UA formulations for topical

application on the skin, represents a separate technological challenge as presented in Strategy C. The derivatization of the UA structure presented here is not an isolated case of using this molecule as a starting compound for further chemical modifications; however, it was the first approach of this kind focused on potentially increasing the bioavailability of UA's active form and bypassing phase II metabolism effects.

9.2. Strategy C

Given the implementation nature of the research conducted within the project "Synthesis of API, development of formulation and *in vivo* studies of a cream containing human gut microbiota postbiotic metabolite – U228 for the topical treatment of atopic dermatitis," funded by the National Centre for Research and Development, the results could not be published and can be found in project reports. Obtained results form the basis for further efforts aimed at commercializing the project, to be undertaken following the conclusion of the work financed under the Lider XII program. Within the scope of Strategy C, I participated in coordination of research with external contractors:

1. Synthex Technologies, responsible for:
    1. The synthesis of the required quantity of UA (500 g) with quality and under conditions meeting the requirements for APIs.
    2. Developing an analytical method (HPLC-MS/MS), identification, and isolation/synthesis of impurities in quantities up to 50 mg.
    3. Studies on forced degradation of UA.
    4. Preparing a report on the work done, and an internal analytical certificate for UA.

2. Pikralida, responsible for:
    1. Studies on the solubility of UA.
    2. Developing an O/W formulation type. Tests of W/O formulations.
    3. Studies on the compatibility of API and excipients and preparing a summary report.
    4. Developing HPLC methods for analyzing the content of API, related substances, and preservatives in the product.
    5. Preparing prototypes of topical formulations.

9.2.1. Semi-technical scale synthesis of UA

The synthesis of UA began with commercially available 2-bromo-5-methoxybenzoic acid, which was subjected to a demethylation reaction in the presence of an excess of $AlCl_3$ in chlorobenzene at 120°C. The resulting crude bromoacid was used in the next step without further purification. Then, the mixture with resorcinol in aqueous sodium hydroxide under copper catalysis led to the formation of UA (Figure 8), with a yield of 60%. The obtained UA, in the form of a yellow powder, underwent a micronization process through sequential mechanical grinding of the material and sieving through sieves up to 150 µm in particle diameter. Levels of resorcinol and 2-bromo-5-hydroxybenzoic acid in UA samples were determined by HPLC-MS/MS quantitative analysis in multiple reaction monitoring mode.

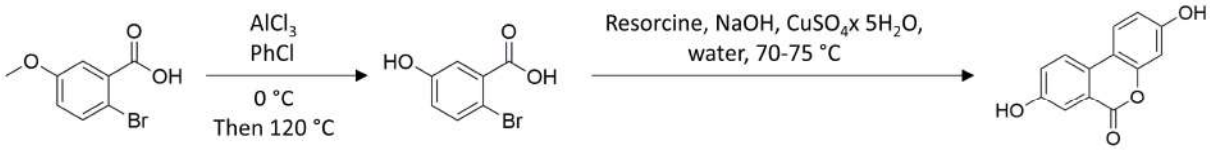


Figure 8 . General synthetic route of UA

Forced degradation studies showed that UA is stable (degradation level did not exceed 10%) over 25 hours in an aqueous environment, 1M HCl, and 30% H_2O_2, while degradation at a level of 14% occurred after 25 hours in 0.01M NaOH and 99% in 0.1M NaOH at room temperature. Increasing the temperature to 50°C did not increase the degradation level of UA. Additional studies confirmed that UA is not susceptible to photolytic degradation in solid form over 45 days, although degradation of a methanol solution of UA under light exposure was confirmed after 6 days.

9.2.2. Development of a formulation containing UA for topical application

The objective of these studies was to develop a therapeutic cream prototype containing UA that takes into account the *in vivo* delivery of the active ingredient to achieve a therapeutic effect. The cream's formulation was designed to minimize the risk of irritation and to be chemically and physically stable during storage. Similar to the API synthesis, an HPLC method was developed and validated for confirming the identity and content of UA in the product.

In order to create an appropriate form of UA application on the skin, the research at this stage began with determining its solubility degree according to the nomenclature of the European Pharmacopoeia in various organic solvents and water. The conducted studies indicate that UA is easily soluble in dimethyl sulfoxide, very slightly soluble in acetone and tetrahydrofuran, and practically insoluble in water, isopropanol, methanol, ethanol, acetonitrile, and dichloromethane. Furthermore, it was demonstrated that the application of ultrasonic waves to the sample allows for the complete dissolution of UA in acetone at a concentration of 1:1000 and in methanol at 1:10000. Subsequently, to select appropriate excipients for the final formulation, the compatibility of UA with excipients such as Eucerin, medium-chain triglyceride oil, cetearyl alcohol, cetyl palmitate, sorbitan stearate, isopropyl myristate, plurol diisostearate, polysorbate 60, a mixture of methyl paraben and propyl paraben (2:1), and benzyl alcohol was investigated. Over a 28-day period, using HPLC-MS/MS methods, no incompatibilities or increased content of related substances were observed at conditions of 40°C and 75% relative humidity, thus all tested excipients were suitable for use in the preparation of a topical formulation containing UA.

The development of the UA cream formulation commenced with testing oil-in-water (O/W) cream formulations, where the continuous phase is the water phase, and the dispersed phase is the oil phase. Similar to the UA synthesis, a separate HPLC method for the analysis of the UA identity, related substances, and preservatives was designed for the formulation. UA was suspended in the water phase, in which preservatives were previously dissolved, and then added to the oil phase in the process of phase combination - emulsification. After selecting the appropriate water content in the cream, a formulation with proper density, homogeneous, easily spreadable on the skin surface, and stable at room temperature was obtained. Five concentrations of this formulation with UA: 0.5%, 1%, 2%, 3%, and 5% with a pH of 5-6 were prepared. Attempts were also made to obtain a formulation based on the reference positive control cream containing 1% of hydrocorisonum, due to difficulties in obtaining a cream of the right consistency combined with unpleasant application of the obtained creams, the development of this type of prototype product was discontinued. Creams of the water-in-oil type, where the continuous phase is the oil phase, were also prepared. Thanks to the use of

appropriate emulsifiers, creams with proper consistency, greasy, glossy, stable at room temperature, and well-spreading on the skin were obtained.

During the stability study of the emulsions over time, it was observed that the water-in-oil emulsion separated after 14 days at 40°C and 75% relative humidity, which led to its exclusion from further developmental studies. During a 6-month incubation at 40°C and 75% relative humidity, no differences in the content of API, resorcinol, and unknown impurities were observed for the two extreme concentrations of the cream compared to the initial formulation, hence the obtained oil-in-water emulsion was considered a stable formulation, suitable for further development work. A study on the permeability of UA from the obtained cream was also conducted on the tissue model of reconstructed human epidermis, EpiDerm Mattek. The experiments showed that within 4 hours of application of 7.44 mg of cream, the percentage of the penetrated substance remained at a level of 0.071-0.124%, while after 24 hours, UA was detected at levels between 5.090% and 10.798% relative to the mass of the applied formulation. These values are close to the permeability results of medicinal formulations containing lidocaine, flurandrenolide, metronidazole, and tacrolimus demonstrated in *in vitro* studies using pig skin in a static Franz diffusion cell system [113].

9.4. Conclusions

In my research, I have presented various strategies for utilizing the bioactive potential of natural products-derived postbiotic metabolites. This work highlights the significance of further research into biologically active metabolites generated by the metabolic activity of gut bacteria. It summarizes the existing knowledge on postbiotic metabolites derived from compounds found in plants traditionally utilized in the treatment of mild mood disorders. In some cases, these postbiotic metabolites, rather than the specialized metabolites found in medicinal plants, may be responsible for the observed therapeutic effect of herbal substances, and may serve as new leading molecules in further drug design and development. My experimental work took a dual approach to the use of postbiotic metabolites, selecting UA molecule as a benchmark compound. Understanding the bioactivity of UA and its limitations due to phase II metabolism *in vivo*, on one hand, I used the UA molecule structure as a basis for further chemical modifications aimed at reducing the glucuronidation process, and on the other hand, these limitations were bypassed by creating a UA formulation intended to exhibit anti-inflammatory effects on the skin. The results I obtained provide directions for further research on postbiotic metabolites, primarily highlighting:

1. **Insufficient knowledge on the antidepressant activity of postbiotic metabolites derived from plants traditionally used in mood disorders** – In publication no. 1, I demonstrated that for some of these plants, the formation and biological activity of postbiotic metabolites have not been adequately researched (and for some plants, not at all). For instance, for postbiotic metabolites derived from hops cones, such as 6-/8-prenylnaringenin or desmethylxanhothumol, neither antidepressant nor anxiolytic activity has been examined, which represents a significant gap in our understanding of the activity of these often centuries-old used plants.
2. **Some postbiotic metabolites derived from plants traditionally used in mood disorders show promising activity in preclinical studies** - Despite the gaps in current knowledge regarding the antidepressant activity of these postbiotic metabolites, some compounds have shown promising results in preclinical studies. These compounds may be interesting candidates for further development and use in treating mood disorders. In some cases, it is the postbiotic metabolites rather than the unmetabolized compounds that are responsible for the observed therapeutic effects, as seen with crocin-derived crocetin.
3. **Limited stability of UA ester derivatives** – Depending on the strategy in drug design, the use of ester bonds can be helpful or impede obtaining postbiotic metabolite derivatives with intended activity. In the synthesis of so-called “prodrugs”, ester bonds are easily hydrolyzed due to the ubiquity of esterases and release the active form of the molecule in the cell.

However, too early hydrolysis of such a bond may limit the usefulness and rationale of using new derivatives. For the synthesis of derivatives with a modified structure compared to the original molecule and stable in host tissues, it seems necessary to synthesize derivatives containing more resistant chemical connections.

4. **High cytotoxicity of UA conjugates with mefenamic acid** - Despite their anti-inflammatory activity, MefUA is also characterized with relatively high cytotoxicity. However, this does not exclude potential development towards applications in, e.g. cancer treatments. Especially considering that the conducted *in vitro* studies were performed using human cancer cells.
5. **UA seems an excellent molecule for further developmental research on its derivatives** – Despite their limitations, ester derivatives showed promising results in *in vitro* studies, reducing the effects of phase II metabolism as in the case of UA esters with diclofenac. Thanks to the well-understood metabolism of UA and its properties and biological activity, it is possible to specifically target changes in its structure and study the impact of specific modifications on its properties and biotransformation *in vitro*/*in vivo*. The obtained results can serve as a basis for planning further research using animal models. Further work on the derivatives obtained so far and the synthesis of new ones seems advisable to overcome the limitations of the original molecule upon oral administration.
6. **The semi-technical scale synthesis of UA allows the production of a product with the requisite purity according to cGMP standards** - By using relatively inexpensive, commercially available reagents, a two-stage synthesis of a sufficient quantity of pure UA for further *in vitro* research and initial clinical trials on a small group of volunteers is feasible.
7. **UA forms stable O/W formulation for skin application at a concentration of 5%** – By using appropriate auxiliary substances and water composition in the finished cream, it is possible to produce a product containing even 5% UA, with proper physicochemical properties enabling its application and distribution on the skin. Conducted *in vitro* studies demonstrated UA's skin penetration ability from the developed formulation.

Considering the experience gathered during the work on UA derivatives, I have started the next stage of these experiments. The conclusions drawn have resulted in the synthesis of new potentially more stable ether derivatives of UA, conjugated with neurotransmitters, whose anti-inflammatory activity and insight into their molecular action have been investigated by me during a six-month internship at the University of Vienna in the Molecular Targets research group under the supervision of Professor Elke Heiß. In a broader perspective, these compounds may be further developed in the context of neurodegenerative and neuroinflammatory diseases, and the studies constitute an excellent introduction to subsequent international projects focused on UADs research. Additionally, established collaboration between Microbiota Lab and Molecular Targets resulted in

another grant application and accepted project, a 6-month internship in Vienna founded by The Walczak Programme, during which the in-depth analysis of the anti-inflammatory activity of hop-derived postbiotic metabolites and their phase II metabolites is planned.

Similarly, the studies presented in Strategy C are currently ongoing, with the formulation containing UA being tested for its efficacy in treating the symptoms of atopic dermatitis *in vivo*. As part of the Lider XII project, a pilot model of atopic dermatitis in Sprague-Dawley rats was developed (approval number WAW2/106/2023). Moreover, using the aforementioned model, a new set of experiments evaluating the anti-inflammatory activity of a UA-containing cream has recently been approved by the local ethical committee (approval number WAW2/036/2024). Rats with topical inflammation induced on the back skin by the application of 2,4-dinitrochlorobenzene will receive either the UA-containing formulation, a placebo or a positive control (hydrocortisone ointment). Subsequently, they will undergo behavioral assessment (frequency of scratching). At the end of the experiment, tissues will be collected, and the levels of inflammatory markers will be examined using immunohistochemistry, qPCR, and Western blot techniques. The results obtained will represent another milestone in the commercialization process of research on the use of UA formulations for the topical treatment of atopic dermatitis.

10. Co-authors' statements

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji "Phytotherapy of mood disorders in the light of microbiota-gut-brain axis." opublikowanej w czasopiśmie Phytomedicine do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko współautora	Udział procentowy	Zakres wkładu
Maciej Korczak	70%	Konceptualizacja, przegląd literatury, organizacja procesu tworzenia manuskryptu, pisanie głównej części manuskryptu oraz edycja gotowego manuskryptu, odpowiedź na uwagi recenzentów, proofreading
Maciej Pilecki	5%	Przegląd literatury, pisanie manuskryptu – sekcja „Microbiota-gut-brain axis"
Sebastian Granica	5%	Przegląd literatury, pisanie manuskryptu – sekcje „Preclinical studies"
Aleksandra Gorczyńska	5%	Przegląd literatury, pisanie manuskryptu – sekcja „Influence of plant extracts on microbial growth"
Karolina A. Pawłowska	5%	Przegląd literatury, pisanie manuskryptu – sekcje „Clinical Studies"
Jakub P. Piwowarski	10%	Konceptualizacja, przegląd literatury, pisanie (sekcja – „Significance of phytotherapy in mild mood disorders" oraz część „Conclusions and perspective") oraz edycja gotowego manuskryptu, odpowiedź na uwagi recenzentów

Czytelny Podpis:

Data:

3.04.24

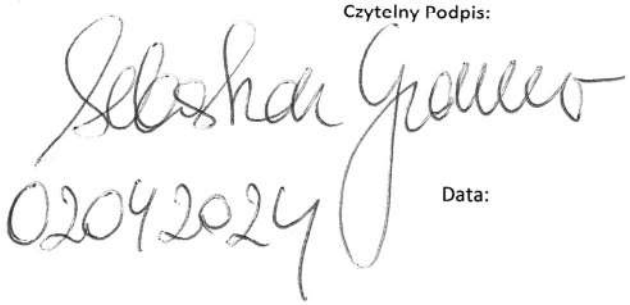
Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji "Phytotherapy of mood disorders in the light of microbiota-gut-brain axis." opublikowanej w czasopiśmie Phytomedicine do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko współautora	Udział procentowy	Zakres wkładu
Maciej Korczak	70%	Konceptualizacja, przegląd literatury, organizacja procesu tworzenia manuskryptu, pisanie głównej części manuskryptu oraz edycja gotowego manuskryptu, odpowiedź na uwagi recenzentów, proofreading
Maciej Pilecki	5%	Przegląd literatury, pisanie manuskryptu – sekcja „Microbiota-gut-brain axis"
Sebastian Granica	5%	Przegląd literatury, pisanie manuskryptu – sekcje „Preclinical studies"
Aleksandra Gorczyńska	5%	Przegląd literatury, pisanie manuskryptu – sekcja „Influence of plant extracts on microbial growth"
Karolina A. Pawłowska	5%	Przegląd literatury, pisanie manuskryptu – sekcje „Clinical Studies"
Jakub P. Piwowarski	10%	Konceptualizacja, przegląd literatury, pisanie (sekcja – „Significance of phytotherapy in mild mood disorders" oraz część „Conclusions and perspective") oraz edycja gotowego manuskryptu, odpowiedź na uwagi recenzentów

Czytelny Podpis:



Data: 02042024

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji "Phytotherapy of mood disorders in the light of microbiota-gut-brain axis." opublikowanej w czasopiśmie Phytomedicine do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko współautora	Udział procentowy	Zakres wkładu
Maciej Korczak	70%	Konceptualizacja, przegląd literatury, organizacja procesu tworzenia manuskryptu, pisanie głównej części manuskryptu oraz edycja gotowego manuskryptu, odpowiedź na uwagi recenzentów, proofreading
Maciej Pilecki	5%	Przegląd literatury, pisanie manuskryptu – sekcja „Microbiota-gut-brain axis"
Sebastian Granica	5%	Przegląd literatury, pisanie manuskryptu – sekcje „Preclinical studies"
Aleksandra Gorczyńska	5%	Przegląd literatury, pisanie manuskryptu – sekcja „Influence of plant extracts on microbial growth"
Karolina A. Pawłowska	5%	Przegląd literatury, pisanie manuskryptu – sekcje „Clinical Studies"
Jakub P. Piwowarski	10%	Konceptualizacja, przegląd literatury, pisanie (sekcja – „Significance of phytotherapy in mild mood disorders" oraz część „Conclusions and perspective") oraz edycja gotowego manuskryptu, odpowiedź na uwagi recenzentów

Czytelny Podpis:

Aleksandra Gorczyńska

Data:

08/04/2024

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji "Phytotherapy of mood disorders in the light of microbiota-gut-brain axis." opublikowanej w czasopiśmie Phytomedicine do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko współautora	Udział procentowy	Zakres wkładu
Maciej Korczak	70%	Konceptualizacja, przegląd literatury, organizacja procesu tworzenia manuskryptu, pisanie głównej części manuskryptu oraz edycja gotowego manuskryptu, odpowiedź na uwagi recenzentów, proofreading
Maciej Pilecki	5%	Przegląd literatury, pisanie manuskryptu – sekcja „Microbiota-gut-brain axis"
Sebastian Granica	5%	Przegląd literatury, pisanie manuskryptu – sekcje „Preclinical studies"
Aleksandra Gorczyńska	5%	Przegląd literatury, pisanie manuskryptu – sekcja „Influence of plant extracts on microbial growth"
Karolina A. Pawłowska	5%	Przegląd literatury, pisanie manuskryptu – sekcje „Clinical Studies"
Jakub P. Piwowarski	10%	Konceptualizacja, przegląd literatury, pisanie (sekcja – „Significance of phytotherapy in mild mood disorders" oraz część „Conclusions and perspective") oraz edycja gotowego manuskryptu, odpowiedź na uwagi recenzentów

Czytelny Podpis:

Karolina Pawłowska

Data:

04.04.2024

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji "Phytotherapy of mood disorders in the light of microbiota-gut-brain axis." opublikowanej w czasopiśmie Phytomedicine do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko współautora	Udział procentowy	Zakres wkładu
Maciej Korczak	70%	Konceptualizacja, przegląd literatury, organizacja procesu tworzenia manuskryptu, pisanie głównej części manuskryptu oraz edycja gotowego manuskryptu, odpowiedź na uwagi recenzentów, proofreading
Maciej Pilecki	5%	Przegląd literatury, pisanie manuskryptu – sekcja „Microbiota-gut-brain axis"
Sebastian Granica	5%	Przegląd literatury, pisanie manuskryptu – sekcje „Preclinical studies"
Aleksandra Gorczyńska	5%	Przegląd literatury, pisanie manuskryptu – sekcja „Influence of plant extracts on microbial growth"
Karolina A. Pawłowska	5%	Przegląd literatury, pisanie manuskryptu – sekcje „Clinical Studies"
Jakub P. Piwowarski	10%	Konceptualizacja, przegląd literatury, pisanie (sekcja – „Significance of phytotherapy in mild mood disorders" oraz część „Conclusions and perspective") oraz edycja gotowego manuskryptu, odpowiedź na uwagi recenzentów

05.04.2024

Czytelny Podpis:

Jakub Piwowarski

Data:

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji „Conjugates of urolithin A with NSAIDs, their stability, cytotoxicity, and anti-inflammatory potential" opublikowanej w Scientific Reports do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgra farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizację projektu.

Imię i nazwisko współautora	Udział procentowy	Zakres wkładu
Maciej Korczak	80%	Projekt badania, synteza, identyfikacja pochodnych, badania *in vitro*, pisanie manuskryptu
Piotr Roszkowski	10%	Synteza, pisanie manuskryptu
Sebastian Granica	5%	Projekt badania, nadzór nad badaniem
Jakub Patryk Piwowarski	5%	Projekt badania, identyfikacja pochodnych, nadzór nad badaniem, pisanie manuskryptu

Czytelny podpis:

Roszkowski Piotr

Data:

15.02.2023

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji "Conjugates of urolithin A with NSAIDs, their stability, cytotoxicity, and anti-inflammatory potential" opublikowanej w Scientific Reports do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko współautora	Udział procentowy	Zakres wkładu
Maciej Korczak	80%	Projekt badania, synteza, identyfikacja pochodnych, badania *in vitro*, pisanie manuskryptu
Piotr Roszkowski	10%	Synteza, pisanie manuskryptu
Sebastian Granica	5%	Projekt badania, nadzór nad badaniem
Jakub Patryk Piwowarski	5%	Projekt badania, identyfikacja pochodnych, nadzór nad badaniem, pisanie manuskryptu

Czytelny Podpis:

p.o. Kierownik Środowiskowego Laboratorium Microbiota Lab
prof. dr hab. n. med. i n. o zdr. Sebastian Granica

Data:

12.07.2021

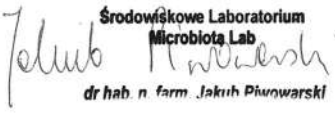
Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji "Conjugates of urolithin A with NSAIDs, their stability, cytotoxicity, and anti-inflammatory potential" opublikowanej w Scientific Reports do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko współautora	**Udział procentowy**	**Zakres wkładu**
Maciej Korczak	80%	Projekt badania, synteza, identyfikacja pochodnych, badania *in vitro*, pisanie manuskryptu
Piotr Roszkowski	10%	Synteza, pisanie manuskryptu
Sebastian Granica	5%	Projekt badania, nadzór nad badaniem
Jakub Patryk Piwowarski	5%	Projekt badania, identyfikacja pochodnych, nadzór nad badaniem, pisanie manuskryptu

Czytelny Podpis:



Data:
12.07.2022

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji " Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function." opublikowanej w Biomedicine & Pharmacotherapy do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko	Procentowy wkład autorski	Zakres zaangażowania przy tworzeniu publikacji
Maciej Korczak	70%	Synteza urolityny A, identyfikacja zsyntetyzowanych związków, analiza UHPLC-MS/MS i półilościowa, badania *in vitro* poprzedzające sekwencjonowanie nowej generacji, eksperymenty western blot, analiza statystyczna otrzymanych wyników, analiza bioinformatyczna wyników otrzymanych z sekwencjonowania nowej generacji, pisanie manuskryptu
Piotr Roszkowski	8%	Synteza pochodnych urolityny A
Weronika Skowrońska	4%	Badania cytotoksyczności testowanych związków
Klaudia Żołdak	4%	Wykonanie badań wpływu testowanych związków na szczelność monowarstw komórek Caco-2
Dominik Popowski	4%	Opracowanie metodologii badań modelu *in vitro* z wykorzystaniem komórek Caco-2
Sebastian Granica	4%	Projekt badania, nadzór nad badaniem
Jakub Piwowarski	6%	Projekt badania, nadzór nad badaniem, pisanie manuskryptu

Czytelny Podpis:
Roszkowski Piotr

Data:
07.12.2023

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji " Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function." opublikowanej w Biomedicine & Pharmacotherapy do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko	Procentowy wkład autorski	Zakres zaangażowania przy tworzeniu publikacji
Maciej Korczak	70%	Synteza urolityny A, identyfikacja zsyntetyzowanych związków, analiza UHPLC-MS/MS i półilościowa, badania *in vitro* poprzedzające sekwencjonowanie nowej generacji, eksperymenty western blot, analiza statystyczna otrzymanych wyników, analiza bioinformatyczna wyników otrzymanych z sekwencjonowania nowej generacji, pisanie manuskryptu
Piotr Roszkowski	8%	Synteza pochodnych urolityny A
Weronika Skowrońska	4%	Badania cytotoksyczności testowanych związków
Klaudia Żołdak	4%	Wykonanie badań wpływu testowanych związków na szczelność monowarstw komórek Caco-2
Dominik Popowski	4%	Opracowanie metodologii badań modelu *in vitro* z wykorzystaniem komórek Caco-2
Sebastian Granica	4%	Projekt badania, nadzór nad badaniem
Jakub Piwowarski	6%	Projekt badania, nadzór nad badaniem, pisanie manuskryptu

Czytelny Podpis:

Weronika Skowrońska

Data:

06.12.2023

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji " Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function." opublikowanej w Biomedicine & Pharmacotherapy do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko	Procentowy wkład autorski	Zakres zaangażowania przy tworzeniu publikacji
Maciej Korczak	70%	Synteza urolityny A, identyfikacja zsyntetyzowanych związków, analiza UHPLC-MS/MS i półilościowa, badania *in vitro* poprzedzające sekwencjonowanie nowej generacji, eksperymenty western blot, analiza statystyczna otrzymanych wyników, analiza bioinformatyczna wyników otrzymanych z sekwencjonowania nowej generacji, pisanie manuskryptu
Piotr Roszkowski	8%	Synteza pochodnych urolityny A
Weronika Skowrońska	4%	Badania cytotoksyczności testowanych związków
Klaudia Żołdak	4%	Wykonanie badań wpływu testowanych związków na szczelność monowarstw komórek Caco-2
Dominik Popowski	4%	Opracowanie metodologii badań modelu *in vitro* z wykorzystaniem komórek Caco-2
Sebastian Granica	4%	Projekt badania, nadzór nad badaniem
Jakub Piwowarski	6%	Projekt badania, nadzór nad badaniem, pisanie manuskryptu

Czytelny Podpis: Klaudia Żołdak

Data: 06.12.2021

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji " Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function." opublikowanej w Biomedicine & Pharmacotherapy do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko	**Procentowy wkład autorski**	**Zakres zaangażowania przy tworzeniu publikacji**
Maciej Korczak	70%	Synteza urolityny A, identyfikacja zsyntetyzowanych związków, analiza UHPLC-MS/MS i półilościowa, badania *in vitro* poprzedzające sekwencjonowanie nowej generacji, eksperymenty western blot, analiza statystyczna otrzymanych wyników, analiza bioinformatyczna wyników otrzymanych z sekwencjonowania nowej generacji, pisanie manuskryptu
Piotr Roszkowski	8%	Synteza pochodnych urolityny A
Weronika Skowrońska	4%	Badania cytotoksyczności testowanych związków
Klaudia Żołdak	4%	Wykonanie badań wpływu testowanych związków na szczelność monowarstw komórek Caco-2
Dominik Popowski	4%	Opracowanie metodologii badań modelu *in vitro* z wykorzystaniem komórek Caco-2
Sebastian Granica	4%	Projekt badania, nadzór nad badaniem
Jakub Piwowarski	6%	Projekt badania, nadzór nad badaniem, pisanie manuskryptu

Czytelny Podpis:

Dominik Popowski

Data:

02/04/2024

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji " Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function." opublikowanej w Biomedicine & Pharmacotherapy do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko	Procentowy wkład autorski	Zakres zaangażowania przy tworzeniu publikacji
Maciej Korczak	70%	Synteza urolityny A, identyfikacja zsyntetyzowanych związków, analiza UHPLC-MS/MS i półilościowa, badania *in vitro* poprzedzające sekwencjonowanie nowej generacji, eksperymenty western blot, analiza statystyczna otrzymanych wyników, analiza bioinformatyczna wyników otrzymanych z sekwencjonowania nowej generacji, pisanie manuskryptu
Piotr Roszkowski	8%	Synteza pochodnych urolityny A
Weronika Skowrońska	4%	Badania cytotoksyczności testowanych związków
Klaudia Żołdak	4%	Wykonanie badań wpływu testowanych związków na szczelność monowarstw komórek Caco-2
Dominik Popowski	4%	Opracowanie metodologii badań modelu *in vitro* z wykorzystaniem komórek Caco-2
Sebastian Granica	4%	Projekt badania, nadzór nad badaniem
Jakub Piwowarski	6%	Projekt badania, nadzór nad badaniem, pisanie manuskryptu

Czytelny Podpis:

Sebastian Granica

Data:

1/12/2023

Oświadczenie współautora artykułu

Wyrażam zgodę na włączenie publikacji " Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function." opublikowanej w Biomedicine & Pharmacotherapy do zbioru powiązanych tematycznie artykułów naukowych stanowiącego rozprawę doktorską mgr. farm. Macieja Korczaka.

Jednocześnie potwierdzam, że przedstawiony poniżej zakres odpowiada mojemu wkładowi w realizacji projektu.

Imię i nazwisko	Procentowy wkład autorski	Zakres zaangażowania przy tworzeniu publikacji
Maciej Korczak	70%	Synteza urolityny A, identyfikacja zsyntetyzowanych związków, analiza UHPLC-MS/MS i półilościowa, badania *in vitro* poprzedzające sekwencjonowanie nowej generacji, eksperymenty western blot, analiza statystyczna otrzymanych wyników, analiza bioinformatyczna wyników otrzymanych z sekwencjonowania nowej generacji, pisanie manuskryptu
Piotr Roszkowski	8%	Synteza pochodnych urolityny A
Weronika Skowrońska	4%	Badania cytotoksyczności testowanych związków
Klaudia Żołdak	4%	Wykonanie badań wpływu testowanych związków na szczelność monowarstw komórek Caco-2
Dominik Popowski	4%	Opracowanie metodologii badań modelu *in vitro* z wykorzystaniem komórek Caco-2
Sebastian Granica	4%	Projekt badania, nadzór nad badaniem
Jakub Piwowarski	6%	Projekt badania, nadzór nad badaniem, pisanie manuskryptu

Czytelny Podpis:

Jakub Piwowarski

Data: 07.12.2023

11. Bibliography

1. Shen B. A New Golden Age of Natural Products Drug Discovery. Cell 10.1016/j.cell.2015.11.031. 2015 Dec;163(6):1297–300.

2. Montinari MR, Minelli S, De Caterina R. The first 3500 years of aspirin history from its roots – A concise summary. Vascular Pharmacology 10.1016/j.vph.2018.10.008. 2019 Feb;113:1–8.

3. Cragg GM, Newman DJ. Natural products: A continuing source of novel drug leads. Biochimica et Biophysica Acta (BBA) - General Subjects 10.1016/j.bbagen.2013.02.008. 2013 Jun;1830(6):3670–95.

4. Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. Journal of Natural Products 10.1021/acs.jnatprod.9b01285. 2020 Mar 27;83(3):770–803.

5. Guo Z. The modification of natural products for medical use. Acta Pharmaceutica Sinica B 10.1016/j.apsb.2016.06.003. 2017 Mar 1;7(2):119–36.

6. Wang S, Dong G, Sheng C. Structural Simplification of Natural Products. Chemical Reviews 10.1021/acs.chemrev.8b00504. 2019 Mar 27;119(6):4180–220.

7. Bruno M, Trucchi B, Monti D, Romeo S, Kaiser M, Verotta L. Synthesis of a Potent Antimalarial Agent through Natural Products Conjugation. ChemMedChem 10.1002/cmdc.201200503. 2013 Feb 10;8(2):221–5.

8. Ha MW, Song BR, Chung HJ, Paek SM. Design and Synthesis of Anti-Cancer Chimera Molecules Based on Marine Natural Products. Marine Drugs 10.3390/md17090500. 2019 Aug 27;17(9):500.

9. Roskoski R. Rule of five violations among the FDA-approved small molecule protein kinase inhibitors. Pharmacological Research 10.1016/j.phrs.2023.106774. 2023 May;191:106774.

10. Zhang MQ, Wilkinson B. Drug discovery beyond the 'rule-of-five.' Current Opinion in Biotechnology 10.1016/j.copbio.2007.10.005. 2007 Dec;18(6):478–88.

11. Abdel-Razek AS, El-Naggar ME, Allam A, Morsy OM, Othman SI. Microbial Natural Products in Drug Discovery. Processes 10.3390/pr8040470. 2020 Apr 16;8(4):470.

12. Le VH, Inai M, Williams RM, Kan T. Ecteinascidins. A review of the chemistry, biology and clinical utility of potent tetrahydroisoquinoline antitumor antibiotics. Natural Product Reports 10.1039/C4NP00051J. 2015;32(2):328–47.

13. Gerwick WH, Fenner AM. Drug Discovery from Marine Microbes. Microbial Ecology 10.1007/s00248-012-0169-9. 2013 May 30;65(4):800–6.

14. Simon JC, Marchesi JR, Mougel C, Selosse MA. Host-microbiota interactions: From holobiont theory to analysis. Microbiome 10.1186/s40168-019-0619-4. 2019 Jan 11;7(1).

15. Quigley EMM. Basic Definitions and Concepts: Organization of the Gut Microbiome. Gastroenterology Clinics of North America 10.1016/j.gtc.2016.09.002. 2017 Mar;46(1):1–8.

16. Ursell LK, Metcalf JL, Parfrey LW, Knight R. Defining the human microbiome. Nutrition Reviews 10.1111/j.1753-4887.2012.00493.x. 2012 Aug;70:S38–44.

17. Chikina A, Matic Vignjevic D. At the right time in the right place: How do luminal gradients position the microbiota along the gut? Cells & Development 10.1016/j.cdev.2021.203712. 2021 Dec 1;168:203712.

18. Ghosh TS, Shanahan F, O'Toole PW. The gut microbiome as a modulator of healthy ageing. Nature Reviews Gastroenterology & Hepatology 10.1038/s41575-022-00605-x. 2022 Sep 25;19(9):565–84.

19. Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. The microbiota–gut–brain axis in obesity. The Lancet Gastroenterology & Hepatology 10.1016/S2468-1253(17)30147-4. 2017 Oct;2(10).

20. Gurung M, Li Z, You H, Rodrigues R, Jump DB, Morgun A, et al. Role of gut microbiota in type 2 diabetes pathophysiology. EBioMedicine 10.1016/j.ebiom.2019.11.051. 2020 Jan;51:102590.

21. Simpson CA, Diaz-Arteche C, Eliby D, Schwartz OS, Simmons JG, Cowan CSM. The gut microbiota in anxiety and depression – A systematic review. Clinical Psychology Review 10.1016/j.cpr.2020.101943. 2021 Feb;83:101943.

22. Wong CC, Yu J. Gut microbiota in colorectal cancer development and therapy. Nature Reviews Clinical Oncology 10.1038/s41571-023-00766-x. 2023 Jul 11;20(7):429–52.

23. Shanahan F, Ghosh TS, O'Toole PW. The Healthy Microbiome—What Is the Definition of a Healthy Gut Microbiome? Gastroenterology 10.1053/j.gastro.2020.09.057. 2021 Jan;160(2):483–94.

24. Eisenstein M. The hunt for a healthy microbiome. Nature 10.1038/d41586-020-00193-3. 2020 Jan 30;577(7792):S6–8.

25. Mosca A, Leclerc M, Hugot JP. Gut Microbiota Diversity and Human Diseases: Should We Reintroduce Key Predators in Our Ecosystem? Frontiers in Microbiology 10.3389/fmicb.2016.00455. 2016 Mar;7.

26. Zhou S, Cui Y, Zhang Y, Zhao T, Cong J. Fecal microbiota transplantation for induction of remission in Crohn's disease: a systematic review and meta-analysis. International Journal of Colorectal Disease 10.1007/s00384-023-04354-4. 2023 Mar 8;38(1):62.

27. Kassam Z, Lee CH, Yuan Y, Hunt RH. Fecal Microbiota Transplantation for Clostridium difficile Infection: Systematic Review and Meta-Analysis. American Journal of Gastroenterology 10.1038/ajg.2013.59. 2013 Apr;108(4):500–8.

28. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nature Reviews Gastroenterology & Hepatology 10.1038/nrgastro.2017.75. 2017 Aug 14;14(8):491–502.

29. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document: The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology and Hepatology 10.1038/nrgastro.2014.66. 2014;11(8):506–14.

30. Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. Nature Reviews Gastroenterology & Hepatology 10.1038/s41575-020-0344-2. 2020 Nov 21;17(11):687–701.

31. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nature Reviews Gastroenterology & Hepatology 10.1038/s41575-021-00440-6. 2021 Sep 4;18(9):649–67.

32. Vinderola G, Sanders ME, Cunningham M, Hill C. Frequently asked questions about the ISAPP postbiotic definition. Frontiers in Microbiology 10.3389/fmicb.2023.1324565. 2024 Jan 10;14.

33. Guo Q, Goldenberg JZ, Humphrey C, El Dib R, Johnston BC. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. Cochrane Database of Systematic Reviews 10.1002/14651858.cd004827.pub5. 2019 Apr 30;

34. Han S, Lu Y, Xie J, Fei Y, Zheng G, Wang Z, et al. Probiotic Gastrointestinal Transit and Colonization After Oral Administration: A Long Journey. Frontiers in Cellular and Infection Microbiology 10.3389/fcimb.2021.609722. 2021 Mar 10;11.

35. Tan J, McKenzie C, Potamitis M, Thorburn AN, Mackay CR, Macia L. The Role of Short-Chain Fatty Acids in Health and Disease. In: 10.1016/B978-0-12-800100-4.00003-9. 2014. p. 91–119.

36. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota–gut–brain communication. Vol. 16, Nature Reviews Gastroenterology and Hepatology. Nature Publishing Group; 2019. p. 461–78.

37. Lavelle A, Sokol H. Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. Nature Reviews Gastroenterology & Hepatology 10.1038/s41575-019-0258-z. 2020 Apr 19;17(4):223–37.

38. Agus A, Planchais J, Sokol H. Gut Microbiota Regulation of Tryptophan Metabolism in Health and Disease. Cell Host & Microbe 10.1016/j.chom.2018.05.003. 2018 Jun 13;23(6):716–24.

39. Vázquez-Gómez G, Petráš J, Dvořák Z, Vondráček J. Aryl hydrocarbon receptor (AhR) and pregnane X receptor (PXR) play both distinct and common roles in the regulation of colon homeostasis and intestinal carcinogenesis. Biochemical Pharmacology 10.1016/j.bcp.2023.115797. 2023 Oct 1;216:115797.

40. Salminen A. Activation of aryl hydrocarbon receptor (AhR) in Alzheimer's disease: role of tryptophan metabolites generated by gut host-microbiota. Journal of Molecular Medicine 10.1007/s00109-023-02289-5. 2023 Mar 9;101(3):201–22.

41. Strandwitz P. Neurotransmitter modulation by the gut microbiota. Brain Research 10.1016/j.brainres.2018.03.015. 2018 Aug;1693:128–33.

42. LeBlanc JG, Milani C, de Giori GS, Sesma F, van Sinderen D, Ventura M. Bacteria as vitamin suppliers to their host: a gut microbiota perspective. Current Opinion in Biotechnology 10.1016/j.copbio.2012.08.005. 2013 Apr;24(2):160–8.

43. Bonakdar M, Czuba LC, Han G, Zhong G, Luong H, Isoherranen N, et al. Gut commensals expand vitamin A metabolic capacity of the mammalian host. Cell Host & Microbe 10.1016/j.chom.2022.06.011. 2022 Aug;30(8):1084-1092.e5.

44. Chatterjee K, Mazumder PM, Sarkar SR, Saha R, Chatterjee A, Sarkar B, et al. Neuroprotective effect of Vitamin K2 against gut dysbiosis associated cognitive decline. Physiology & Behavior 10.1016/j.physbeh.2023.114252. 2023 Oct;269:114252.

45. Pham VT, Dold S, Rehman A, Bird JK, Steinert RE. Vitamins, the gut microbiome and gastrointestinal health in humans. Nutrition Research 10.1016/j.nutres.2021.09.001. 2021 Nov;95:35–53.

46. Wahlström A, Sayin SI, Marschall HU, Bäckhed F. Intestinal Crosstalk between Bile Acids and Microbiota and Its Impact on Host Metabolism. Cell Metabolism 10.1016/j.cmet.2016.05.005. 2016 Jul 12;24(1):41–50.

47. Thomas JP, Modos D, Rushbrook SM, Powell N, Korcsmaros T. The Emerging Role of Bile Acids in the Pathogenesis of Inflammatory Bowel Disease. Frontiers in Immunology 10.3389/fimmu.2022.829525. 2022 Feb 3;13.

48. Di Lorenzo F, De Castro C, Silipo A, Molinaro A. Lipopolysaccharide structures of Gram-negative populations in the gut microbiota and effects on host interactions. FEMS Microbiology Reviews 10.1093/femsre/fuz002. 2019 May 1;43(3):257–72.

49. Van Den Berg C, De Neeling AJ, Schot CS, M Hustinx WN, Wemer J, De Wildt DJ. Delayed Antibiotic-Induced Lysis of Escherichia coli in vitro is Correlated with Enhancement of LPS Release Delayed Antibiotic-Induced Lysis of Escherichia coli In Vitro is Correlated with Enhancement of LPS Release. Scandinavian Journal of Infectious Diseases 10.3109/00365549209054648. 2009;24(5).

50. Horowitz A, Chanez-Paredes SD, Haest X, Turner JR. Paracellular permeability and tight junction regulation in gut health and disease. Nature Reviews Gastroenterology & Hepatology 10.1038/s41575-023-00766-3. 2023 Jul 25;20(7):417–32.

51. Lu YC, Yeh WC, Ohashi PS. LPS/TLR4 signal transduction pathway. Cytokine 10.1016/j.cyto.2008.01.006. 2008 May;42(2):145–51.

52. Hamjane N, Mechita MB, Nourouti NG, Barakat A. Gut microbiota dysbiosis -associated obesity and its involvement in cardiovascular diseases and type 2 diabetes. A systematic review. Microvascular Research 10.1016/j.mvr.2023.104601. 2024 Jan 1;151:104601.

53. Kesika P, Suganthy N, Sivamaruthi BS, Chaiyasut C. Role of gut-brain axis, gut microbial composition, and probiotic intervention in Alzheimer's disease. Life Sciences 10.1016/j.lfs.2020.118627. 2021 Jan;264:118627.

54. Rudzki L, Maes M. The Microbiota-Gut-Immune-Glia (MGIG) Axis in Major Depression. Molecular Neurobiology 10.1007/s12035-020-01961-y. 2020 Oct 22;57(10):4269–95.

55. Zhang Y, Wang Y, Ke B, Du J. TMAO: how gut microbiota contributes to heart failure. Translational Research 10.1016/j.trsl.2020.08.007. 2021 Feb;228:109–25.

56. Tomas-Barberan FA, Selma M V, Espín JC. Polyphenols' Gut Microbiota Metabolites: Bioactives or Biomarkers? Journal of Agricultural and Food Chemistry 10.1021/acs.jafc.8b00827. 2018 Apr;66(14).

57. Zhang X, Han Y, Huang W, Jin M, Gao Z. The influence of the gut microbiota on the bioavailability of oral drugs. Acta Pharmaceutica Sinica B 10.1016/j.apsb.2020.09.013. 2021 Jul 1;11(7):1789–812.

58. Spanogiannopoulos P, Bess EN, Carmody RN, Turnbaugh PJ. The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. Nature Reviews Microbiology 10.1038/nrmicro.2016.17. 2016 May 14;14(5):273–87.

59. Alexander JL, Wilson ID, Teare J, Marchesi JR, Nicholson JK, Kinross JM. Gut microbiota modulation of chemotherapy efficacy and toxicity. Nature Reviews Gastroenterology & Hepatology 10.1038/nrgastro.2017.20. 2017 Jun 8;14(6):356–65.

60. Qian Y, Jiang S, Zhu Z, Wang Q, Su S, Tao J, et al. Simultaneous quantification and semi-quantification of amentoflavone and its metabolites in human intestinal bacteria by liquid chromatography Orbitrap high-resolution mass spectrometry. Biomedical Chromatography 10.1002/bmc.3990. 2017 Nov;31(11).

61. Murota K, Nakamura Y, Uehara M. Flavonoid metabolism: the interaction of metabolites and gut microbiota. Bioscience, Biotechnology, and Biochemistry 10.1080/09168451.2018.1444467. 2018 Apr 3;82(4):600–10.

62. Zhang Y, Geng J, Hong Y, Jiao L, Li S, Sun R, et al. Orally Administered Crocin Protects Against Cerebral Ischemia/Reperfusion Injury Through the Metabolic Transformation of Crocetin by Gut Microbiota. Frontiers in Pharmacology 10.3389/fphar.2019.00440. 2019 Apr;10.

63. Yang J, Qian D, Guo J, Jiang S, Shang EX, Duan JA, et al. Identification of the major metabolites of hyperoside produced by the human intestinal bacteria using the ultra performance liquid chromatography/ quadrupole-time-of-flight mass spectrometry. Journal of Ethnopharmacology 10.1016/j.jep.2013.02.029. 2013 May;147(1):174–9.

64. Duda-Chodak A, Tarko T, Satora P, Sroka P. Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. European Journal of Nutrition 10.1007/s00394-015-0852-y. 2015 Apr;54(3).

65. Stoupi S, Williamson G, Drynan JW, Barron D, Clifford MN. A comparison of the *in vitro* biotransformation of (-)-epicatechin and procyanidin B2 by human faecal microbiota. Molecular Nutrition & Food Research 10.1002/mnfr.200900123. 2010 Jun;54(6).

66. Chen J, Zhong K, Jing Y, Liu S, Qin S, Peng F, et al. Procyanidin B2: A promising multi-functional food-derived pigment for human diseases. Food Chemistry 10.1016/j.foodchem.2023.136101. 2023 Sep;420:136101.

67. Holt RR, Lazarus SA, Sullards MC, Zhu QY, Schramm DD, Hammerstone JF, et al. Procyanidin dimer B2 [epicatechin-(4β-8)-epicatechin] in human plasma after the consumption of a flavanol-rich cocoa. The American Journal of Clinical Nutrition 10.1093/ajcn/76.4.798. 2002 Oct;76(4):798–804.

68. Barnett CF, Moreno-Ulloa A, Shiva S, Ramirez-Sanchez I, Taub PR, Su Y, et al. Pharmacokinetic, partial pharmacodynamic and initial safety analysis of (-)-epicatechin in healthy volunteers. Food and Function 10.1039/c4fo00596a. 2015 Mar 1;6(3):824–33.

69. Mena P, Bresciani L, Brindani N, Ludwig IA, Pereira-Caro G, Angelino D, et al. Phenyl-γ-valerolactones and phenylvaleric acids, the main colonic metabolites of flavan-3-ols: synthesis, analysis, bioavailability, and bioactivity. Natural Product Reports 10.1039/C8NP00062J. 2019 May 1;36(5):714–52.

70. Ruotolo R, Minato I, La Vitola P, Artioli L, Curti C, Franceschi V, et al. Flavonoid-Derived Human Phenyl-γ-Valerolactone Metabolites Selectively Detoxify Amyloid-β Oligomers and Prevent Memory Impairment in a Mouse Model of Alzheimer's Disease. Molecular Nutrition & Food Research 10.1002/mnfr.201900890. 2020 Mar 16;64(5).

71. Huang Z, Xu Y, Wang Q, Gao X. Metabolism and mutual biotransformations of anthraquinones and anthrones in rhubarb by human intestinal flora using UPLC-Q-TOF/MS. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 10.1016/j.jchromb.2018.10.008. 2019 Jan 1;1104:59–66.

72. Iglesias-Aguirre CE, Vallejo F, Beltrán D, Aguilar-Aguilar E, Puigcerver J, Alajarín M, et al. Lunularin Producers versus Non-producers: Novel Human Metabotypes Associated with the Metabolism of Resveratrol by the Gut Microbiota. Journal of Agricultural and Food Chemistry 10.1021/acs.jafc.2c04518. 2022 Aug 31;70(34):10521–31.

73. Li F, Han Y, Wu X, Cao X, Gao Z, Sun Y, et al. Gut Microbiota-Derived Resveratrol Metabolites, Dihydroresveratrol and Lunularin, Significantly Contribute to the Biological Activities of Resveratrol. Frontiers in Nutrition 10.3389/fnut.2022.912591. 2022 May 11;9.

74. Zanoli P, Zavatti M. Pharmacognostic and pharmacological profile of Humulus lupulus L. Journal of Ethnopharmacology 10.1016/j.jep.2008.01.011. 2008 Mar 28;116(3):383–96.

75. Liu M, Hansen P, Wang G, Qiu L, Dong J, Yin H, et al. Pharmacological Profile of Xanthohumol, a Prenylated Flavonoid from Hops (Humulus lupulus). Molecules 10.3390/molecules20010754. 2015 Jan 7;20(1):754–79.

76. Pohjanvirta R, Nasri A. The Potent Phytoestrogen 8-Prenylnaringenin: A Friend or a Foe? International Journal of Molecular Sciences 10.3390/ijms23063168. 2022;23(6).

77. Paraiso IL, Plagmann LS, Yang L, Zielke R, Gombart AF, Maier CS, et al. Reductive Metabolism of Xanthohumol and 8-Prenylnaringenin by the Intestinal Bacterium Eubacterium ramulus. Molecular Nutrition & Food Research 10.1002/mnfr.201800923. 2019 Jan;63(2).

78. Setchell KDR, Brown NM, Lydeking-Olsen E. The Clinical Importance of the Metabolite Equol—A Clue to the Effectiveness of Soy and Its Isoflavones. The Journal of Nutrition 10.1093/jn/132.12.3577. 2002 Dec;132(12):3577–84.

79. Lampe JW. Emerging Research on Equol and Cancer. The Journal of Nutrition 10.3945/jn.109.118323. 2010 Jul;140(7):1369S-1372S.

80. Cerdá B, Llorach R, Cerón JJ, Espín JC, Tomás-Barberán FA. Evaluation of the bioavailability and metabolism in the rat of punicalagin, an antioxidant polyphenol from pomegranate juice. European Journal of Nutrition 10.1007/s00394-003-0396-4. 2003 Jan;42(1):18–28.

81. Piwowarski JP, Granica S, Zwierzyńska M, Stefańska J, Schopohl P, Melzig MF, et al. Role of human gut microbiota metabolism in the anti-inflammatory effect of traditionally used ellagitannin-rich plant materials. Journal of Ethnopharmacology 10.1016/j.jep.2014.06.032. 2014 Aug;155(1):801–9.

82. Kiss AK, Piwowarski JP. Ellagitannins, Gallotannins and their Metabolites- The Contribution to the Anti-Inflammatory Effect of Food Products and Medicinal Plants. Current Medicinal Chemistry 10.2174/0929867323666160919111559. 2016 Sep 23;25(37):4946–67.

83. Landete JM. Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health. Food Research International 10.1016/j.foodres.2011.04.027. 2011 Jun;44(5):1150–60.

84. Zhang M, Cui S, Mao B, Zhang Q, Zhao J, Zhang H, et al. Ellagic acid and intestinal microflora metabolite urolithin A: A review on its sources, metabolic distribution, health benefits, and

biotransformation. Critical Reviews in Food Science and Nutrition 10.1080/10408398.2022.2036693. 2023 Sep 21;63(24):6900–22.

85. Iglesias-Aguirre CE, García-Villalba R, Beltrán D, Frutos-Lisón MD, Espín JC, Tomás-Barberán FA, et al. Gut Bacteria Involved in Ellagic Acid Metabolism To Yield Human Urolithin Metabotypes Revealed. Journal of Agricultural and Food Chemistry 10.1021/acs.jafc.2c08889. 2023 Feb 25;

86. Zhang X, Fang Y, Yang G, Hou X, Hai Y, Xia M, et al. Isolation and characterization of a novel human intestinal Enterococcus faecium FUA027 capable of producing urolithin A from ellagic acid. Frontiers in Nutrition 10.3389/fnut.2022.1039697. 2022 Nov 9;9.

87. García-Villalba R, Giménez-Bastida JA, Cortés-Martín A, Ávila-Gálvez MÁ, Tomás-Barberán FA, Selma MV, et al. Urolithins: a comprehensive update on their metabolism, bioactivity, and associated gut microbiota. Molecular Nutrition & Food Research 10.1002/mnfr.202101019. 2022 Feb;(February):2101019.

88. Cortés-Martín A, Selma MV, Tomás-Barberán FA, González-Sarrías A, Espín JC. Where to Look into the Puzzle of Polyphenols and Health? The Postbiotics and Gut Microbiota Associated with Human Metabotypes. Molecular Nutrition & Food Research 10.1002/mnfr.201900952. 2020 May;64(9).

89. Cortés-Martín A, García-Villalba R, González-Sarrías A, Romo-Vaquero M, Loria-Kohen V, Ramírez-De-Molina A, et al. The gut microbiota urolithin metabotypes revisited: the human metabolism of ellagic acid is mainly determined by aging. Food and Function 10.1039/c8fo00956b. 2018;9(8):4100–6.

90. Eduardo Iglesias-Aguirre C, Romo-Vaquero M, Victoria Selma M, Carlos Espín J. Unveiling metabotype clustering in resveratrol, daidzein, and ellagic acid metabolism: Prevalence, associated gut microbiomes, and their distinctive microbial networks. Food Research International 10.1016/j.foodres.2023.113470. 2023 Nov;173:113470.

91. Bobowska A, Granica S, Filipek A, Melzig MF, Moeslinger T, Zentek J, et al. Comparative studies of urolithins and their phase II metabolites on macrophage and neutrophil functions. European Journal of Nutrition 10.1007/s00394-020-02386-y. 2021 Jun 22;60(4):1957–72.

92. Mc Cormack BA, Olivares CN, Madanes D, Ricci AG, Bilotas MA, Barañao RI. Effect of urolithins A and B on ectopic endometrial growth in a murine model of endometriosis. Food & Function 10.1039/d1fo01702k. 2021;12(20):9894–903.

93. Giménez-Bastida JA, Ávila-Gálvez MÁ, Espín JC, González-Sarrías A. The gut microbiota metabolite urolithin A, but not other relevant urolithins, induces p53-dependent cellular senescence in human colon cancer cells. Food and Chemical Toxicology 10.1016/j.fct.2020.111260. 2020 May 1;139.

94. D'Amico D, Andreux PA, Valdés P, Singh A, Rinsch C, Auwerx J. Impact of the Natural Compound Urolithin A on Health, Disease, and Aging. Trends in Molecular Medicine 10.1016/j.molmed.2021.04.009. 2021;27(7):687–99.

95. Komatsu W, Kishi H, Yagasaki K, Ohhira S. Urolithin A attenuates pro-inflammatory mediator production by suppressing PI3-K/Akt/NF-κB and JNK/AP-1 signaling pathways in lipopolysaccharide-stimulated RAW264 macrophages: Possible involvement of NADPH

oxidase-derived reactive oxygen species. European Journal of Pharmacology 10.1016/j.ejphar.2018.06.023. 2018 Aug;833:411–24.

96. Gong QY, Cai L, Jing Y, Wang W, Yang DX, Chen SW, et al. Urolithin A alleviates blood-brain barrier disruption and attenuates neuronal apoptosis following traumatic brain injury in mice. Neural Regeneration Research 10.4103/1673-5374.335163. 2022;17(9):2007.

97. Boakye YD, Groyer L, Heiß EH. An increased autophagic flux contributes to the anti-inflammatory potential of urolithin A in macrophages. Biochimica et Biophysica Acta (BBA) - General Subjects 10.1016/j.bbagen.2017.10.006. 2018 Jan;1862(1):61–70.

98. Ahsan A, Liu M, Zheng Y, Yan W, Pan L, Li Y, et al. Natural compounds modulate the autophagy with potential implication of stroke. Acta Pharmaceutica Sinica B 10.1016/j.apsb.2020.10.018. 2021 Jul 1;11(7):1708–20.

99. Ryu D, Mouchiroud L, Andreux PA, Katsyuba E, Moullan N, Nicolet-Dit-Félix AA, et al. Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents. Nature Medicine 10.1038/nm.4132. 2016 Aug 1;22(8):879–88.

100. Pinto CatarinaJG, Ávila-Gálvez MÁ, Lian Y, Moura-Alves P, Nunes dos Santos C. Targeting the aryl hydrocarbon receptor by gut phenolic metabolites: A strategy towards gut inflammation. Redox Biology 10.1016/j.redox.2023.102622. 2023 Feb;102622.

101. Van Breemen RB, Chen L, Tonsing-Carter A, Banuvar S, Barengolts E, Viana M, et al. Pharmacokinetic Interactions of a Hop Dietary Supplement with Drug Metabolism in Perimenopausal and Postmenopausal Women. Journal of Agricultural and Food Chemistry 10.1021/acs.jafc.0c01077. 2020 May 6;68(18):5212–20.

102. Klimas R, Mikus G. Morphine-6-glucuronide is responsible for the analgesic effect after morphine administration: a quantitative review of morphine, morphine-6-glucuronide, and morphine-3-glucuronide. British Journal of Anaesthesia 10.1093/bja/aeu186. 2014 Dec;113(6):935–44.

103. González-Sarrías A, Giménez-Bastida JA, Núñez-Sánchez MÁ, Larrosa M, García-Conesa MT, Tomás-Barberán FA, et al. Phase-II metabolism limits the antiproliferative activity of urolithins in human colon cancer cells. European Journal of Nutrition 10.1007/s00394-013-0589-4. 2014 Apr 1;53(3):853–64.

104. Bialonska D, Kasimsetty SG, Khan SI, Daneel F. Urolithins, intestinal microbial metabolites of pomegranate ~ellagitannins, exhibit potent antioxidant activity in a cell-based assay. Journal of Agricultural and Food Chemistry 10.1021/jf9025794. 2009;57(21):10181–6.

105. James SL, Abate D, Abate KH, Abay SM, Abbafati C, Abbasi N, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. The Lancet 10.1016/S0140-6736(18)32279-7. 2018 Nov;392(10159).

106. Thumann TA, Pferschy-Wenzig EM, Moissl-Eichinger C, Bauer R. The role of gut microbiota for the activity of medicinal plants traditionally used in the European Union for gastrointestinal disorders. Journal of Ethnopharmacology 10.1016/j.jep.2019.112153. 2019 Dec 5;245:112153.

107. Jarrar YB, Kim DH, Lee SJ, Shin JG. Inhibition of 20-hydroxyeicosatetraenoic acid (20-HETE) glucuronidation by non-steroidal anti-inflammatory drugs in human liver microsomes and

recombinant UDP-glucuronosyltransferase enzymes. Prostaglandins, Leukotrienes and Essential Fatty Acids 10.1016/j.plefa.2020.102055. 2020 Feb;153:102055.

108. Joo J, Kim YW, Wu Z, Shin JH, Lee B, Shon JC, et al. Screening of non-steroidal anti-inflammatory drugs for inhibitory effects on the activities of six UDP-glucuronosyltransferases (UGT1A1, 1A3, 1A4, 1A6, 1A9 and 2B7) using LC-MS/MS. Biopharmaceutics & Drug Disposition 10.1002/bdd.1933. 2015 May;36(4):258–64.

109. Sohail R, Mathew M, Patel KK, Reddy SA, Haider Z, Naria M, et al. Effects of Non-steroidal Anti-inflammatory Drugs (NSAIDs) and Gastroprotective NSAIDs on the Gastrointestinal Tract: A Narrative Review. Cureus 10.7759/cureus.37080. 2023 Apr 3;

110. Lavis LD. Ester Bonds in Prodrugs. ACS Chemical Biology 10.1021/cb800065s. 2008 Apr 1;3(4):203–6.

111. Shao Y, Wolf PG, Guo S, Guo Y, Gaskins HR, Zhang B. Zinc enhances intestinal epithelial barrier function through the PI3K/AKT/mTOR signaling pathway in Caco-2 cells. The Journal of Nutritional Biochemistry 10.1016/j.jnutbio.2017.01.013. 2017 May;43:18–26.

112. Shao YX, Lei Z, Wolf PG, Gao Y, Guo YM, Zhang BK. Zinc supplementation, via GPR39, upregulates PKCζ to protect intestinal barrier integrity in Caco-2 cells challenged by Salmonella enterica serovar typhimurium. Journal of Nutrition 10.3945/jn.116.243238. 2017 Jul 1;147(7):1282–9.

113. Alonso C, Carrer V, Espinosa S, Zanuy M, Córdoba M, Vidal B, et al. Prediction of the skin permeability of topical drugs using in silico and in vitro models. European Journal of Pharmaceutical Sciences 10.1016/j.ejps.2019.05.023. 2019 Aug;136:104945.

11. Reprint of publications constituting the doctoral dissertation

Publication No. 1

Phytotherapy of mood disorders in the light of microbiota-gut-brain axis.

Maciej Korczak [a], Maciej Pilecki [b], Sebastian Granica [a], Aleksandra Gorczyńska [a], Karolina Aleksandra Pawłowska [a], Jakub Patryk Piwowarski [a]

[a] Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland

[b] Department of Psychiatry, Collegium Medicum, Jagiellonian University, Cracow, Poland

Phytomedicine. 2023 Mar

111:154642

Phytomedicine 111 (2023) 154642

Contents lists available at ScienceDirect

Phytomedicine

journal homepage: www.elsevier.com/locate/phymed

Review

Phytotherapy of mood disorders in the light of microbiota-gut-brain axis.

Maciej Korczak [a], Maciej Pilecki [b], Sebastian Granica [a], Aleksandra Gorczynska [a], Karolina A. Pawłowska [a], Jakub P. Piwowarski [a,*]

[a] Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland
[b] Department of Psychiatry, Collegium Medicum, Jagiellonian University, Cracow, Poland

ARTICLE INFO

Keywords:
Microbiota
Mood disorders
Anxiety
Depression
Microbiota-gut-brain axis

ABSTRACT

Background: Clinical research in natural product-based psychopharmacology has revealed a variety of promising herbal medicines that may provide benefit in the treatment of mild mood disorders, however failed to unambiguously indicate pharmacologically active constituents. The emerging role of the microbiota-gut-brain axis opens new possibilities in the search for effective methods of treatment and prevention of mood disorders.
Purpose: Considering the clinically proven effectiveness juxtaposed with inconsistencies regarding the indication of active principles for many medicinal plants applied in the treatment of anxiety and depression, the aim of the review is to look at their therapeutic properties from the perspective of the microbiota-gut-brain axis.
Method: A literature-based survey was performed using Scopus, Pubmed, and Google Scholar databases. The current state of knowledge regarding *Hypericum perforatum, Valeriana officinalis, Piper methysticum, Passiflora incarnata, Humulus lupulus, Melissa officinalis, Lavandula officinalis,* and *Rhodiola rosea* in terms of their antimicrobial activity, bioavailability, clinical effectiveness in depression/anxiety and gut microbiota – natural products interaction was summarized and analyzed.
Results: Recent studies have provided direct and indirect evidence that herbal extracts and isolated compounds are potent modulators of gut microbiota structure. Additionally, some of the formed postbiotic metabolites exert positive effects and ameliorate depression-related behaviors in animal models of mood disorders. The review underlines the gap in research on natural products – gut microbiota interaction in the context of mood disorders.
Conclusion: Modification of microbiota-gut-brain axis by natural products is a plausible explanation of their therapeutic properties. Future studies evaluating the effectiveness of herbal medicine and isolated compounds in treating mild mood disorders should consider the bidirectional interplay between phytoconstituents and the gut microbiota community.

Significance of phytotherapy in mild mood disorders

The burden of mental disorders continues to grow with significant impacts on health and human rights as well as has major social and economic consequences in all countries of the world. According to the World Health Organization (WHO), an estimated 971 million people are currently affected by mental disorders worldwide with an increase of more than 13% over the 10-year period since 2007. In 2017 depression was identified among three main chronic disabilities (along with back pain and headache disorders) with the prevalence of 264 million, while the prevalence of anxiety was estimated at the level of 284 million (James, 2018). Health systems have not yet adequately responded to the burden of diseases associated with mental health. Therefore, the gap between the need for treatment and its provision is wide worldwide. In

Abbreviations: 6PN, 6-prenylnaringenin; 8PN, 8-prenylnaringenin; BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; CNS, central nervous system; DAS, Corah's Dental Anxiety Scale; DDXN, *O*-desmethyl-α,β-dihydroxanthohumol; DMX, desmethylxanthohumol; DSM, Diagnostic and Statistical Manual of Mental Disorders; DOPAC, 4-dihydroxyphenylacetic acid; DXN, α,β-dihydroxanthohumol; GAD, generalized anxiety disorder; GABA, gamma-aminobutyric acid, HAM-A - The Hamilton Anxiety Rating Scale; HAM-D, Hamilton Depression Rating Scale; HPA axis, hypothalamic-pituitary-adrenal axis; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; LSD, lysergic acid diethylamide; MADRS, Montgomery-Asberg Depression Rating Scale; MDD, major depressive disorder; MGB axis, microbiota-gut-brain axis; OTU, Operational Taxonomic Units; SCFA, short-chain fatty acids; SSRIs, selective serotonin reuptake inhibitors; STAI, State-Trait Anxiety Inventory; VAS, Visual Analogue Scale; WHO, World Health Organization; XN, xanthohumol.

* Corresponding author: Jakub P. Piwowarski, ul. Banacha 1, 02-097 Warsaw, Poland.
E-mail address: jpiwowarski@wum.edu.pl (J.P. Piwowarski).

https://doi.org/10.1016/j.phymed.2023.154642
Received 22 January 2022; Received in revised form 22 November 2022; Accepted 1 January 2023
Available online 4 January 2023

low- and middle-income countries, between 76% and 85% of people with mental disorders receive no or insufficient treatment (Wang et al., 2007).

The majority of mental disorders require professional psychiatric and psychotherapeutic interventions, however in the case of mild mood disorders such as mild depression and anxiety, self-care can be sufficient and effective (Pilkington and Wieland, 2020). WHO defines self-care as "the ability of individuals, families, and communities to promote health, prevent disease, maintain health, and to cope with illness and disability with or without the support of a health-care provider" (WHO Regional Office for South-East Asia, 2017). The WHO has proposed a global perspective on self-care in traditional medicine which encompasses a range of approaches and activities, including herbal and other traditional remedies for disease management (WHO Regional Office for South-East Asia, 2017). Data from a nationally representative sample of 2055 people interviewed during 1997–1998 revealed that 57% of those suffering anxiety attacks, and 54% of those with severe depression reported using herbal medicine and complementary and alternative medicine therapies during the previous 12 months to treat their disorder. Interviews of 82 psychiatric North American patients hospitalized for acute care for various psychiatric disorders revealed that 44% had used herbal medicine (mainly for psychiatric purposes) during the previous 12 months (Sarris et al., 2011).

Research in the area of natural product-based psychopharmacology has revealed a variety of promising medicines that may provide benefits in the treatment of depression and anxiety disorders. Although conventional pharmacotherapies and psychological interventions are frontline approaches, natural product-based medicines may offer additional safe and effective treatment options or can become a source of novel lead compounds, which development can result in the introduction of therapies based on alternative modes of action. In the past decade, no substantial advances have occurred in the development of new classes of synthetic antidepressant and anxiolytic medications, with selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines currently remaining first-line treatments in depression and anxiety disorders, respectively (Gartlehner et al., 2016; Sarris, 2018). However, especially in the case of depression, a significant percentage of patients do not experience full remission with the pharmacological treatment. Benzodiazepines are a common class of drugs used to cure anxiety disorders. Although these drugs are effective, benzodiazepines treatment leads to several problems: a significant percentage of patients are non-responders (approximately 25%), the development of tolerance and potential dependence as well as sedation, resulting in cognitive and psychomotor impairment (Cowley et al., 1991; Uzun et al., 2010). For this reason, the development of safe alternative therapies based on novel pharmacological targets is still desired (Koen and Stein, 2011). It is worth noting that in the case of mild depressive symptoms, the effectiveness of drugs is no greater than placebo, while their use is subject to health risks associated with possible side effects (Kirsch, 2019).

The role of natural products in the treatment of various psychiatric disorders has become more established over the past decade, with phytotherapeutic preparations such as St. John's wort (*Hypericum perforatum* l.), valerian (*Valeriana officinalis* l.), and Kava (*Piper methysticum* G. Forst.) possessing respectable clinical evidence confirmed by meta-analyses and indicated well-established use in EMA monographs (Ng et al., 2017; Nicolussi et al., 2020; Pittler and Ernst, 2003; Sarris, 2018; White, 2018). Other medicinal plant materials such as purple passionflower (*Passiflora incarnata* l.), hops (*Humulus lupulus* l.), lemon balm (*Melissa officinalis* l.) are applied due to their long traditional use supported by controlled clinical trials partially confirming their activity, however referring to some inconsistencies, which prevent their registration as a medicinal product of well-established use (Leach and Page, 2015; Miroddi et al., 2013; Sarris, 2018; Sarris et al., 2013; Shakeri et al., 2016). In recent years a renaissance in the research of antidepressant, anxiolytic and antiaddictive potential of psilocybin and psilocin (serotonin 2A receptor (5-HT_{2A}R) agonists) present in various fungi species belonging to *Psilocybe* and *Panaeolus* species has reemerged, giving very promising results in clinical studies (dos Santos and Hallak, 2020).

There are numerous pharmacological findings for certain natural products published, which propose similar clinical effects to the synthetic antidepressant and anxiolytic drugs (Schulz, 2006). However, the discussion on the compounds responsible for the observed activity of the whole extracts is still ongoing. The common problem faced by most of the plant extracts applied in mood disorders is that despite fully confirmed clinical effects and postulated mechanisms of anti-depressant and/or anxiolytic activity (involving nonselective inhibition of the neuronal reuptake of serotonin, dopamine, norepinephrine, gamma-aminobutyric acid (GABA), and l-glutamate, decreased degradation of neurochemicals and sensitization of and increased binding to various receptors), in the majority of cases the compounds responsible for their activity remain not fully identified in vivo (EMA/HMPC/150,848/2015; EMEA/HMPC/101,303/2008). This leads to controversies regarding the active pharmaceutical ingredient monitoring in clinical trials and consequence determination of its pharmacokinetic parameters. Certain constituents of plant extracts are not capable of crossing the blood-brain barrier or they display in vitro effects at very high human-equivalent doses, which makes the extrapolation of pre-clinical data on humans problematic (Sarris, 2018).

In some cases, the compounds present in the plant material, despite having active pharmacophores, are not applied in sufficient amounts to exert biological activity in vivo (Miroddi et al., 2013). The lack of precise identification of chemical constituents in plant preparations that are necessary to achieve specific pharmacological action affects the process of plant material standardization, which in turn does not allow obtaining and application in clinical trials a plant material preparation exerting optimal antidepressant and/or anxiolytic activity. These factors are commonly considered as a major cause of heterogeneity and discrepancies in results of all clinical and interventional trials conducted for complex natural mixtures leading to the common ambiguity in their metanalyses (Beaubrun and Gray, 2000).

As occurs in all herbal medicines, the synergy between compounds may be a vital aspect of efficacy (Williamson, 2001). Due to the lack of unambiguous identification of active principles, it is commonly postulated that various bioactive constituents can contribute to the reported clinical effects, probably synergistically. However, no studies that would confirm this hypothesis for natural products applied in mood disorders have been conducted so far. Thus, currently the synergism concept can also be considered to function as a rationalization strategy for natural products with not yet identified active principles (Caesar and Cech, 2019).

Much attention has been paid to the role of gut microbiota in the biotransformation of xenobiotics ingested by humans. It is discussed that gut microbiota significantly influences the efficacy and toxicity of synthetic drugs and natural products used to treat different diseases (Wilson and Nicholson, 2017). Recent findings consequently show that gut microbiota is also crucial for normal healthy brain function. Bidirectional communication between the brain and the gut has long been recognized. It is suggested that gut microbiota is an important player in how the body influences the brain, contributes to normal healthy homeostasis, and influences the risk of disease, including anxiety and mood disorders (Ameringen et al., 2019; Foster and McVey Neufeld, 2013).

Microbiota-gut-brain axis

Depression constitutes a large group of mental disorders with a varied clinical picture and severity that cause significant health, social and economic complications (World Health Organization, 2017). Observations about the relationship of adverse life events in childhood with the risk of developing depression in adulthood are one of the first attempts to determine the importance of environmental factors in the etiology of depression (Freud, 1917).

Nowadays, the importance of inflammatory processes activated by stress and other environmental factors in the etiology of depression is growing (Gałecki and Talarowska, 2018). The most popular and probable etiological hypothesis of depression is monoaminergic deficiency with a decrease of serotonin, noradrenaline, and dopamine levels in the synaptic cleft in several areas of the central nervous system (Hirschfeld, 2000). Its complement and extension is the etiological model of depression, which indicates the importance of stress experienced in early life, in predisposed individuals may lead to dysfunction of the hypothalamic-pituitary-adrenal axis (HPA axis), resulting in a number of adverse structural, hormonal and immunological changes affecting the functioning of the central nervous system (CNS) (Slavich and Irwin, 2014).

The researchers' attention is currently focused on the importance of intensifying inflammatory phenomena and autoimmune distortions of the intestinal barrier function and the microbiota-gut-brain axis (MGB axis) due to the stress response in the pathogenesis of depression (Cryan et al., 2019; Simpson et al., 2021). Although inconclusive, significant differences in gut microbiota composition in healthy and depressed people were found (Sanada et al., 2020). Despite all limitations and inconsistencies, variations in several taxa can be linked with the occurrence of psychiatric disorders. For instance, the increased relative abundances of *Enterobacteriaceae, Streptococcaceae, Eggerthella, Olsenella, Lactobacillus, Erysipelotrichaceae incertae sedis, Holdemania, Streptococcus, Desulfovibrio, Paraprevotella* and decreased levels of *Prevotellacae, Sutterella, Clostridium cluster XIVa, Escherichia/Shigella,* and *Coprococcus* are associated with depression. At the same time, the Enterobacteriales, *Bacteroidaceae, Escherichia/Shigella, Bacteroides, Tyzerella* enrichment and depletion of Firmicutes, Mollicutes, *Prevotellaceae, Ruminococcaceae, Subdoligranulum, Coprococcus,* and *Dialister* are correlated with anxiety (Simpson et al., 2021). In terms of gut microbiota diversity, researchers obtained inconsistent results in studies focused on major depressive disorder (MDD), yet patients with a generalized anxiety disorder (GAD) are marked with lower microbiota richness compared to healthy participants. However, the limited number of studies evaluating changes in the microbiome structure of GAD patients makes it difficult to clearly define modification in their intestinal bacteria composition.

In depression and anxiety disorders, a higher relative abundance of pro-inflammatory microbiota species and a lower abundance of short-chain fatty acids (SCFA) producing bacteria is observed (Simpson et al., 2021). Indirect evidence for the importance of microbiota in the etiology of depression are studies on fecal transplantation from depressed patients to laboratory animals with reduced microbiota counts or without microbiota (Kelly et al., 2016; Liang et al., 2018). How the composition or function of the microbiota influences the risk of developing depression remains a separate issue. Recent years have brought much evidence of two-way communication between the gut microbiota and the CNS via neuronal, metabolic, endocrine, and immune signaling (Simpson et al., 2021). The intestinal microbiota affects the functions of the HPA axis, the immune system, regulates the blood-brain barrier, the synthesis of neurotransmitters, neurogenesis, and influences the brain's proper development (Liang et al., 2018). Changes in the intestinal microbiota composition can lead to the creation or intensification of systemic inflammatory processes and directly and indirectly modulate inflammatory pathways and microglial activation in CSN via the cytokine mechanism (Carlessi et al., 2021). In terms of neuroimmunophysiology, two major advances in understanding have emerged: enteric microbiota actively participate in neuroglial communication in the gastrointestinal tract, and enteric glia can orchestrate neuroimmune regulation of host defense in the intestinal mucosa (Sharkey et al., 2018).

Stress, which is one of the important etiological factors of depression, also affects intestinal microbiota composition. Studies on animal models indicate that the experience of stress modifies the composition of the microbiota and contributes to the intensification of inflammatory processes and immune responses (Rieder et al., 2017). The resulting increase in intestinal permeability can cause fragments of bacteria or toxins and other specific activating substances, and non-specific immune mechanisms can enter the circulation, affecting the brain's work (Maes et al., 2012). The research conducted so far does not allow for an unambiguous answer to the question of to what extent the change in the microbiota composition is an etiological factor and to what extent it accompanies depression. There is no evidence of modification of the microbiota composition as a therapeutic agent in humans. Studies on animal models and healthy volunteers indicate the importance of diet or supplementation in modifying the composition of the microbiota in the prevention and treatment of depression (Cheng et al., 2019). The factors correlating with the correct intestinal microbiota composition include high consumption of fiber and fermented products, a Mediterranean diet, and plant-based diets. The Mediterranean diet reduces the risk of neurodegenerative disorders, mental illness, cancer, and cardiovascular disease. This diet has also been shown to correlate with a reduced risk of depression. The positive effects of the Mediterranean diet can be related to its anti-inflammatory properties as well as the observed changes at the level of the intestinal microbiota (Cryan et al., 2019).

The gut contains a nervous system as complex as that of the spinal cord and is the body's largest repository of immune cells. It is a major endocrine tissue and is home to a vast, diverse, and complex microbiota (Sharkey et al., 2018). The intestinal epithelium serves as a semi-permeable barrier restricting the movement of water, ions, and macromolecules from the gut lumen to the underlying mucosa. The epithelial lining of the gastrointestinal tract is the frontline of host defense and is all that separates host organism from commensal bacteria, protozoa, and fungi that live mutualistically within the gut lumen (Bäckhed et al., 2005). The intestinal epithelium actively contributes to the interplay between the intestinal microbiota and the luminal contents that regulate local effector responses to maintain homeostasis. The enteric epithelium is dynamically regulated by the mucosal immune system, the enteric nervous system, and enteroendocrine and stromal cells. The aim of this complex orchestration is to efficiently adjust to the challenges of the luminal environment, including the ingested food and xenobiotics as well as microbial communities that form an ecological network in the gut (Powell et al., 2017). Enteric nerves and glial cells, closely associated with the intestinal epithelium, play a critical role in maintaining epithelial integrity and intestinal homeostasis. The role of enteric nerves in the maintenance of epithelial permeability involves direct control of the physical barrier, which they accomplish by altering the distribution of tight junction proteins, the activation of electrogenic ion movement to initiate fluid and mucin secretion, and the stimulation of the immune barrier (Neunlist et al., 2013). The gut receives extrinsic innervation that contributes to the maintenance of homeostasis. Stimulation of the vague nerve elicits a well-described anti-inflammatory 'reflex' and immunomodulatory actions in the gut (Browning et al., 2017). The brain and gut are closely linked more than most other organ systems. Interestingly, patients with intestinal diseases associated with inflammation and disrupted barrier (including inflammatory bowel disease (IBD), coeliac disease, intestinal motility disorders and functional gastrointestinal disorders) often have comorbid neurological and/or psychiatric issues (Sharkey et al., 2018). Significant similarities between the risk factors of depressive and anxiety disorders and the importance of the discussed issues in the etiology of other mental disorders, such as schizophrenia or autism, indicate the promising directions of novel therapeutic targets identification.

The discussed role of the microbiota-gut-brain axis opens up new possibilities in the search for effective methods of treatment and prevention of mood disorders. Considering the inconsistencies regarding indication of pharmacologically active substances for most of the medicinal plants applied in the treatment of anxiety and depression, the aim of the review is to look at their pharmacological activity from the perspective of the microbiota-gut-brain axis.

Influence of plant extracts on microbial growth

Given that microbiota plays a role in the metabolism of drugs, it can be expected that this relationship also works the other way around, and plant extracts influence the composition and abundance of microorganisms inhabiting the intestine. Before analyzing the data on chosen medicinal plants regarding their complex interaction with gut microbiota communities, it is important to summarize the hitherto research on their anti-microbial activities determined by classical microbiological methods. Table S1 summarizes the data from available publications on the antibacterial activity of both isolated components and crude extracts from plants included in this review. It turns out that many of them exhibit antimicrobial activity against pathogens that disrupt intestinal homeostasis. Eradication of pathogens from the intestinal environment gives a chance for the gut re-colonization by strains beneficial for human health, which will produce the desired postbiotic metabolites.

Interestingly, herbal extracts can influence bacterial growth more intensively than powdered raw material, as it is with *Rhodolia rosea* (Kosakowska et al., 2018). Additionally, extract preparation methods should also be considered in the context of plant – microbiota interaction since different extracts are characterized by distinctive antimicrobial activity. For instance, aqueous extract of aerial parts of St. John's wort has stronger inhibitory activity on microbial growth than 96% ethanolic extract (Süntar et al., 2016). Some isolated compounds, such as hyperforin, act more selectively against gram-positive than gram-negative bacteria (Reichling et al., 2001). This specific activity towards some groups of bacteria can create niches for the growth of particular microbes. Unfortunately, the antimicrobial activity on commensal or pathogenic microorganisms in the gut microbiota of *Passiflora incarnata* extracts has not been studied sufficiently yet.

Natural products and gut microbiota

The bilateral interaction between herbal substances and gut microbiota proposes a new, alternative health-beneficial mechanism of action for naturally occurring substances. Considering the impact of natural products, herbs, and isolated plant compounds on human gut microbiota, one should take into account those materials' influence on gut microbiota structure, especially alpha and beta diversity. The first parameter measures the diversity within one sample, its variety, richness, and evenness, and the latter indicates how different the samples from separated environments are (Willis, 2019). Contemporarily two metagenomic analyses, 16 s rRNA (especially applicable in taxonomic studies) and shotgun sequencing, provide detailed insight into the complex gut microbiota community composition (Durazzi et al., 2021). However, due to plenty of data obtained during the high-throughput sequencing of microbiota samples, instead of analyzing the particular DNA sequences, scientists use Operational Taxonomic Units (OTUs), closely related, clustered sequences. This procedure enables the reduction of output data and simplifies data management. The microbiota composition within one sample is measured using alpha diversity estimators, while beta diversity describes differences between samples. Commonly used estimators for measuring the species richness are the number of OTUs, Chao1 index, and ACE index, while the Shannon and Simpson indices provide information about the diversity of bacterial communities. The often-used term is relative abundance, indicating one species/OTUs commonness. Additionally, in respect of beta diversity, the phylogenetic similarity of different microbial communities can be analyzed using the weighted or unweighted UniFrac algorithm coupled with principal coordinates analysis (Lozupone et al., 2011). This algorithm outlines similarities in bacterial communities habituated by distinct bacteria, but, in some cases, closely phylogenetically related.

Herbal medicines used in treating mood disorders exhibit a multidirectional mechanism of action, and their effect on the gut microbiota community emerges as a potential new one. Moreover, given that the reasons of the beneficial effects of lower doses of psychedelic substances such as ayahuasca, lysergic acid diethylamide (LSD), or psilocybin remain elusive, changes in gut microbiota structure may be essential to our thorough understanding of this phenomenon (Kuypers, 2019). Clinical interventions are undoubtedly the most valuable source of gut microbiota-plant interactions information; however, due to their complexity and inter-individual variability, preclinical studies are necessary to investigate this subject (Booijink et al., 2010). Several experimental platforms, starting from in vitro experiments like fecal fermentation, in vivo studies using rodents or pigs, and ending with germ-free animals colonized with human gut microbiota, are used. Each method implicates its advantages and disadvantages, and selecting a method should be conditioned by the subject of investigation (Thumann et al., 2019). Unfortunately, herbal substances used in treating mental stress and mood disorders are not sufficiently studied in the context of the gut-brain axis. Especially the relationship between *Piper methysticum* and the intestinal microbiome has not been investigated yet. Undoubtedly, the influence of herbal medicinal products on gut microbiota and consequently the modulation of mood and emotions is a fascinating and promising area of research. Constituents presented in herbal medicine products may act as a prebiotic and promote beneficial bacterial growth, e.g., short fatty acid producers (Peterson et al., 2018). Antidepressant activity of fructo-oligosaccharides from *Morinda officinalis* on mice exposed to chronic unpredictable mild stress is linked to both their conversion to profitable metabolites and modulation of gut microbiota structure (Chi et al., 2020). While prebiotic supplementation alone does not significantly reduce depression score in patients with depressive disorder, it can benefit mood improvement (Leyrolle et al., 2021; Liu et al., 2019).

Due to the gut ecosystem's complexness and lack of stability, it remains unclear what the healthy microbiome actually is (Bäckhed et al., 2012). This fact poses a question of measuring the beneficial changes in gut microbiota structure after natural products administration. In this light, studies evaluating the impact of medical plants on intestinal bacteria focus on reversing changes in the abundance of individual taxa during disease rather than achieving a specific microbiome structure. The most frequently performed preclinical studies are carried out in the context of changes in microbiota structures on rodent models of a high-fat diet, colitis, or *ex vivo* fermentation with human fecal samples. This fact creates the need for new studies explicitly focused on the plants-microbiota-mood disorders triangle of interactions.

Most herbal medicines and isolated constituents influence bacteria communities, significantly increasing alpha and beta diversity and changing their structure, consequently creating separate gut ecosystems (Table 1). It seems vital because the loss of microbiota diversity is one of the most apparent dysbiosis in numerous human diseases (Mosca et al., 2016). The changes in relative abundances of particular gut bacteria after natural products administration are repeatedly conflicting, so one substance can promote the growth of taxon found depleted in patients with psychiatric disorders and at the same time lower the level of another potentially beneficial genus.

Herbal medicines applied in mild mood disorders

Hypericum perforatum l

Clinical trials

One of the best-known and thoroughly studied herbal medicinal products used in treating mood disorders is Hyperici herba. Eight meta-analyses investigating the anti-depressant efficacy of *H. perforatum* were retrieved and summarized in Table S2. All the reports consecutively showed the effectiveness of *H. perforatum* preparations and single compounds originating from this plant material as effective in treating depression. Both responses to treatment and remission of the disease were reported. The evidence examined in the meta-analyses indicated a significant decrease of symptoms compared to placebo and equal effect of *H. perforatum* preparations compared to SSRIs and second-line anti-

Table 1
Modulation of gut microbiota by natural products.

Extract/ compound	Tested model	Effect	Dose/ duration	Ref.
α,β-dihydro-xanthohumol (*Humulus lupulus)*	Male, 9-week-old C57BL/6 J mice fed with high-fat diet	↑Proteobacteria, *Enterobacteriaceae, Erysipelotrichaceae, Paenibacillaceae* ↓Bacteroidetes, Tenericutes, *Eubacteriaceae, Clostridiaceae, Halobacteroidacae, Lactobacillaceae, Anaeroplasmataceae, Enterococcaceae, Porphyromonadaceae, Rikenellaceae*	30 mg/kg p.o. for 13 weeks	(Zhang et al., 2020)
Bornyl acetate (*Valeriana officinalis)*	Male, 8-week-old Spraque-Dawley rats treated with 5-fluorouracil	↑*Lactobacillaceae, Lactobacillus* ↓*Helicobacteraceae, Enterobacteriaceae, Porphyromondaceae, Streptococcaceae, Bacteroidaceae, Ruminococcus, Helicobacter, Escherichia, Parabacteroides, Bacteroides*	2, 4 or 8 mg/kg p.o. for 12 days	(Zhang et al., 2017)
Citral (*Melissa officinalis)*	Male KM mice	↑Observed species index, Chao1 index, Simpson index, Shannon index ↑*Lactobacillus, Parabacteroides* ↓*Bacteroides*	0.2 ml of prepared citral (2 ml citral mixed with 100 ml of 2 g/l Tween-80 solution) p.o. for 28 days	(Wang et al., 2019)
Crocin-I (*Crocus sativus*)	Male, 6-week-old C57BL/6 J mice treated with corticosterone	↑Shannon Index ↑Bacteroidetes, *Parabacteroides, Prevotella, Allobaculum* ↓Firmicutes, Proteobacteria, *Odoribacter, Ruminococcus.* ↓Firmicutes/Bacteroidetes	20 or 40 mg/kg p.o. for 3 weeks	(Xie et al., 2019)
Crocin-I (*Crocus sativus*)	Male, 6-week-old C57BL/6 J mice exposed to chronic restraint stress	↓Shannon index, Simpson index ↑*Peptococcaceae, Anaerotruncus, Mucispirillum, Lactobacillus ruminococcus,* l. *lactobacillus, Bacteroidacea bacteroides* ↓Bacteroidetes, Proteobacteria, *Porphyromonadaceae parabacteroides, A. sutterella, R. ruminococcus*	40 mg/kg p.o. for 6 weeks	(Xiao et al., 2020)
Geraniol (*Lavandula officinalis*)	People diagnosed with irritable bowel syndrome	↑*Collinsella, Faecalibacterium*	8 mg/kg p.o. for 4 weeks	(Rizzello et al., 2018)
Humulus lupulus extract	Female, C57BL/6 7-month-old mice	↓*Akkermansia muciniphila*	400 mg/kg p.o. 12 weeks	(Hamm et al., 2019)
Humulus lupulus (spent hops)	5 week old crossbreed pigs [Piétrain x (German Landrace × German Edelschwein)]	↓*Streptococcus, Clostridium Cluster XIVa*	Diet supplemented with 1% spent hops for 4 weeks	(Fiesel et al., 2014)
Humulus lupulus cultivar „Pacific Gem" supercritical CO2 extract	In vitro fermentation of mixed fecal samples sourced from 10 healthy volunteers	↑Proteobacteria, *Enterobacteriaceae, Clostridiaceae, Akkermansia* ↓Bacteroidetes, Actinobacteria, Firmicutes, *Coriobacteriaceae, Bacteroides, Collinsella, Clostridium, Eubacterium, Desulfovibrio, Bifidobacterium, Blautia, Dorea, Veillonella*	10 – 5000 μg/ml	(Blatchford et al., 2019)
Hypericum perforatum extract (8.94% total flavonoids, 0,026% hyperoside, 0.323% hypericin)	Female, 6–8-week-old ovariectomized Sprague–Dawley rats	↑Firmicutes/Bacteroidetes ↑Firmicutes, Verrucomicrobia, *Lactobacillus, Romboutsia, Faecalitalea, Akkermansia* ↓Bacteroidetes, Elusimicrobia, Gemmatimonadetes, *Bacteroides, Elusimicrobium, Oscillibacter, Acinetobacter*	300 mg/kg p.o. for 12 weeks	(l. Chen et al., 2021)
Isoorientin (*Passiflora incarnata)*	Male, 5-week-old BALB/c mice were treated with benzo[*a*] pyrene	↑*Faecalibaculum, Lactobacillus, Enterococcus, Blautia* ↓Acinetobacter, *Veillonella, Alloprevotella*	20 mg/kg p.o. for 42 days	(He et al., 2019)
Isoorientin (*Passiflora incarnata)*	Male, 5-week-old BALB/c mice	↑*Marvinbryantia* ↓ *Odoribacter, Anaerotruncus, Rikenella*	15 mg/kg p.o. for 30 days	(Yuan et al., 2018)
Isoquercetin (*Hypericum perforatum)*	Male, 6–8-week-old C57BL6 mice fed with high-fat diet	↑Firmicutes	Diet supplemented with 0.05% isoquercetin for 12 weeks	(Tan et al., 2018)
Isoxanthoflav containing 95% isoxanthohumol (*Humulus lupulus*)	Male, 7-week-old C57BL/GJ mice fed with high-fat diet	↑Verrucomicrobia, *Akkermansia muciniphila* ↓Firmicutes, *Clostridium*	Diet supplemented with 0.01%, 0.03% and 0.1% isoxanthoflav for 4 and 8 weeks	(Yamashita et al., 2020)
Isoxanthohumol (*Humulus lupulus*)	Male, 7-week-old C57BL/6 J mice fed with high-fat diet	↑ Verrucomicrobia, Bacteroidetes, Proteobacteria, *Akkermansia muciniphila, Blautia, E. coli, Bacteroides, Prevotella, Eubacterium* ↓Firmicutes, *Oscillospira, Lactococcus, Dehalobacterium, Anaerotruncus, Ruminococcus*	20, 60 and 180 mg/kg p.o.	(Fukizawa et al., 2020)
Linalool (*Lavandula officinalis*)	Male KM mice	↑Observed species, Shannon and Chao1 indexes in cecum. ↑Chao1 index in colon ↓Observed species, Shannon and Simpson indexes in colon. ↑Firmicutes/Bacteroidetes ratio in cecum	0.2 ml of prepared linalool (2 ml linalool mixed with 100 ml of 2 g/l Tween-80 solution) p.o. for 28 days.	(Wang et al., 2019)

(*continued on next page*)

Table 1 (*continued*)

Extract/ compound	Tested model	Effect	Dose/ duration	Ref.
		↓Firmicutes/Bacteroidetes in colon ↑*Lactobacillus* in cecum and colon. ↑*Parabacteroides* in colon. ↓*Bacteroides* in cecum and colon		
Limonene (*Lavandula officinalis*)	Male KM mice	↑Observed species, Shannon, Simspon and Chao1 indexes in cecum and colon ↑Firmicutes/Bacteroidetes ratio in cecum ↓Firmicutes/Bacteroidetes ratio in colon ↑*Lactobacillus* in cecum and colon. ↑*Parabacteroides* in colon. ↓*Bacteroides* in cecum and colon	0.2 ml of prepared limonene (2 ml limonene mixed with 100 ml of 2 g/l Tween-80 solution) p.o. for 28 days.	(Wang et al., 2019)
Melissa officinalis extract (2.76 +/- 0.05 mg of rosmarinic acid per 100 mg dried material)	Male, 12-week-old, C57Bl/6 J ob/ob mice	↑Chao-1 index ↑*Porphyromonadaceae*	500 mg p.o. for 2 weeks	(Brochot et al., 2019)
Procyanidin B2 (*Hypericum perforatum*)	Male, 2-month-old New Zealand white rabbits provided with high-fat diet	↓ Firmicutes/Bacteroidetes ↓*Allobaculum* ↑*Ruminococcus, Bacteroidetes, Akkermansia*	150 mg/kg p.o. for 12 weeks	(Xing et al., 2019)
Procyanidin B2 Extracted from fresh litchi (89,57% purity) (*Hypericum perforatum*)	Male, 3-week-old ApoE−/− mice fed with high-fat diet	↓Firmicutes/Bacteroidetes ↑Verrucomicrobia, *Akkermansia, unclassified_f_Prevotellaceae, Coriobacteriaceae_UCG-002*	110 mg/kg p.o. for 12 weeks	(Yang et al., 2021)
Quercetin (*Hypericum perforatum*)	In vitro fermentation of mixed fecal samples sourced from healthy volunteers	↑ Bacteroides, Firmicutes ↓*Bifidobacterium*	10, 30, or 100 μg/ml for 24 or 48 h	(Parkar et al., 2013)
Quercetin (*Hypericum perforatum*)	In vitro fermentation of mixed fecal samples sourced from healthy volunteers	↑Actinobacteria. Firmicutes ↓Bacteroidetes	0.15 g/l for 10h	(Huang et al., 2016)
Quercetin (*Hypericum perforatum*)	Male, 5-week-old Wistar rats	↓The total amount of OTUs ↓*Lactobacillus, Unclassified, Clostridiaceae, Sutterella*	150 mg/kg p.o for 14 days	(Yu et al., 2019)
Quercetin (*Hypericum perforatum*)	Male, 7 weeks old C57BL/6 J mice provided with high-fat diet	↑Bacteroidetes, Bacteroidia, Erysipelotrichi, BetaProteobacteria, *Flavobacterium, Allobaculum, Sutturella* ↓Proteobacteria, DeltaProteobacteria, *Desulfovibrio, Helicobacter* ↓Firmicutes/Bacteroidetes	Diet supplemented with 0.05% quercetin for 16 weeks	(Porras et al., 2017)
Quercetin (*Hypericum perforatum*)	Wistar rats fed with high-fat sucrose diet	↓Firmicutes/Bacteroidetes ↓Erysipelotrichi, Erysipelotrichaceae, Bacillus, Eubacterium cylindroides	30 mg/kg p.o. for 6 weeks	(Etxeberria et al., 2015)
Quercetin (*Hypericum perforatum)*	28–56-day-old ApoE−/− mice provided with high cholesterol diet	↓*Phascolarctobacterium, Anaerovibrio*	100 mg/kg p.o. for 12 weeks	(Wu et al., 2019)
Quercetin (*Hypericum perforatum*	90-day- old *Ldlr*–/– C57BL/6 mice provided with high-fat diet	↑ *Akkermansia, Bacteroides, Parabacteroides, Ruminococcus* ↓ *Lactobacillus*	100 μg p.o. for 4 weeks	(Nie et al., 2019)
Quercetin (*Hypericum perforatum*	Male, six-week-old, Sprague Dawley rats treated with streptozotocin	↑ Actinobacteria, Bifidobacteriales, Enterobacteriales, *Prevotellaceae, Enterobacteriaceae, Bifidobacteriaceae, Prevotella, Paraprevotella, Escherichia/Shigella, Bifidobacterium*	50 mg/kg for 12 weeks	(Xie et al., 2020)
Quercetin (*Hypericum perforatum*)	Male, Kunming mice treated with antibiotics cocktail	↑ Chao1 Index, Simpson Index ↑Defferribacteres, *Mucispirillum, Roseburia, Coprococcus_1, Rumini-Clostridium_9, Tyzzerella, RuminiClostridium, Eubacterium xylanophilum, Oscillibacter, Anaerovorax, Lachnospiraceae FCS020 group* ↓Proteobacteria, GammaProteobacteria and Erysipelotrichia, *Escherichia-shigella*	Diet supplemented with 0.2% quercetin for 10 days	(Shi et al., 2020)
Quercetin Onion preparation, quercetin aglycone 97.8% of total polyphenols (*Hypericum perforatum*)	Female ICR mice treated with dextran sodium sulfate	↑ Chao1 index, Shannon index ↓Simpson index	Diet supplemented with 0.21% onion preparation for 7 days	(Hong and Piao, 2018)

(*continued on next page*)

Table 1 (*continued*)

Extract/ compound	Tested model	Effect	Dose/ duration	Ref.
Quercetin (*Hypericum perforatum*)	Female C57BL/6 mice infected with *Citrobacter rodentium*	↑ Chao1 Index, Shannon index Ace index ↓Simpson index ↑*Bacteroides, Bifidobacterium, Lactobacillus, Clostridia* ↓*Fusobacterium, Enterococcus*	30 mg/kg p.o. for 2 weeks	(Lin et al., 2019)
Quercetin (*Hypericum perforatum*)	Male, 14-month-old ICR mice	↓*Epsilonbacteraeota*	Diet supplemented with 0.08% quercetin p.o. for 21 days	(Yang et al., 2020)
Quercetin (*Hypericum perforatum*)	15-month-old ICR mice fed with regular diet or diet including dietary advanced glycation end products	↑ACE index, Chao1 index ↓ *Proteobacteria, Tenericutes, Prevotella, Ruminococcus,*	Diet supplemented with 0.08% quercetin for 16 weeks	(Yang et al., 2020a)
Quercetin (*Hypericum perforatum*)	Male, 7-week-old Sprague-Dawley rats fed with high-fat diet	↑*Ruminococcaceae*, Akkermansiaceae, *Bacteroides, Eubacterium* ↓*Prevotellaceae, Alloprevotella, Desulfovibrio, Phascolarctobacterium*	Diet supplemented with 0.1% quercetin for 12 weeks	(Peng et al., 2020)
Quercetin (*Hypericum perforatum*)	Male, 4-week-old C57BL/6 mice fed with low-fat or high-fat diet	↑ Bacteroidetes, *Parabacteroides* ↓Firmicutes, *Lactobacillus* ↓Firmicutes/Bacteroidetes	Diet supplemented with 1% quercetin for 16 weeks	(Pei et al., 2021)
Quercetin (*Hypericum perforatum*)	Female and male newborn ICR mice exposed to $PM_{2.5}$	↑ *Odoribacter, Streptococcus, Lactobacillus, Roseburia* ↓*Flavonifractor, Oscillibacter, Bacteroides, Alistipes, Proteus, Enterobacter, Akkermansia*	50, 100 or 200 mg/kg p.o. for 35 days	(Liu et al., 2020)
Quercetin (*Hypericum perforatum*)	In vitro fermentation of mixed fecal samples sourced from healthy volunteers	↑Firmicutes, *Bifidobacterium, Enterococcus, Streptococcus, Collinsella* ↓Bacteroidetes, *Bacteroides, Parabacteroides, Prevotella, Rikenellaceae spp, Butyricimonas, Odoribacter*	0.15 g/l for 10h	(Xue et al., 2016)
Rosmarinic acid (*Melissa officinalis, Lavandula officinalis)*	Male, Sprague-Dawley rats fed with high-fat diet and treated with streptozocin	↑Actinobacteria, Proteobacteria, Verrucomicrobia, Fusobacteria, Bifidobacteriales, Burkholderiales, Coriobacteriales, Enterobacteriales, Erysipelotrichales, Lactobacillales, Selenomonadales, Fusobacteriales, *Anaerostipes, Bacteroides, Bifidobacterium, Blautia, Collinsella, Coprococcus, Faecalibacterium, Oscillospira, Parabacteroides, Peptostreptococcaceae incertaesedis, Phascolarctobacterium, Sutterella, Turicibacter* ↓ *Anaerotruncus, Desulfovibrio, Flavonifractor, Lachnospiraceae (Unclassified), Lachnospiraceae (uncultured), Oscillibacter, Prevotella, Ruminococcaceae, incertaesedis, Ruminococcaceae (unclassified), Ruminococcaceae (uncultured), Ruminococcus*	30 mg/kg p.o. for 8 weeks	(Ou et al., 2018)
Rosmarinic acid (*Melissa officinalis, Lavandula officinalis)*	Male, Wistar rats	↑*Lactobacillus*, ↓Firmicutes, Bacteroidetes, *Bacteroides, Clostridium leptum*,	1 and 10 mg/kg p.o. in nanoparticles for 14 days	(Madureira et al., 2016)
Rutin (*Hypericum perforatum)*	Male, 5week-old C57BL/6 mice treated with 2% w/v dextran sodium sulfate	↑*Rikenellaceae*, unassigned *Lachnospiraceae, Ruminococcus,* unassigned *Clostridiales, Oscillospira, Lactobacillus, Streptococcus, Coprococcus,* unassigned *Erysipelotrichaceae, Bifidobacterium* ↓*Bacteroides*, unassigned *Enterococcaceae*	Diet supplemented with 0.025% for 3 weeks	(Power et al., 2016)
Rutin (*Hypericum perforatum*)	In vitro fermentation of mixed fecal samples sourced from healthy volunteers	↑ Bacteroides, Firmicutes ↓*Bifidobacterium*	10, 30, or 100 μg/ml for 24 or 48 h	(Parkar et al., 2013)
Saffron aqueous extract (*Crocus sativus)*	C57BL/6 N mice treated with 2.5% w/v dextran sodium sulfate	↓Proteobacteria, Cyanobacteria	10,20 mg/kg p.o. for 11 days	(Banskota et al., 2021)
Salidroside (*Rhodiola rosea*)	Balb/c mice treated with furan	↑Chao 1 index, observed species ↑ Verrucomicrobia, Candidatus_Melainabacteria, Bacteroidetes, *Akkermansia, Roseburia* ↓Proteobacteria, Candidatus Saccharibacteria, *Rhodospirillaceae, Blautia*	10, 20 or 40 mg/kg for 15 days	(Yuan et al., 2019)
Salidroside (*Rhodiola rosea*)	SAMP8 mice	↑*Norank_f_Muribaculaceae, Alloprevotella, Parasutterella* ↓*Prevotellaceae, Lachnospiraceae_NK4A136_group, Unclassified_f_Lachnospiraceae, Alistipes, Norank_f_Lachnospiraceae, Odoribacter, Rikenellaceae_RC9_gut_group, Ruminococcaceae_UCG-014* and *RuminiClostridium_9*	50 mg/kg p.o. for 3 months	(Xie et al., 2020)

(*continued on next page*)

Table 1 (*continued*)

Extract/ compound	Tested model	Effect	Dose/ duration	Ref.
		↑*Bacteroidetes/Firmicutes*		
Salidroside (*Rhodiola rosea*)	Male C57BL/6 mice provided with high-fat diet	↑*norank_f_Lachnospiraceae, Ruminiclostridium* ↓*Lactobacillus, AlloPrevotella*	20 mg/kg p.o. for 4 weeks	(Li et al., 2020)
Terpinen-4-ol (*Lavandula officinalis)*	Male C57BL/6 mice treated with 2.5% w/v dextran sodium sulfate	↑*Lactobacillus* ↓*E. coli*	5, 10, 20 mg/kg p.o. for 21 days	(Zhang et al., 2017)
Ursolic acid (*Melissa officinalis*)	Male hamsters fed with high cholesterol diet	↑Bacteroidetes, *Bacteroidales_S24–7_group, Rikenellaceae_RC9_gut_group, Bifidobacterium* ↓Firmicutes, *Ruminococcaceae* ↓Firmicutes/Bacteroidetes	Diet supplemented with 0.2% or 0.4% ursolic acid for 6 weeks	(Hao et al., 2020)
Ursolic acid (*Melissa officinalis*)	C57BL/6 mice gaved with CCL_4	↑Chao1 index, Shannon index ↑Firmicutes, Bacteroidetes, *Lachnospiraceae* ↓Verrucomicrobia, *Akkermansia*	40 mg/kg p.o. twice a week for 4 weeks	(Wan et al., 2020)
Ursolic acid (*Melissa officinalis*)	C57BL/6 mice gaved with CCL_4	↑Chao1 index, Shannon index ↑Firmicutes, Actinobacteria, *Lactobacillus, Bifidobacterium, Ruminiclostridium*	40 mg/kg p.o. twice a week for 4 weeks	(Wan et al., 2019)
Ursolic acid (*Melissa officinalis*)	Male, Spraque-Dawley rats gaved with CCL_4	↓Number of OTUs ↑Proteobacteria, Firmicutes, Actinobacteria, Tenericutes	40 mg/kg p.o. for 4 weeks	(Zhang et al., 2019b)
Ursolic acid (*Melissa officinalis*)	Male, 4-week-old Kunming mice treated with chlortetracycline	↑*Serratia, Bradyrhizobium* ↓ Burkholderiales, Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria	150 mg/kg p.o. for 2 weeks	(Peng et al., 2021)
Xanthohumol (*Humulus lupulus*)	Male, 9-week-old C57BL/6 J mice fed with high-fat diet	↓Tenericutes, *Eubacteriaceae, Clostridiaceae, Halobacteroidacea, Lactobacillaceae, Anaeroplasmataceae*	30 mg/kg p.o. for 13 weeks	(Y. Zhang et al., 2020)
Xanthohumol (*Humulus lupulus*)	Male, 8–10 week-old Tac:SW mice fed with high-fat diet	↓Chao1 index, Shannon index ↑*Verrucomicrobiaceae, Akkermansia muciniphila, Parabacteroides goldsteinii, Alistipes finegoldi,* unknown *Bacteroides* ↓*Porphyromonadaceae, Lactobacillaceae, Lachnospiraceae*	60 mg/kg p.o. for 10 weeks	(Logan et al., 2021)

depressants.

Since the publication of these meta-analyses, the new evidence has been published in five trials (Di Pierro et al., 2018; Eatemadnia et al., 2019; Seifritz et al., 2016; Warren et al., 2019; Yechiam et al., 2019) summarized in Table S3. Four of them applied comprehensively standardized extracts as the interventions. Four studies were randomized, double-blind, and controlled. Two trials investigated short-term or single administration (Warren et al., 2019; Yechiam et al., 2019) and gathered evidence on the early-onset efficacy against some symptoms of depression and/or anxiety. One trial showed evidence supporting the efficacy of *H. perforatum* in alleviating menopausal symptoms and depression with very low doses of the preparation (Eatemadnia et al., 2019). One publication retrieved was a subgroup analysis of data acquired by (Szegedi et al., 2005), which focused on participants with the diagnosis of moderate depression accordingly to DSM-IV (Seifritz et al., 2016). According to this re-analysis, WS 5570 extract is an effective treatment in this subgroup with the significant response and remission rates compared to paroxetine.

Preclinical studies

Many studies were performed focused on the metabolism and bioavailability of compounds present in extracts of *Hypericum perforatum* (Caccia, 2005; Caccia and Gobbi, 2010; Schulz et al., 2005). The most important studies and reviews on the subject were summarized in Table S4. It was shown in rodents (rats or mice) that several natural products including hypericin, hyperforin, amentoflavone, 12,II8-biapigenin and simple flavonoids (quercetin, isorhamnetin) can be absorbed and are present in plasma (Caccia and Gobbi, 2010; Fox et al., 2001). In the case of hyperforin and 12,II8-biapigenin was detected in the brain tissue of investigated animals. There are also several reports involving human volunteers. The administration of *Hypericum* extract shows that hypericin, pseudohypericin, quercetin or isorhamnetin can access circulation in the range from 3.3 to 89.2 μg/l (Caccia, 2005; Caccia and Gobbi, 2010).

Several studies on the permeability of compounds contained in Hypericum extract were performed (Table S5). The analysis of the extract using Caco-2 cell monolayers showed that protohypericin, hypericin, quercetin, hyperoside, isoquercitrin, quercitrin and rutin could pass through cells from apical to the basolateral side (Kamuhabwa et al., 1999; Verjee et al., 2019). Additionally, it was proven that hypericins can accumulate in cells (Kamuhabwa et al., 1999). The report involving amentoflavone using BCEC cells showed that the compound can pass monolayers and can also be detected in cell lysates (Gutmann et al., 2002) (Table S5).

Hypericum perforatum and gut microbiota

The preclinical studies indicate the plausible impact of Hyperici herba constituents on the intestinal microbiome. The beneficial effect of *Hypericum perforatum* extract on gut microbiota structure was recently proved using an animal model of estrogen deficiency in ovariectomized rats (l. Chen et al., 2021). Despite the use of a model different than the animal model of depression, the results obtained from this study should arouse curiosity due to the co-occurrence of mood disorders with menopause and the associated decrease in estrogen levels (Albert and Newhouse, 2019; Llaneza et al., 2012). Administration of *Hypericum perforatum* extract induced a change in the relative abundance of several genres, including increased levels of *Lactobacillus, Akkermansia,* or Firmicutes and depletion of *Bacteroides* bacteria. The elevated level of the first one was found in depressed patients; nonetheless, species of *Lactobacillus* are commonly used as a probiotic with a potentially advantageous activity on mood (Kazemi et al., 2019; Romijn et al., 2017; Simpson et al., 2021; Slykerman et al., 2017). The abundance of the

second one was found decreased in mice exposed to chronic mild social defeat stress (McGaughey et al., 2019). Furthermore, *Akkermansia muciniphila* is a key regulator of the intestinal mucus layer, increasing its thickness and functionality of the gut barrier, which abnormal functionality is linked with mood disorders as well (Zhai et al., 2019). Additionally, the growth-promoting effect on *Akkermansia* was observed in the rabbit model of obesity after oral supplementation with one of the *Hypericum perforatum* constituents – procyanidin B2, which reinforces the hypothesis of the beneficial effect of St. John's Wort on *Akkermansia* levels (Xing et al., 2019). The lower abundance of Firmicutes and a higher level of *Bacteroides* were found in patients with GAD; hence a reversal of this trend could contribute to a positive action of *Hypericum perforatum* extract, especially since isoquercetin or rutin, both presented in Hyperici herba, exhibit a similar effect on Firmicutes level (Parkar et al., 2013; Simpson et al., 2021; Tan et al., 2018). In a mouse model of colitis, rutin promotes *Coprococcus* genus; bacteria decreased in patients with anxiety and, what was recently observed, crucial for the beneficial effects of prebiotic supplementation on mood in obese patients (Leyrolle et al., 2021; Power et al., 2016).

Undoubtfully, quercetin, another compound found in *Hypericum perforatum*, is one of the most excessively studied naturally occurring substances in terms of its impact on gut microbiota (Table 1). As expected, obtained results differ significantly depending on the model used, duration of administration, and concentration of quercetin tested. However, due to its widespread occurrence in several plants, it is questionable whether this flavonol is responsible for the beneficial activity of Hyperici herba. Thus, the reason why some herbal medicinal products exhibit antidepressant effects, while others do not, should be searched among more specialized and plant-specific substances.

Crocus sativus l

Clinical trials

Four meta-analyses investigating the anti-depressant and anxiolytic efficacy of *C. sativus* were retrieved and summarized in Table S2. The accumulated evidence allowed for the analyses of the influence of both response to treatment and remission of depression. The results of three meta-analyses of trials which included participants with depression, indicated a significant decrease of symptoms compared to placebo and equal effect of *C. sativus* preparations compared to SSRIs. Results of one meta-analysis showed substantial differences in subgroups only. Additionally, the influence on anxiety symptoms was also analyzed quantitatively but did not show conclusive results compared to placebo.

Since the publication of these meta-analyses, the new evidence has been published in five trials (Ahmadpanah et al., 2019; Akhondzadeh et al., 2020; Lopresti et al., 2019; Moghadam et al., 2021; Salek et al., 2021) summarized in Table S3. Four of these studies were conducted in Iran. The trials investigated varied *C. sativus* preparations among participants with different medical backgrounds. Standardization of plant material was performed comprehensively only in one trial (Lopresti et al., 2019), whereas in one study efficacy of a single compound (crocin) was investigated (Salek et al., 2021). All studies were randomized, double-blind, and controlled (four employed placebo as a control, while in one study, authors used sertraline). Narrow test samples characterized these clinical trials, e.g., elderly participants, overweight women, or participants already receiving the therapy, among whom the use of *C. sativus* was tested as an adjuvant. Although the evidence acquired by testing in such particular groups is valuable, the efficacy should also be investigated among other possible recipients of such interventions, emphasizing the quality control of the preparations. All retrieved studies reported a significant decrease in symptoms measured with BDI, BAI, HAM-D, and MADRS questionnaires.

Preclinical studies

The absorption of natural products present in extracts from *Crocus sativus* leads to the detection of several metabolites in the circulation, tissues, urine, and feces (Almodóvar et al., 2020; Girme et al., 2021; Shakya et al., 2020) (Table S4). After oral administration of Crocus extract, the analysis of fluids confirmed the presence of crocetin and crocin derivatives (Chryssanthi et al., 2011; Xi et al., 2007). In general, crocetin is considered as a major metabolite of crocins occurring in the raw material which are a product of hydrolysis in the gastrointestinal tracts (Moratalla-López et al., 2019). The absorption in the gut may lead to the glucuronidation and other transformations involving II phase enzymatic systems. The studies involving rodents (rats or mice) proved that crocins and crocetin derivatives are present in plasma in concentrations up to 10 μg/ml (Girme et al., 2021) (Table S4). The administration of pure crocetin or *Crocus* extract to human volunteers leads to the detection of crocetin up to 280 ng/ml (Umigai et al., 2011).

In vitro investigations on the bioavailability of compounds from *Crocus* showed that crocin-1, crocetin, picrocrocin and trans-4GG crocetin can pass cell monolayers using both caco-2 and BCEC models (Kyriakoudi et al., 2015; Lautenschläger et al., 2015). It suggests compounds can migrate to the circulation in the gut and can also penetrate the blood-brain barrier (Table S5). The biotransformation of saffron extract using artificial gastrointestinal fluids showed that both crocins and crocetin can be degraded (Kyriakoudi et al., 2013). However, the study did not include the chemical analysis of produced metabolites.

Crocus sativus and gut microbiota

When it comes to the investigation of antidepressant activity of plant-derived substances, scientists use diverse rodent models, starting from chronic stress procedures or maternal deprivation and ending with genetically modified mice characterized with depressed behavior (Pollak et al., 2010). One of the compounds examined for its antidepressant activity is crocin-I, a carotenoid presented in *Crocus sativus* (Xiao et al., 2020). Researchers found that crocin-I not only alleviates the depression symptoms but also influences the gut microbiota composition in mice exposed to chronic restraint stress. What one can find intriguing, in contrast to most reviewed in this paper herbal medicinal products and naturally occurring compounds, in preclinical study crocin-I decreased alpha diversity indices. Contrary to these results, a study conducted on mice treated with corticosterone presented that administration of crocin-I increased alpha diversity and, in some cases, resulted in opposite effects to those performed on depressed mice. For example, in the mouse model of depression, crocin-I suppressed the growth of Bacteroidetes, while after administration of corticosterone, crocin-I increased the relative abundance of those bacteria (Xie et al., 2019). In both studies the cecal microbiota composition was analyzed; thus, crocin effects on mice gut microbiota may be model-dependent.

Valeriana officinalis l

Clinical trials

The main focus of hitherto published meta-analyses was on using valerian preparations in sleep disturbances, whereas meta-analyses on anxiolytic efficacy of *V. officinalis* were not found. Thus, all clinical trials covering this therapeutic use that have been published since 2000 were summarized in Table S3. Inclusion criteria varied among all six trials recognized (Ahmadi et al., 2017; Andreatini et al., 2002; Farah et al., 2019; Gharib et al., 2015; Jacobs et al., 2005; Roh et al., 2019). One study included participants with diagnosed GAD (Andreatini et al., 2002), whereas others incorporated subjects with a wide range of medical backgrounds. Trials were conducted mainly in Brazil and Iran. Five out of six studies were randomized, placebo- and/or benzodiazepine-controlled trials, and all were double-blinded. Interventions included oral intake of diversified valerian preparations, four precisely standardized, while the other two unspecified. The duration of the analyzed studies was also heterogenic, varying from singular administration to four weeks. Outcomes of these trials on anxiety were measured using varied diagnostic questionnaires (HAM-A, STAI, VAS, BAI, DAS) and produced inconclusive results. In two trials

measuring anxiety symptoms with the HAM-A questionnaire, after a 4-week administration of well-standardized herbal preparation, acquired results showed a promising reduction of HAM-A scores compared to placebo (Ahmadi et al., 2017; Andreatini et al., 2002). In summary, the qualitative heterogeneity of available trials is substantial. Therefore, to perform a reliable meta-analysis or draw any conclusions on the efficacy, it may be necessary to examine more evidence as soon it becomes available.

Preclinical studies

Animal studies focused on the bioavailability and disposition of compounds contained in valerian are summarized in Table S4. Two studies involving rodents (rats) using pure valeric or valerenic acid were performed. The results showed that both compounds present in the plant material and its extracts can reach circulation. In the case of valeric acid top concentration was achieved in the portal vein at the level of ca. 24 μM (Onyszkiewicz et al., 2020). In the systemic circulation the concentrations were significantly lower (ca. 0.3 to 7 μM) (Onyszkiewicz et al., 2020). The tissues analysis proved that the compound could access several organs including the brain (ca. 0.12 μM). For valerenic acid the experiments showed that the administration of 20 mg/kg b.w. results in the presence of an unchanged compound at ca. 2.4 μg/ml in the rats' plasma (Sampath et al., 2012).

The investigation of the oral administration of valerian extract involving human volunteers showed that valerenic acid is present in the serum at a concentration up to 2.8 μg/ml (Anderson et al., 2005).

Some in vitro experiments on the absorption and metabolism of compounds contained in valerian root were also performed (Table S5). It was proven using the rat liver perfusion model that valerenic acid undergoes I and II phase reactions resulting in the occurrence of hydroxy derivatives and conjugates with glucuronic acid (Maier-Salamon et al., 2009). The investigation of permeability of valerenic acid and its derivatives in the rat glioma cells model showed that all compounds can cross cell monolayers in vitro suggesting their ability to cross the blood-brain barrier in vivo (Neuhaus et al., 2008).

Valeriana officinalis and gut microbiota

Of the compounds presented in Valerianae radix, bornyl acetate significantly modulated the gut ecosystem in rats model of intestinal mucositis induced by 5-fluorouracil (Zhang et al., 2017). Administration of bornyl acetate decreased the relative abundance of *Escherichia*; genus depleted in patients with the MDD but overrepresented in patients with GAD. It also reduced the level of *Enterobacteriaceae* and *Streptococcaceae*, taxa positively associated with depression (Simpson et al., 2021). In addition, researchers found that bornyl acetate increased the relative abundance of *Lactobacillus.* In the context of the gut-brain axis, a study with different *Valeriana* species, *Valeriana jatamansi,* was carried out. It was presented that the total iridoids content of this plant exhibited an antidepressant activity in an unpredictable mild stress mouse model and at the same time increased the gut microbiome richness and diversity (Wang et al., 2020). With growing evidence of the vital role of the gut microbiota in mood disorders and anxiety, the similarly designed study could shed light on the nature of *Valeriana officinalis* anxiolytic activity.

Lavandula officinalis chaix

Clinical trials

Six meta-analyses on the efficacy of L. *officinalis* essential oil in treating anxiety have been published since 2000 (summarized in Table S2). The authors of the most recent report (Salehi-Pourmehr et al., 2020) showed that aromatherapy alleviates the physical and psychological symptoms of menopause. Other meta-analyses were focused on anxiety only, both in the form of aromatherapy and oral administration. Although the research on aromatherapy showed favorable results of its efficacy in the short-term treatment of anxiety, the authors discussed that the blinding of the studies on aromatherapy might be considered insufficient and resulting in overestimating the results due to the characteristic scent of lavender oil. Lavender oil may also be administered orally (silexan). Hitherto meta-analyses showed its efficacy in the long-term treatment of anxiety and superiority compared to other routes of administration in long-term therapy (Sayed et al., 2020), as well as a significant reduction of HAM-A scores –comparable with paroxetine and superior to lorazepam and placebo (Yap et al., 2019). Other comparisons of oral administration with other routes did not show significant differences in available data.

Eight recently published clinical trials were not included in the meta-analyses and thus were summarized in Table S3. Five studies were conducted in Iran. Although the majority of trials researched aromatherapy for participants undergoing various surgical procedures (Abbaszadeh et al., 2020; Babatabar Darzi et al., 2020; Karan, 2019; Shammas et al., 2021; Stanley et al., 2020), three trials investigated the efficacy of orally administered L. *officinalis* (Araj-Khodaei et al., 2020; Bazrafshan et al., 2020; Farshbaf-Khalili et al., 2018). Two of these involved participants with diagnosed depression, and one study investigated anxiety in menopausal women. Studies on aromatherapy showed inconclusive results; some reported a beneficial reduction of anxiety scores (Abbaszadeh et al., 2020; Stanley et al., 2020), whereas others showed no efficacy (Babatabar Darzi et al., 2020; Karan, 2019; Shammas et al., 2021). The methodologies of these studies were diversified; both placebo and non-placebo (no intervention) controls were used. Moreover, only one trial was reported using chemically standardized herbal preparation. Research on orally administered lavender included encapsulated plant material in two trials and infusion in one trial. The authors reported incomplete standardization of used plant material. Trials were controlled with non-placebo, placebo, and fluoxetine. In conclusion, more evidence is necessary to fully support the clinical efficacy of L. *officinalis* preparations in specific indications.

Preclinical studies

Several studies showed that compounds contained in the essential oil from lavender are bioavailable after oral administration (Table S4). The administration of lavender essential oil, linalool or linalyl acetate to rats proved that both compounds can be detected in plasma or different organs in the concentration range from 11 ng/ml to 2.20 μg/ml depending on the dose and experiments design (Nöldner et al., 2011). The highest cumulation of linalool was detected in the fat of investigated animals (up to 3.96 μg/ml). The experiments with human volunteers showed that oral administration of essential oil rich in linalool and linalyl acetate allowed detection of linalool in plasma in the concentration range from 2.2 to 9.0 ng/ml, compared to < 2.0 ng/ml for linalyl acetate (Doroshyenko et al., 2013).

Based on the literature research, it seems clear that linalool and/or linalyl acetate are considered as active constituents of lavender. However, this plant material also contains several other groups of phytochemicals e.g., phenolic acids or flavonoids, which contribute to the general effect of lavender was not investigated in a detailed manner.

The research databases did not contain any information on in vitro studies focused on the bioavailability and disposition of natural products present in lavender oil or lavender extracts.

Lavandula officinalis and gut microbiota

The impact of *Lavandula officinalis* constituents on gut microbiota was tested both in human trials and mice models. Results obtained from a study conducted with patients suffering from irritable bowel syndrome (IBS) indicate that oral administration of geraniol alleviates colitis symptoms and modulates gut microbiota composition by stimulating the growth of *Collinsella* and *Faecalibacterium* (Rizzello et al., 2018). The deficiency of *Faecalibacterium* bacteria is linked with the severity of depressive symptoms, and one species, potential psychobiotic, *Faecalibacterium prausnitzii*, shows anxiolytic and antidepressant-like effects in rats (Hao et al., 2019; Jiang et al., 2015). Despite apparent differences between IBS and mood disorder, co-occurrence of these diseases is

significantly associated, and prebiotics or probiotics might serve as valuable approaches in their treatment (Mykletun et al., 2010; Pusceddu et al., 2018). Another compound present in lavender oil, terpinene-4-ol, not only ameliorates inflammatory reactions in mice model of colitis but also increases the abundance of *Lactobacillus* and *Escherichia coli*.

Interestingly, the latter one expresses the ability to generate serotonin and noradrenalin (Cryan and Dinan, 2012). Depletion of those both neurotransmitters was found in patients with major depression and bipolar disorder (Briley and Chantal, 2011; Fakhoury, 2016; Mahmood and Silverstone, 2001; Wiste et al., 2008). Two subsequent constituents of lavender oil, linalool, and limonene, exhibit similar pattern in the shaping of mice gut microbiota, increasing richness and diversity in the cecum and relative abundance of *Lactobacillus* in cecum and colon, *Parabacteroides (*producers of main inhibitory neurotransmitter γ-aminobutyric acid (Strandwitz et al., 2019)) in the colon and decreasing *Bacteroides* in cecum and colon (Wang et al., 2019). One should note their ambiguous impact on Firmicutes/Bacteroidetes ratio, which was found increased in the cecum and reduced in the colon after administering linalool or limonene. The gut microbiome community is diverse across the gastrointestinal tract and is affected by several factors: oxygen and nutrient source availability, pH, or mucus structure (Donaldson et al., 2016). For instance, it was presented that bacterial communities vary between particular murine gastrointestinal organs with higher levels of *Lachnospiraceae* and *Ruminococcaceae (*belonging to Firmicutes phylum) and depletion of *Bacteroidaceae* and *Prevotellaceae (*belonging to Bacteroidetes phylum) in cecum compared to the colon (Gu et al., 2013). Moreover, natural products' organ-specific enrichment of gut bacteria should be considered in light of new studies presenting the regional interaction between microbiota and immune cells (James et al., 2020).

Passiflora incarnata/pallida l

Clinical trials

A Cochrane meta-analysis on anxiolytic efficacy of *P. incarnata* was retrieved (Miyasaka et al., 2007). Two clinical trials, including participants with diagnosed GAD, neurosis, or anxiety, met the inclusion criteria of the authors of the report. However, due to differences in methodologies and lack of evidence acquired in trials controlled with placebo and anti-depressants, the standard treatment for anxiety, unequivocal conclusions on efficacy could not be reached.

Four clinical trials have been reported recently (Aslanargun et al., 2012; da Cunha et al., 2021; Movafegh et al., 2008; Ngan and Conduit, 2011) and were not included in the meta-analysis. The main characteristics of these studies were summarized in Table S3. Three studies aimed to investigate the efficacy of single administration of *P. incarnata* preparations in lowering surgery-related anxiety. All studies were placebo-controlled, randomized, and at least double-blinded. However, thorough standardization was not reported in one of the trials (da Cunha et al., 2021). The outcomes of these studies were measured with different questionnaires. All evidence collected indicated a significant improvement or lack of the increase of anxiety symptoms. The fourth study aimed to investigate how *P. incarnata* infusion effectively alleviates minor sleep problems among healthy volunteers. The trial was also placebo-controlled, randomized, and double-blinded, but with a cross-over design. The intervention comprised of week-long administration of herbal preparation or placebo and a week-long wash-out period. One of the outcomes measured was the influence of state and trait anxiety (STAI), but the obtained evidence did not show any significant differences compared to placebo.

In summary, the conclusions of the Cochrane meta-analysis regarding the long-term anxiolytic treatment with *P. incarnata* preparations were not yet addressed. However, some evidence of passionflower's efficacy for acute alleviation of anxiety has been collected. The clinical significance of this type of intervention might be furtherly investigated in the future.

Preclinical studies

While doing the literature research no preclinical studies involving animals or human volunteers were reported in the context of the bioavailability of compounds contained in extracts from *Passiflora.*

The literature review showed that one in vitro study focused on the bioavailability of natural products present in extracts from passionflower was performed (Table S5). The ability to cross caco-2 cell monolayers by C-glycosides of apigenin and luteolin was checked. The results showed that all compounds could pass cell monolayers in performed experiments. It was also proven that the passage through the endothelium led to the formation of several metabolites, products of I and II phase metabolism, including hydroxy and methoxy derivatives and conjugates with sulphuric or glucuronic acid (Tremmel et al., 2021).

Passiflora incarnata and gut microbiota

Among the compounds presented in *Passiflorae herba*, the impact on the gut microbiome was investigated for isoorientin. In the mouse model of colonic injury, besides the attenuation of colonic damage and promoting influence on potentially beneficial *Lactobacillus*, isoorientin enlarges the relative population of *Enterococcus* (He et al., 2019). These results are in line with a study presenting the antiinflammatory and antidepressive activity of *Enterococcus faecalis 2001* on mice treated with dextran sulfate sodium to induce colitis (Takahashi et al., 2019). Thus, it is proposed that the shortage of bacteria belonging to the *Enterococcus* genus may play a key role in developing symptoms of comorbid depression and colitis. Furthermore, the beneficial effect of isoorientin on the gut microbiota in treating both diseases is reinforced because isoorientin decreased the relative abundance of *Veillonella*, bacteria overexpressed ($p = 0.075$) in patients with comorbidities of IBS and depressive disorder (Liu et al., 2016). Also, decreased level of *Alloprevotella* after administration of isoorientin may contribute to its desirable effect since the elevated level of these bacteria was recently found in patients suffering from MDD (Zhang et al., 2021). Other research examining the effects of isoorientin on BALB/c mice demonstrated a decline in *Anaerotruncus* levels after administering this flavone. Interestingly, it was found that *Anaerotruncus* bacteria are positively correlated with depression symptoms in female patients, and a decrease of *Anaerotruncus* may contribute to the antidepressive mechanism of action of isoorientin Chen et al., 2021b).

Rhodiola rosea l

Clinical trials

Currently, there are no meta-analyses investigating the anxiolytic and anti-depressant efficacy of *R. rosea.* We retrieved the results of eight clinical trials published since 2000, which are summarized in Table S3. Three were conducted in the USA, two in the UK, and other ones in different countries. Four studies included participants with mild to moderate depression diagnosed accordingly to DSM-IV criteria (Darbinyan et al., 2007; Gao et al., 2020; Mao et al., 2015; Ross, 2014); two investigated subjects with anxiety (Bystritsky et al., 2008; Cropley et al., 2015); one – chronic fatigue (Lekomtseva et al., 2017); one – chronic stress (Edwards et al., 2012). Four studies were randomized, placebo-controlled, and double-blind. All studies investigated long-term oral use of the herbal preparation (from 2 to 12 weeks). Moreover, intervention in six studies was conducted with comprehensively standardized *R. rosea* extracts WS® 1375 or SHR-5. Outcome measurements were conducted mostly with questionnaires dedicated to screening depression and anxiety, such as HAM-D, BDI, HAM-A, and STAI. Two trials with the most comprehensive methodological approach (Darbinyan et al., 2007; Ross, 2014) showed that SHR-5 extract (in two doses: 340 or 680 mg/day) was significantly more effective than placebo in decreasing BDI and HAM-D scores. A dose-dependent effect was also observed in some datasets. The other trials also showed some promising

results, however less conclusive. Some trials employed baseline comparisons only, non-placebo, flexible dosing, or did not specify the qualitative criteria of the herbal preparations. In summary, growing evidence on the anti-depressant activity of *R. rosea* has been established in the last fifteen years. However, to justify the clinical relevance of rhodiola preparations, it is necessary to perform meta-analyses and possibly more high-quality randomized clinical trials.

Preclinical studies

Several reports on the bioavailability of constituents present in *Rhodiola* were found (Table S4). However, no information on the bioavailability after the extract administration was reported in the reviewed databases. The researches show that rosavin and salidroside are considered as two most important constituents which are responsible for the observed bioactivity of extracts and raw plant material. Studies on rats showed that rosavin after oral administration reaches circulation at a concentration up to ca. 325 ng/ml (Zhang et al., 2019). Rosavin is also present in the urine. The oral or intravenous administration of salidroside to rats showed that this compound can be detected in the plasma up to 6.5 μg/ml (*per os*) and 28 μg/ml (*i.v.*) (Chang et al., 2007; Yu et al., 2008). It was also proved that salidroside could reach tissues (up to 2.7 ng/ml) (Zhang et al., 2013). One study involving dogs showed the *i.v. the* administration this compound results in the concentration of ca. 100 μg/ml in the plasma (Mao et al., 2007). No studies involving the administration of Rhodiola to healthy volunteers were found in the literature.

The research databases did not contain any information on in vitro studies focused on the bioavailability and disposition of natural products present in *Rhodiola* extracts.

Rhodiola rosea and gut microbiota

Salidroside, a phenolic glycoside presented in *Rhodiola rosea*, was found a potent modulator of the gut microbiome in distinct rodent studies (Li et al., 2020; Xie et al., 2020 ; Yuan et al., 2019). Except for its promoting effect on the general bacterial richness and previously mentioned *Akkermansia* in a rodent model of liver injury, salidroside inhibits the growth of *Lactobacillus* bacteria in mice provided with a high-fat diet. Oral supplementation of natural products used in mood disorders can either increase or decrease the relative abundance of the *Lactobacillus* or *Lactobacillacae* family. However, in presented studies, rodents fed with a high-fat diet without additional treatment, the addition of isolated compounds resulted only in a decreased level of these taxa (Table 1). Studies focused on obesity-related microbiota indicate that the favorable effect of *Lactobacillus* genus on body weight is strain-dependent (Crovesy et al., 2017; Million et al., 2012); therefore, the inhibitory activity on obesity-associated species might explain the negative impact of natural products on *Lactobacillus* genus abundance and be notably helpful in the context of obesity and depression comorbidity (Luppino et al., 2010).

Melissa officinalis l

Clinical trials

One meta-analysis on anxiolytic and anti-depressant efficacy of orally administered *M. officinalis* was retrieved and summarized in Table S2. Although the heterogeneity of analyzed trials was high, the authors were able to show the significant decrease of symptoms of acute anxiety and depression, as well as chronic depression compared to placebo. However, the evidence of the latter was retrieved from one trial only. Effects of herbal preparations on chronic anxiety were not significant. The authors underlined the need for cautious interpretation of the results due to divergent methodologies and a small number of trials. Consequently, further investigations in high-quality trials need to establish the clinical significance of interventions using M. officinalis to treat anxiety or depression.

Since the publication of this meta-analysis, new evidence has been published in the trial summarized in Table S3 (Araj-Khodaei et al., 2020). The methodology of this trial included the use of thoroughly standardized herbal preparation, the inclusion of participants with depression diagnosed accordingly to DSM-V criteria; randomized, fluoxetine-controlled, parallel design; and standardization of plant material. The evidence acquired in this study indicated that the tested herbal preparation and fluoxetine had an equal effect on reducing symptoms of depression measured with the HAM-D questionnaire.

Preclinical studies

Based on the literature review only one study focused on the presence of metabolites from lemon balm after oral administration was performed (Table S4). The investigation of the application of *Melissa* extract and pure rosmarinic acid to healthy volunteers showed that this compound could reach systemic circulation up to 142.2 nmol/l (Noguchi-Shinohara et al., 2015). No information on the bioavailability of essential oil components were found in the accessed sources.

The in vitro investigation of the biotransformation of rosmarinic acid by human gastrointestinal enzymes showed that the compound can be decomposed to caffeic acid derivatives and other simple chemicals (Zorić et al., 2016) (Table S5).

Melissa officinalis and gut microbiota

Melissa officinalis extract and two compounds present in the *Melissa officinalis* folium, citral and ursolic acid increased Chao1 index in the rodent gut microbiota suggesting elevated species richness of bacteria community (Brochot et al., 2019; Wan et al., 2019, 2020; Wang et al., 2019). What is more, the administration of ursolic acid and another constituent found in lemon balm, rosmarinic acid, resulted in a higher relative abundance of *Bifidobacterium* (Hao et al., 2020; Ou et al., 2018; Wan et al., 2019). It should be noted that despite the promising outcomes from preclinical studies, the beneficial role of *Bifidobacterium* supplementation, given in formulation with other psychobiotic or alone, in mood disorders has not been fully confirmed yet (Aizawa et al., 2019; Akkasheh et al., 2016; Desbonnet et al., 2010; Kazemi et al., 2019; Romijn et al., 2017; Tian et al., 2019). In a rat model of diabetes, rosmarinic acid alone induced considerable changes in gut microbiota structure. Except for its growth-promoting effect on *Bifidobacterium*, treatment with rosmarinic acid boosted levels of numerous taxa e.g., *Anaerostipes, Coprococcus, Faecalibacterium,* or *Sutterella* (Ou et al., 2018). *Anaerostipes* bacteria are inversely associated with depression scores in patients without physician-diagnosed mood disorders (Taylor et al., 2020). By contrast, opposite results were found in the group of depressed patients (Zheng et al., 2016). Researchers highlighted that the presented discrepancy might have resulted from different mental health statuses of recruited subjects and countries where the studies were conducted (the United States versus China), which in turn is linked with diet and country-specific gut microbiome communities (Taylor et al., 2020). Since *Coprococcus* and *Sutterella* bacteria were found reduced in patients suffering from MDD, rosmarinic acid emerges as a promising agent in the normalization of “depressed microbiota” in patients (Sanada et al., 2020; Simpson et al., 2021).

Moreover, rosmarinic acid, also presented in lavender flowers, inhibits the growth of potentially unfavorable bacteria: *Desulfovibrio* and *Flavonifractor*. The increased prevalence of the first one is associated both with gastrointestinal inflammation and the occurrence of depressive disorder, while the higher levels of *Flavonifractor* are presented in patients with affective disorders (Coello et al., 2021; Simpson et al., 2021).

Humulus lupulus l

Clinical trials

The main focus of hitherto published meta-analyses was on using *H. lupulus* preparations in sleep disturbances and meta-analyses on anxiolytic efficacy were not found. We retrieved the results of four

clinical trials published since 2000, which investigated hop preparations among participants with different medical backgrounds as summarized in Table S3. The types of herbal preparations were varied, as well. Two studies aimed to investigate the efficacy of *H. lupulus* preparations in long-term anxiolytic treatment (Franco et al., 2012; Kyrou et al., 2017), the third trial examined preparation of a single compound (β-eudesmol) in the acute stress model (TSST) (Ohara et al., 2018); and the fourth study investigated mainly menopause-related symptoms, with the inclusion of anxiety, depression, and stress measured with Greene Scale (Aghamiri et al., 2016). Three studies were randomized, placebo-controlled, and double-blind. In these trials, statistically significant results indicating anxiolytic efficacy were acquired with the DASS-21 questionnaire and Greene Scale.

On the other hand, the two remaining studies using the STAI questionnaire did not influence trait anxiety and reported inconclusive results on state anxiety. All studies reported divergent standardization approaches. This aspect should be improved in future experiments in order to facilitate conclusions about specific chemical groups or constituents relevant to the activity. In summary, the evidence supporting the effectiveness of hop preparations in anxiety is scarce and does not fully substantiate the use of *H. lupulus* in such indications. More data should be obtained in studies with a thoroughly planned methodology, including comprehensive standardization of examined plant materials.

Preclinical studies

The research on the bioavailability of natural products contained in hop was performed involving animal (mice or rats) and human models (Table S4). The administration of the extract showed that several compounds including co-humulone, α-acids humulone, adhumulone, adlupulone, β-acids lupulone, xanthohumol (XN), isoxanthohumol, 6-prenylnaryngenin and 8- prenylnaringenin can be detected in blood and urine of experimental animals or human volunteers (Calvo-Castro et al., 2018; Legette et al., 2014; Salviati et al., 2021). Depending on the experimental design the concentrations of compounds ranged from ca. 1 ng/ml to ca. 40 ng/ml. higher concentrations were measured in the case of the administration of pure compounds including isoxanthohumol/xanthohumol or 6-/8-prenylnaryngenins (Martinez et al., 2014). In this case the reports show that quantities of detected compounds were up to 1 μg/ml for naringenin derivatives and up to 178 μg/ml for xanthohumol (Legette et al., 2014; Martinez et al., 2014).

Several reports on the bioavailability of natural products contained in hops involving in vitro models were found during the search of databases (Table S5). It was proven that both constituents of volatile oil and alpha-acids could cross Caco-2 monolayers. However, no metabolites were detected (Cattoor et al., 2010; Heinlein et al., 2014). The analysis of the metabolism of humulone derivatives by animal microsomes showed that enzymatic transformation leads to the production of several metabolites including products of oxidation, desaturation reduction and glucuronidation (Cattoor et al., 2013; Salviati et al., 2021). The treatment of 8-prenylnaryngenin with human or animal liver microsomes showed that the glucuronidation at a different site of the molecule could take place (Fang et al., 2019). Two reports showing the possible metabolism of xanthohumol, isoxanthohumol or 8-prenylnaryngenin by isolated microorganisms proved that different types of metabolites could be produced including sulphates and glucuronides (Kim et al., 2019; Paraisoet al., 2019). One study focused on the gut microbiota metabolism of xanthohumol was performed in vitro which showed that the compound could be biotransformed to isoxanthohumol and 8-prenylnaryngenin (Hanske et al., 2010) (Table S5).

Humulus lupulus and gut microbiota

Compounds present in *Humulus lupulus* flos are well-known factors that both undergo gut bacteria metabolism and modify microbiome structure. In the study conducted with fecal fermentation of samples obtained from healthy subjects, substantial changes were observed in the relative abundance of several taxa, including increased levels of *Akkermansia* or reduction in *Desulfovibrio* and *Veillonella* after addition of *Humulus lupulus extract* to batch culture (Blatchford et al., 2019). As the opposite changes are linked with the occurrence of mood disorders, rebalancing the relative abundance of these taxa can reinforce the positive effects of *Humulus lupulus* extract administration (Sanada et al., 2020; Simpson et al., 2021). Similar to results obtained from fecal fermentation with the addition of *Humulus lupulus* extract, increased *Akkeremansia* levels were observed in animal studies using isoxanthohumol, one of the constituents found in *Humulus lupulus* (Fukizawa et al., 2020; Yamashita et al., 2020). Furthermore, the decline in *Anaerotruncus* bacteria after isoxanthohumol administration is another favorable change in gut microbiota structure in the context of the gut-brain axis and mood disorders (Y. Chen et al., 2021; Fukizawa et al., 2020). However, one study carried out on female, C57BL/6 mice treated with *Humulus lupulus* extract presented opposite outcomes, suggesting the need to investigate gender role in modulation of the microbiome by plant extracts (Hamm et al., 2019).

Interestingly, the ability to shape gut microbiome structure is not limited to hops' flavonoids, but also their bacteria metabolites are potent modulators of a microbial community. For instance, administration of xanthohumol or its metabolite, α, β-dihydro-xanthohumol to mice fed with a high-fat diet caused promotion of *Akkermansia* or depletion of Tenericutes, *Eubacteriaceae* and *Enterococcaceae* (Logan et al., 2021; Y. Zhang et al., 2020). The decreased level of Tenericutes is ambiguously linked with mental health problems. On the one hand, a lower abundance of Tenericutes was found in patients with GAD (Chen et al., 2019). On the other hand, phylum Tenericutes was found to be enriched in patients with treatment-resistant depression (Fontana et al., 2020). The disorder-specific nuances in microbiota composition may be vital to fully understand the role of gut bacteria in maintaining mental wellbeing. The overrepresentation of two other taxa, *Eubacteriaceae* and *Enterococcaceae,* was associated with depression; thus, the inhibitory effect of xanthohumol derivative on these bacteria can contribute to the beneficial activity of hops during the treatment of mood disorders (R.T. Liu et al., 2020; Zheng et al., 2016).

Studies focused on the modulation of gut microbiota structure by hops are not restricted to rodent models. In fact, in many areas, the pig model is more comparable to investigating gastrointestinal tract function and microbiota than rodent (Heinritz et al., 2013; Sciascia et al., 2016). The advantageous effect of diet supplemented with spent hops was investigated in pigs and, except for a decrease in expression of pro-inflammatory genes in the gastrointestinal tract, the reduction in *Streptococcus* was observed (Fiesel et al., 2014). *Streptococcus* bacteria are another microbes found increased in patients with MDD and, interestingly, streptococcal infection is associated with diagnoses of depression (Leslie et al., 2008; Lin et al., 2017; Rong et al., 2019).

Piper methysticum G. forst

Clinical trials

Four meta-analyses on the efficacy of *P. methysticum* in treating anxiety have been published since 2000 (summarized in Table S2). The authors of three meta-analyses focused on trials researching well-standardized kava extracts, all placebo-controlled and randomized. Additionally, analyzed trials included participants with diagnosed anxiety disorders. Three older meta-analyses reported that the efficacy of kava extracts in reducing HAM-A scores is significantly superior compared to placebo, whereas in the most recent meta-analysis (Ooi et al., 2018), the effect favoring kava was not statistically significant.

Since the publication of this review, new evidence has been published in the trial summarized in Table S3 (Sarris et al., 2020). The methodology of this trial included the use of thoroughly standardized herbal preparation, the inclusion of participants with diagnosed GAD, and a randomized, placebo-controlled, parallel design. The data acquired in this study indicated that the tested extract is not significantly more effective than a placebo.

Preclinical studies

The investigation of the bioavailability of compounds occurring in kava-kava showed that several compounds could be detected in fluids of animals and human volunteers after administering the extract or pure compounds (Table S4). The experiments involving rodents (rats or mice) proved that the application of the kava-kava extract leads to the occurrence of kavain, dihydrokavain, methysticin, dihydromethysticin and desmetoxymethysticin in the urine and plasma. The highest concentration was observed for kavain (up to 7.8 μg/ml in the plasma) (Ferreira et al., 2020; Mathews et al., 2005; Wang et al., 2018). The analysis of tissues of rats after the administration of pure kavain showed that the highest content of this compound was recorded in kidneys and liver (2370–3150 ng-Eq/g) (Mathews et al., 2005). One study involving human volunteers was performed showing that the administration of kava-kava extract led to the presence of 6-phenyl-3-hexen-2-one at the level of 0.6 μg/ml (Zou et al., 2005).

The in vitro investigations showed that flavokawains and cardamonin could be metabolized by human liver macrosomes (Zenger et al., 2015) (Table S5). Several metabolites, including helichrysetin and flavokawain A 2′-*O*-glucuronide were detected during the experiments (Zenger et al., 2015). The study involving the caco-2 cell model showed that kavalactones could pass cell monolayers. Mo products of endothelial metabolism were investigated (Matthias et al., 2007).

Piper methysticum and gut microbiota

As mentioned above, the impact of kava-kava and its constituents on gut microbiota structure has not been sufficiently investigated yet.

Question of metabolites of postbiotic origin

The following scientific question which needs to be asked when considering the bidirectional relationship between herbal medicines and gut microbiota is the impact of the bacterial catabolic activity on naturally occurring compounds. According to International Scientific Association for Probiotics and Prebiotics consensus postbiotics are defined as “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host” (Salminen et al., 2021). Taking that, purified metabolites such as crocin-I, quercetin or salidroside alone should not be named postbiotics, as they do not contain at least fragments of inanimate microbes.

Do the gut microbiota-derived metabolites exhibit mood modulating activity, or are they are just biomarkers? The question about their role in human homeostasis and interindividual differences in natural products metabolites remains unanswered (Cortés-Martín et al., 2020; Tomas-Barberan et al., 2018). The discrepancy between patients’ gut bacteria catabolic activity can profoundly affect expected outcomes of the administration of herbal medicines. For instance, the formation of bioactive urolithins, postbiotic metabolites derived from ellagitannins, is conditioned by the structure of the gut microbiome and the presence of specific metabotypes. Contrary to people possessing metabotypes A and B, the population with metabotype 0 does not express the capability to produce urolithins as final ellagic acid metabolites, which severely limits the potential beneficial effects of administration of ellagitannin-rich plants in this group (Cortés-Martín et al., 2018). The concept of metabotypes can uncloud reasons of diverse effects of diet interventions; however, the instability of the gut microbiome cast doubt on the implementation of metabotype-specified long-term herbal products interventions.

The biological activity of metabolites of postbiotic origin poses an alternative mechanism of action of well-known substances, yet in studies examining their properties concentrations used are often higher than those obtained after plant extracts administration.

The common metabolism of a natural substance is the formation of its aglycones from glycosides, e.g. hyperoside to quercetin or isovitexin to apigenin (Yang et al., 2013; Zheng et al., 2019) (Table 2). These aglycones can be furtherly metabolized. Bacteria transform quercetin into several organic acids, such as 4-dihydroxyphenylacetic acid (DOPAC), a dopamine metabolite (Duda-Chodak et al., 2015; Yang et al., 2013). In the context of mood disorders, studies focused on quercetin metabolites offer noteworthy results. The gut microbiota synthesis of DOPAC is associated with mental quality of life, and orally administered DOPAC induced an antidepressant effect in mice exposed to forced swimming test (Martínez-Hernández et al., 2021; Valles-Colomer et al., 2019). Moreover, it was proved that kaempferol and quercetin’s anxiolytic mechanism of action depends on gut microbiota functioning, with no effect after intraperitoneal administration or after antibiotic therapy, and with statistically significant effect after oral administration deprived of antibiotic intervention. Additional tests using corresponding gut metabolites, p-hydroxyphenylacetic and DOPAC, provided intraperitoneally proved that kaempferol and quercetin act as prodrugs (Vissiennon et al., 2012). Similarly, gut microbiota biotransformation is likely to be essential for the beneficial effects of crocin (Shakya et al., 2020; Zhang et al., 2019).

Metabolites and intermetabolites of isoorientin (luteolin and eriodicytol) and isovitexin (apigenin) ameliorate depression-related behaviors in preclinical studies as well (Gadotti and Zamponi, 2019; Hattori et al., 1988; Ishisaka et al., 2011; Li et al., 2016, 2015; Weng et al., 2016; Yi et al., 2008; Zhang et al., 2020; Zhang et al., 2019). The first intermetabolite obtained from in vitro colonic fermentation of rosmarinic acid, caffeic acid, was found to partially inhibit the decrease

Table 2
Formation of postbiotic metabolites.

Plant	Compound	Metabolites	Ref.
Hypericum perforatum	Hyperoside	quercetin, 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxybenzoic acid, dehydroxylated hyperoside, hydroxylated hyperoside, acetylated hyperoside	(Yang et al., 2013)
Hypericum perforatum	Isoquercetin	Quercetin	(Yuan et al., 2016)
Hypericum perforatum	Quercetin	Several phenolic acids e.g., 3,4-dihydroxyphenylacetic acid	(Duda-Chodak et al., 2015)
Hypericum perforatum	Amentoflavone	Methylated and/or hydroxylated amentoflavone	(Qian et al., 2017)
Hypericum perforatum	Procyanidin B2	Epicatechin and several phenolic acids	(Stoupi et al., 2010)
Hypericum perforatum	Rutin	Quercetin, quercetin-3-glucoside	(Riva et al., 2020)
Humulus lupulus	(iso) Xanthohumol	8-prenylnaringenin, 6-prenylnaringenin, desmethylxanthohumol, desmethyl-α,β-dihydroxanthohumol, α,β-dihydroxanthohumol	(Paraisoet al., 2019; Possemiers et al., 2006)
Crocus sativus	Crocin	Crocetin	(Shakya et al., 2020;Zhang et al., 2019)
Passiflora incarnata	Isovitexin	Apigenin	(Zheng et al., 2019)
Passiflora incarnata	Isoorientin	6-glucosyleriodictyol, eriodictyol, luteolin, 3,4-dihydroxyphenylpropionic acid, phloroglucinol	(Hattori et al., 1988)
Rhodiola rosea	Salidroside	Tyrosol	(Olennikov et al., 2020)
Rhodiola rosea	Rosarin, rosavin, rosin	Cinnamyl alcohol	(Olennikov et al., 2020)
Rhodiola rosea	Arbutin	Hydrochinone	(Olennikov et al., 2020)
Melissa officinalis, *Lavandula officinalis*	Rosmarinic acid	Several phenolic acids e.g., caffeic acid, 3- (3′,4′-dihydroxyphenyl) propionic acid, 3- (4′-hydroxypehyl) propionic acid	(Mosele et al., 2014)

of noradrenaline in the cortex and hippocampus of mice exposed to CUMS (Huang et al., 2019). Procyanidin B2, a compound present in *Hypericum perforatum* l., undergoes microbial cleavage of the C_4—C_8 bond and the formation of potentially antidepressant epicatechin, which, similarly to quercetin, is furtherly metabolized to smaller organic acids (Martínez-Damas et al., 2021; Rothenberg and Zhang, 2019; Stoupi et al., 2010).

Ones of the well-known gut-derived metabolites with substantial biological activity are prenylated flavonoids, 8-prenylnaringenin (8PN), and 6-prenylnaringenin (6PN), enzymatically demethylated from isoxanthohumol (IX) presented in *Humulus lupulus*. 8PN, thanks to its potent estrogenic activity, alleviates menopausal symptoms, osteoporosis or even inhibits tumor growth (Štulíková et al., 2018). 8PN can be furtherly metabolized by the intestinal bacterium *Eubacterium ramulus* to desmethylxanthohumol (DMX) and *O*-desmethyl-α,β-dihydroxanthohumol (DDXN) (Paraisoet al., 2019; Possemiers et al., 2006). Another microbial metabolite of XN is α,β-dihydroxanthohumol (DXN), devoid of estrogenic activity, however, alleviating insulin resistance and cognitive decline in mice fed with a high-fat diet (Miranda et al., 2018). In addition, DXN emerges as a promising therapeutic agent in cancer treatment (Logan et al., 2019). Despite the use of *Humulus lupulus* flos in treating mental stress, the bacterial metabolites of hops are not adequately studied in the context of psychiatric disorders. Analogously to *Humulus lupulus* flos, the lack of studies focused on the antidepressant or anxiolytic activity of metabolites of postbiotic origin formed after administering *Rhodolia rosea, Piper methysicum,* and *Valeriana officinallis* remains a gap in our understanding of their mechanism of action.

Conclusions and perspective

Despite the clinically confirmed effectiveness of phytotherapy in treating mild mood disorders, there is a tremendous gap between pharmacokinetic parameters of native plant extract constituents and effects confirmed in vivo. The consistent lack of unambiguously identified biologically active principles in the majority of reviewed medicinal plants as well as lack of consistency between epidemiological, clinical, and interventional studies often prevents their registration as well-established use medicinal products. The gaps in research on botanical drugs can be linked to the problems related to their patent protection and commercial development. Medical use of plant materials originating from the cultural heritage as well as the universal access to the medicinal plants in the societies are important arguments for conducting further research within the support of public funds.

The collected literature data provide direct and indirect evidence that gut microbiota can contribute to natural products activity in the therapy of mild mood disorders. The significant role of the microbiota-gut-brain axis in mood modulation indicates the necessity of consideration phytotherapeutics' interaction with gut microbiota including the contribution of the gut barrier, blood-brain barrier, and immune system. Of particular importance is the conversion of natural products contained in medicinal plants by gut microbiota to metabolites of different structure and pharmacological properties. Due to the absence of these metabolites in initial herbal products, they have not been considered in vitro studies, as well as were not targeted in pharmacokinetic analyses. The question whether these metabolites could cross BBB and influence brain functions directly or express their activity peripherally at the MGB level remains open. Modulation of gut microbiota composition and metabolism as well formation of postbiotic metabolites was confirmed for several natural products and has been indicated as a crucial, but often disregarded factor. Such variables as tested extract composition as well as interindividual differences in the gut microbiota composition are often not addressed in preclinical and clinical studies. Another problem is the scarceness of in vivo studies using animal models of mood disorders considering the impact of natural products on the gut-microbiota-brain axis. Although it must be pointed that the translation of in vivo animal studies due to significant interspecies differences in gut microbiota composition should be done with caution. The question which has been often stated is whether postbiotic metabolites of natural products are pharmacologically active agents or just biomarkers of specific gut microbiota metabotypes. Especially in the context of recent findings in the field of microbial endocrinology, interaction with pathways responsible for neurotransmitters metabolism becomes conceivable.

As an emerging field, research on physiological processes linked to gut microbiota faces several challenges and limitations. Although many studies attempt to define the healthy microbiota parameters, till now there is no clear definition of eubiosis with indicated universal parameters. The same refers to the term disbiosis, which is thoroughly used in literature to describe the imbalanced condition of the microbial communities (Brüssow, 2020). As gut microbiome is a very complex environment composed of Bacteria, Fungi, Archea and Viruses mixed with heterogenous digested food matrix, isolation of any active components for causative and mechanistic analyses is a very challenging task. Using techniques such as metabolomics allows identification of relevant metabolites in blood, stool and urine and may indicate how alterations in the gut microbiota, environment, diet, and xenobiotics intake influence postbiotic metabolites levels. However, unstable metabolites produced to act in situ and/or subjected to rapid deactivation remain difficult to target and identify (Han et al., 2021). Despite the substantial progress which has been made in growing diverse microorganisms of the microbiota, the large number of species residing in the human gut remain uncultured, which is an obstacle for understanding their biological roles (Nayfach et al., 2019). A likely reason for this failure is the absence in artificial media of key growth factors that can be provided by neighboring microorganisms in situ. As the conseqence, the metabolic processes of isolated single microorganism can significantly differ from those expressed in vivo. What is more, some metabolic pathways may be conducted through subsequent participation of different microbial species, what additionally complicates their simulation outside the organism.

The ability to shape fecal microbiota composition and long-lasting colonize the mucosal layer by probiotics is the subject of ongoing discussion (Kristensen et al., 2016; Sanders, 2016; Suez et al., 2019). How long does the modulating effect of natural products on gut microbiota persists is questioned as well. As an example may serve a study conducted with subjects suffering from IBS treated with geraniol. After four weeks of treatment, a change in the relative abundances of *Collinsella* and *Bifidobacterium* was observed; however, it did not reach a significant difference compared to baseline after additional four weeks of follow-up (Rizzello et al., 2018). Nonetheless, further studies, including follow-ups are required to obtain more data regarding the persistence of gut microbiota changes resulting from natural products administration.

Future in vivo and clinical studies should target pharmacokinetic analysis beyond the plant specialized metabolites by the inclusion of their potential postbiotic metabolites as well as their phase I and II metabolites. The proven existence of different metabotypes necessitates the inclusion of gut microbiota composition screening in the study/trial metadata. Research on the gut microbiota and herbal medicine may be confounded by factors such as tremendous individual compositional variations, diet and xenobiotics. The composition and functionality of the gut microbiota is subjected to constant change in patients throughout their life. There is an unmet need for large longitudinal studies combining indepth phylogenetic analysis with a comprehensive phenotypic characterization using 'omics (meta-genomics, metabolomics, transcriptomics and meta-transcriptomics).

Translation of basic research into clinically relevant effects in humans is obviously a priority. However, applying the results of basic and pre-clinical research on processes associated with microbiota to humans has limitations. For example, fixation of mammalian tissues for microscopic observations undoubtedly interferes with the regulation of mitochondrial dynamics, and may give limited or misleading results (Liang et al., 2021). These limitations may also be related to the

host-specific interactions with microbiota. Due to the interspecies differences in postbiotic metabolites production, the humanized gnotobiotic animals should be considered as the best compromise in vivo animal studies. Finally, the specific stereochemistry of postbiotic metabolites should be respected, when they are obtained by chemical synthesis and applied in vitro and in vivo experiments.

The hereby diagnosed role of gut microbiota enables the discovery of factors determining the therapeutic effects of plant materials applied in mild mood disorders. This complex interplay of natural products with gut microbiota should be viewed as a result of long-lasting evolution. Structural features of microbiota postbiotic metabolites can be seen as a direct footprint of certain microbial enzymatic pathways resulting in a wide range of potentially active pharmacophores and a high degree of biologically compatible stereochemistry, which opens a completely new perspective on finding active metabolites and/or alternative molecular targets. From the clinical point of view it also opens a potential of probiotic interventions to boost the production of postbiotic metabolites with the desired bioactivity.

CRediT authorship contribution statement

Maciej Korczak: Conceptualization, Writing – original draft, Writing – review & editing. **Maciej Pilecki:** Writing – original draft. **Sebastian Granica:** Writing – original draft. **Aleksandra Gorczynska:** Writing – original draft. **Karolina A. Pawłowska:** Writing – original draft. **Jakub P. Piwowarski:** Conceptualization, Writing – review & editing, Writing – original draft, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Project financially supported by Polish National Science centre research grant Preludium Bis No. UMO-2019/35/O/NZ7/00619.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2023.154642.

References

Abbaszadeh, R., Tabari, F., Asadpour, A., 2020. The Effect of Lavender Aroma on Anxiety of Patients Having Bone Marrow Biopsy. Asian Pacific J. Cancer Prev. 21, 771–775.
Aghamiri, V., Mirghafourvand, M., Mohammad-Alizadeh-Charandabi, S., Nazemiyeh, H., 2016. The effect of Hop (Humulus lupulus L.) on early menopausal symptoms and hot flashes: a randomized placebo-controlled trial. Complement. Ther. Clin. Pract. 23, 130–135.
Ahmadi, M., Khalili, H., Abbasian, L., Ghaeli, P., 2017. Effect of Valerian in Preventing Neuropsychiatric Adverse Effects of Efavirenz in HIV-Positive Patients: a Pilot Randomized, Placebo-Controlled Clinical Trial. Ann. Pharmacother. 51, 457–464.
Ahmadpanah, M., Ramezanshams, F., Ghaleiha, A., Akhondzadeh, S., Sadeghi Bahmani, D., Brand, S., 2019. Crocus Sativus L. (saffron) versus sertraline on symptoms of depression among older people with major depressive disorders–a double-blind, randomized intervention study. Psychiatry Res 282, 112613.
Aizawa, E., Tsuji, H., Asahara, T., Takahashi, T., Teraishi, T., Yoshida, S., Koga, N., Hattori, K., Ota, M., Kunugi, H., 2019. Bifidobacterium and Lactobacillus Counts in the Gut Microbiota of Patients With Bipolar Disorder and Healthy Controls. Front. Psychiatry 9, 730.
Akhondzadeh, S., Mostafavi, S., Keshavarz, S.A., Mohammadi, M.R., Hosseini, S., Eshraghian, M.R., 2020. A placebo controlled randomized clinical trial of Crocus sativus L. (saffron) on depression and food craving among overweight women with mild to moderate depression. J. Clin. Pharm. Ther. 45, 134–143.
Akkasheh, G., Kashani-Poor, Z., Tajabadi-Ebrahimi, M., Jafari, P., Akbari, H., Taghizadeh, M., Memarzadeh, M.R., Asemi, Z., Esmaillzadeh, A., 2016. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. Nutrition 32, 315–320.
Albert, K.M., Newhouse, P.A., 2019. Estrogen, Stress, and Depression: cognitive and Biological Interactions. Annu. Rev. Clin. Psychol. 15, 399–423.
Almodóvar, P., Briskey, D., Rao, A., Prodanov, M., Inarejos-García, A.M., 2020. Bioaccessibility and Pharmacokinetics of a Commercial Saffron (Crocus sativus L.) Extract. Evidence-based Complement. Altern. Med. 2020, 1575730.
Ameringen, M., Turna, J., Patterson, B., Pipe, A., Mao, R.Q., Anglin, R., Surette, M.G., 2019. The gut microbiome in psychiatry: a primer for clinicians. Depress. Anxiety 36, 1004–1025.
Anderson, G.D., Elmer, G.W., Kantor, E.D., Templeton, I.E., Vitiello, M.V., 2005. Pharmacokinetics of valerenic acid after administration of valerian in healthy subjects. Phyther. Res. 19, 801–803.
Andreatini, R., Sartori, V.A., Seabra, M.L.V., Leite, J.R., 2002. Effect of valepotriates (valerian extract) in generalized anxiety disorder: a randomized placebo-controlled pilot study. Phyther. Res. 16, 650–654.
Araj-Khodaei, M., Noorbala, A.A., Yarani, R., Emadi, F., Emaratkar, E., Faghihzadeh, S., Parsian, Z., Alijaniha, F., Kamalinejad, M., Naseri, M., 2020. A double-blind, randomized pilot study for comparison of Melissa officinalis L. and Lavandula angustifolia Mill. with Fluoxetine for the treatment of depression. BMC Complement. Med. Ther. 20, 207.
Aslanargun, P., Cuvas, O., Dikmen, B., Aslan, E., Yuksel, M.U., 2012. Passiflora incarnata Linneaus as an anxiolytic before spinal anesthesia. J. Anesth. 26, 39–44.
Babatabar Darzi, H., Vahedian-Azimi, A., Ghasemi, S., Ebadi, A., Sathyapalan, T., Sahebkar, A., 2020. The effect of aromatherapy with rose and lavender on anxiety, surgical site pain, and extubation time after open-heart surgery: a double-center randomized controlled trial. Phyther. Res. 34, 2675–2684.
Bäckhed, F., Fraser, C.M., Ringel, Y., Sanders, M.E., Sartor, R.B., Sherman, P.M., Versalovic, J., Young, V., Finlay, B.B., 2012. Defining a Healthy Human Gut Microbiome: current Concepts, Future Directions, and Clinical Applications. Cell Host Microbe 12, 611–622.
Bäckhed, F., Ley, R.E., Sonnenburg, J.L., Peterson, D.A., Gordon, J.I., 2005. Host-Bacterial Mutualism in the Human Intestine. Science 307 (80–), 1915–1920.
Banskota, S., Brim, H., Kwon, Y.H., Singh, G., Sinha, S.R., Wang, H., Khan, W.I., Ashktorab, H., 2021. Saffron pre-treatment promotes reduction in tissue inflammatory profiles and alters Microbiome composition in experimental colitis mice. Molecules 26, 3351.
Bazrafshan, M.-R., Jokar, M., Shokrpour, N., Delam, H., 2020. The effect of lavender herbal tea on the anxiety and depression of the elderly: a randomized clinical trial. Complement. Ther. Med. 50, 102393.
Beaubrun, G., Gray, G.E., 2000. A Review of Herbal Medicines for Psychiatric Disorders. Psychiatr. Serv. 51, 1130–1134.
Blatchford, P., Parkar, S., Hopkins, W., Ingram, J., Sutton, K., 2019. Dose-Dependent Alterations to In Vitro Human Microbiota Composition and Butyrate Inhibition by a Supercritical Carbon Dioxide Hops Extract. Biomolecules 9, 390.
Booijink, C.C.G.M., El-Aidy, S., Rajilić-Stojanović, M., Heilig, H.G.H.J., Troost, F.J., Smidt, H., Kleerebezem, M., De Vos, W.M., Zoetendal, E.G., 2010. High temporal and inter-individual variation detected in the human ileal microbiota. Environ. Microbiol. 12, 3213–3227.
Briley, M., Chantal, M., 2011. The importance of norepinephrine in depression. Neuropsychiatr. Dis. Treat. 7, 9–13.
Brochot, A., Azalbert, V., Landrier, J., Tourniaire, F., Serino, M., 2019. A Two-Week Treatment with Plant Extracts Changes Gut Microbiota, Caecum Metabolome, and Markers of Lipid Metabolism in ob/ob Mice. Mol. Nutr. Food Res. 63, 1900403.
Browning, K.N., Verheijden, S., Boeckxstaens, G.E., 2017. The Vagus Nerve in Appetite Regulation, Mood, and Intestinal Inflammation. Gastroenterology 152, 730–744.
Brüssow, H., 2020. Problems with the concept of gut microbiota dysbiosis. Microb. Biotechnol. 13, 423–434.
Bystritsky, A., Kerwin, L., Feusner, J.D., 2008. A Pilot Study of Rhodiola rosea (Rhodax ®) for Generalized Anxiety Disorder (GAD). J. Altern. Complement. Med. 14, 175–180.
Caccia, S., 2005. Antidepressant-Like Components of Hypericum perforatum Extracts: an Overview of Their Pharmacokinetics and Metabolism. Curr. Drug Metab. 6, 531–543.
Caccia, S., Gobbi, M., 2010. St. Johns Wort Components and the Brain: uptake, Concentrations Reached and the Mechanisms Underlying Pharmacological Effects. Curr. Drug Metab. 10, 1055–1065.
Caesar, L.K., Cech, N.B., 2019. Synergy and antagonism in natural product extracts: when 1 + 1 does not equal 2. Nat. Prod. Rep. 36, 869–888.
Calvo-Castro, L.A., Burkard, M., Sus, N., Scheubeck, G., Leischner, C., Lauer, U.M., Bosy-Westphal, A., Hund, V., Busch, C., Venturelli, S., Frank, J., 2018. The Oral Bioavailability of 8-Prenylnaringenin from Hops (Humulus Lupulus L.) in Healthy Women and Men is Significantly Higher than that of its Positional Isomer 6-Prenylnaringenin in a Randomized Crossover Trial. Mol. Nutr. Food Res. 62, 1700838.
Carlessi, A.S., Borba, L.A., Zugno, A.I., Quevedo, J., Réus, G.Z., 2021. Gut microbiota–brain axis in depression: the role of neuroinflammation. Eur. J. Neurosci. 53, 222–235.
Cattoor, K., Dresel, M., De Bock, L., Boussery, K., Van Bocxlaer, J., Remon, J.P., De Keukeleire, D., Deforce, D., Hofmann, T., Heyerick, A., 2013. Metabolism of hop-derived bitter acids. J. Agric. Food Chem. 61, 7916–7924.
Cattoor, K.O., Bracke, M., Deforce, D., De Keukeleire, D., Heyerick, A., 2010. Transport of hop bitter acids across intestinal Caco-2 cell monolayers. J. Agric. Food Chem. 58, 4132–4140.
Chang, Y.W., Yao, H.T., Hsieh, S.H., Lu, T.J., Yeh, T.K., 2007. Quantitative determination of salidroside in rat plasma by on-line solid-phase extraction integrated with high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 857, 164–169.

Chen, L., Liu, Y., Tang, Z., Shi, X., Song, Z., Cao, F., Wei, P., Li, M., Li, X., Jiang, D., Yan, Y., Yang, N., 2021a. Improvements in estrogen deficiency-induced hypercholesterolemia by Hypericum perforatum L. extract are associated with gut microbiota and related metabolites in ovariectomized (OVX) rats. Biomed. Pharmacother. 135, 111131.

Chen, Y., Bai, J., Wu, D., Yu, S., Qiang, X., Bai, H., Wang, H., Peng, Z., 2019. Association between fecal microbiota and generalized anxiety disorder: severity and early treatment response. J. Affect. Disord. 259, 56–66.

Chen YH, Xue F, Yu SF, Li XS, Liu L, Jia YY, Yan WJ, Tan QR, Wang HN, Peng ZW. Gut microbiota dysbiosis in depressed women: The association of symptom severity and microbiota function. J Affect Disord. 202, 282, 391-400.

Cheng, L.-H., Liu, Y.-W., Wu, C.-C., Wang, S., Tsai, Y.-C., 2019. Psychobiotics in mental health, neurodegenerative and neurodevelopmental disorders. J. Food Drug Anal. 27, 632–648.

Chi, L., Khan, I., Lin, Z., Zhang, J., Lee, M.Y.S., Leong, W., Hsiao, W.L.W., Zheng, Y., 2020. Fructo-oligosaccharides from Morinda officinalis remodeled gut microbiota and alleviated depression features in a stress rat model. Phytomedicine 67, 153157.

Chryssanthi, D.G., Lamari, F.N., Georgakopoulos, C.D., Cordopatis, P., 2011. A new validated SPE-HPLC method for monitoring crocetin in human plasma-Application after saffron tea consumption. J. Pharm. Biomed. Anal. 55, 563–658.

Coello, K., Hansen, T.H., Sørensen, N., Ottesen, N.M., Miskowiak, K.W., Pedersen, O., Kessing, L.V., Vinberg, M., 2021. Affective disorders impact prevalence of Flavonifractor and abundance of Christensenellaceae in gut microbiota. Prog. Neuro-Psychopharmacology Biol. Psychiatry 110, 110300.

Cortés-Martín, A., García-Villalba, R., González-Sarrías, A., Romo-Vaquero, M., Loria-Kohen, V., Ramírez-de-Molina, A., Tomás-Barberán, F.A., Selma, M.V., Espín, J.C., 2018. The gut microbiota urolithin metabotypes revisited: the human metabolism of ellagic acid is mainly determined by aging. Food Funct 9, 4100–4106.

Cortés-Martín, A., Selma, M.V., Tomás-Barberán, F.A., González-Sarrías, A., Espín, J.C., 2020. Where to Look into the Puzzle of Polyphenols and Health? The Postbiotics and Gut Microbiota Associated with Human Metabotypes. Mol. Nutr. Food Res 64, 1900952.

Cowley, D.S., Dager, S.R., Roy-Byrne, P.P., Avery, D.H., Dunner, D.L., 1991. Lactate vulnerability after alprazolam versus placebo treatment of panic disorder. Biol. Psychiatry 30, 49–56.

Cropley, M., Banks, A.P., Boyle, J., 2015. The Effects of Rhodiola rosea L. Extract on Anxiety, Stress, Cognition and Other Mood Symptoms. Phyther. Res. 29, 1934–1939.

Crovesy, L., Ostrowski, M., Ferreira, D.M.T.P., Rosado, E.L., Soares-Mota, M., 2017. Effect of Lactobacillus on body weight and body fat in overweight subjects: a systematic review of randomized controlled clinical trials. Int. J. Obes. 41.

Cryan, J.F., Dinan, T.G., 2012. Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. Nat. Rev. Neurosci. 13, 701–712.

Cryan, J.F., O'Riordan, K.J., Cowan, C.S.M., Sandhu, K.V., Bastiaanssen, T.F.S., Boehme, M., Codagnone, M.G., Cussotto, S., Fulling, C., Golubeva, A.V., Guzzetta, K. E., Jaggar, M., Long-Smith, C.M., Lyte, J.M., Martin, J.A., Molinero-Perez, A., Moloney, G., Morelli, E., Morillas, E., O'Connor, R., Cruz-Pereira, J.S., Peterson, V.L., Rea, K., Ritz, N.L., Sherwin, E., Spichak, S., Teichman, E.M., van de Wouw, M., Ventura-Silva, A.P., Wallace-Fitzsimons, S.E., Hyland, N., Clarke, G., Dinan, T.G., 2019. The Microbiota-Gut-Brain Axis. Physiol. Rev. 99, 1877–2013.

da Cunha, R.S., Amorim, K.S., Gercina, A.C., de Oliveira, A.C.A., dos Santos Menezes, L., Groppo, F.C., Souza, L.M.A., 2021. Herbal medicines as anxiolytics prior to third molar surgical extraction. A randomized controlled clinical trial. Clin. Oral Investig. 25, 1579–1586.

Darbinyan, V., Aslanyan, G., Amroyan, E., Gabrielyan, E., Malmström, C., Panossian, A., 2007. Clinical trial of Rhodiola rosea L. extract SHR-5 in the treatment of mild to moderate depression. Nord. J. Psychiatry 61, 343–348.

Desbonnet, L., Garrett, L., Clarke, G., Kiely, B., Cryan, J.F., Dinan, T.G., 2010. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience 170, 1179–1188.

Di Pierro, F., Risso, P., Settembre, R., 2018. Role in depression of a multi-fractionated versus a conventional Hypericum perforatum extract. Panminerva Med 60, 156–160.

Donaldson, G.P., Lee, S.M., Mazmanian, S.K., 2016. Gut biogeography of the bacterial microbiota. Nat. Rev. Microbiol. 14, 20–32.

Doroshyenko, O., Rokitta, D., Zadoyan, G., Klement, S., Schläfke, S., Dienel, A., Gramatté, T., Lück, H., Fuhr, U., 2013. Drug cocktail interaction study on the effect of the orally administered lavender oil preparation silexan on cytochrome p450 enzymes in healthy volunteers. Drug Metab. Dispos. 41, 987–993.

dos Santos, R.G., Hallak, J.E.C., 2020. Therapeutic use of serotoninergic hallucinogens: a review of the evidence and of the biological and psychological mechanisms. Neurosci. Biobehav. Rev. 108, 423–434.

Duda-Chodak, A., Tarko, T., Satora, P., Sroka, P., 2015. Interaction of dietary compounds, especially polyphenols, with the intestinal microbiota: a review. Eur. J. Nutr. 54, 325–341.

Durazzi, F., Sala, C., Castellani, G., Manfreda, G., Remondini, D., De Cesare, A., 2021. Comparison between 16S rRNA and shotgun sequencing data for the taxonomic characterization of the gut microbiota. Sci. Rep. 11, 3030.

Eatemadnia, A., Ansari, S., Abedi, P., Najar, S., 2019. The effect of Hypericum perforatum on postmenopausal symptoms and depression: a randomized controlled trial. Complement. Ther. Med. 45, 109–113.

Edwards, D., Heufelder, A., Zimmermann, A., 2012. Therapeutic Effects and Safety of Rhodiola rosea Extract WS® 1375 in Subjects with Life-stress Symptoms - Results of an Open-label Study. Phyther. Res. 26, 1220–1225.

Etxeberria, U., Arias, N., Boqué, N., Macarulla, M.T., Portillo, M.P., Martínez, J.A., Milagro, F.I., 2015. Reshaping faecal gut microbiota composition by the intake of trans-resveratrol and quercetin in high-fat sucrose diet-fed rats. J. Nutr. Biochem. 26, 651–660.

Fakhoury, M., 2016. Revisiting the Serotonin Hypothesis: implications for Major Depressive Disorders. Mol. Neurobiol. 53, 2778–2786.

Fang, J.B., Nikolić, D., Lankin, D.C., Simmler, C., Chen, S.N., Ramos Alvarenga, R.F., Liu, Y., Pauli, G.F., Van Breemen, R.B., 2019. Formation of (2 R)- And (2 S)-8-Prenylnaringenin Glucuronides by Human UDP-Glucuronosyltransferases. J. Agric. Food Chem. 67, 11650-11656.

Farah, G.J., Ferreira, G.Z., Danieletto-Zanna, C.F., Luppi, C.R., Jacomacci, W.P., 2019. Assessment of Valeriana officinalis l. (Valerian) for Conscious Sedation of Patients During the Extraction of Impacted Mandibular Third Molars: a Randomized, Split-Mouth, Double-Blind, Crossover Study. J. Oral Maxillofac. Surg. 77, 1796.e1–1796.e8.

Farshbaf-Khalili, A., Kamalifard, M., Namadian, M., 2018. Comparison of the effect of lavender and bitter orange on anxiety in postmenopausal women: a triple-blind, randomized, controlled clinical trial. Complement. Ther. Clin. Pract. 31, 132–138.

Ferreira, J.V., Braga, A.V., Machado, R., de, R., Michel, D., Pianetti, G.A., El-Aneed, A., César, I.C., 2020. Liquid chromatography-tandem mass spectrometry bioanalytical method for the determination of kavain in mice plasma: application to a pharmacokinetic study. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1137, 121939.

Fiesel, A., Gessner, D.K., Most, E., Eder, K., 2014. Effects of dietary polyphenol-rich plant products from grape or hop on pro-inflammatory gene expression in the intestine, nutrient digestibility and faecal microbiota of weaned pigs. BMC Vet. Res. 10, 196.

Fontana, A., Manchia, M., Panebianco, C., Paribello, P., Arzedi, C., Cossu, E., Garzilli, M., Montis, M.A., Mura, A., Pisanu, C., Congiu, D., Copetti, M., Pinna, F., Carpiniello, B., Squassina, A., Pazienza, V., 2020. Exploring the Role of Gut Microbiota in Major Depressive Disorder and in Treatment Resistance to Antidepressants. Biomedicines 8, 311.

Foster, J.A., McVey Neufeld, K.-A., 2013. Gut–brain axis: how the microbiome influences anxiety and depression. Trends Neurosci 36, 305–312.

Fox, E., Murphy, R.F., McCully, C.L., Adamson, P.C., 2001. Plasma pharmacokinetics and cerebrospinal fluid penetration of hypericin in nonhuman primates. Cancer Chemother. Pharmacol. 47, 41–44.

Franco, L., Sánchez, C., Bravo, R., Rodríguez, A.B., Barriga, C., Romero, E., Cubero, J., 2012. The Sedative Effect of Non-Alcoholic Beer in Healthy Female Nurses. PLoS ONE 7, e37290.

Freud, S., 1917. Trauer Und Melancholie.

Fukizawa, S., Yamashita, M., Wakabayashi, K., Fujisaka, S., Tobe, K., Nonaka, Y., Muryama, N., 2020. Anti-obesity effect of a hop-derived prenylflavonoid isoxanthohumol in a high-fat diet-induced obese mouse model. Biosci. Microbiota, Food Heal. 39, 175–182.

Gadotti, V.M., Zamponi, G.W., 2019. Anxiolytic effects of the flavonoid luteolin in a mouse model of acute colitis. Mol. Brain 12, 114.

Gałecki, P., Talarowska, M., 2018. Inflammatory theory of depression. Psychiatr. Pol. 52, 437–447.

Gao, L., Wu, C., Liao, Y., Wang, J., 2020. Antidepressants effects of Rhodiola capsule combined with sertraline for major depressive disorder: a randomized double-blind placebo-controlled clinical trial. J. Affect. Disord. 265, 99–103.

Gartlehner, G., Gaynes, B.N., Amick, H.R., Asher, G.N., Morgan, L.C., Coker-Schwimmer, E., Forneris, C., Boland, E., Lux, L.J., Gaylord, S., Bann, C., Pierl, C.B., Lohr, K.N., 2016. Comparative Benefits and Harms of Antidepressant, Psychological, Complementary, and Exercise Treatments for Major Depression: an Evidence Report for a Clinical Practice Guideline From the American College of Physicians. Ann. Intern. Med. 164, 331.

Gharib, M., Samani, L.N., Panah, Z.E., Naseri, M., Bahrani, N., Kiani, K., 2015. The Effect of Valeric on Anxiety Severity in Women Undergoing Hysterosalpingography. Glob. J. Health Sci. 7, 358–363.

Girme, A., Pawar, S., Ghule, C., Shengule, S., Saste, G., Balasubramaniam, A.K., Deshmukh, A., Hingorani, L., 2021. Bioanalytical method development and validation study of neuroprotective extract of kashmiri saffron using ultra-fast liquid chromatography-tandem mass spectrometry (Uflc-ms/ms): in vivo pharmacokinetics of apocarotenoids and carotenoids. Molecules 26, 1815.

Gu, S., Chen, D., Zhang, J.-N., Lv, X., Wang, K., Duan, L.-P., Nie, Y., Wu, X.-L., 2013. Bacterial Community Mapping of the Mouse Gastrointestinal Tract. PLoS ONE 8, e74957.

Gutmann, H., Bruggisser, R., Schaffner, W., Bogman, K., Botomino, A., Drewe, J., 2002. Transport of amentoflavone across the blood-brain barrier in vitro. Planta Med 68, 804–807.

Hamm, A.K., Manter, D.K., Kirkwood, J.S., Wolfe, L.M., Cox-York, K., Weir, T.L., 2019. The Effect of Hops (Humulus lupulus L.) Extract Supplementation on Weight Gain, Adiposity and Intestinal Function in Ovariectomized Mice. Nutrients 11, 3004.

Han, S., Van Treuren, W., Fischer, C.R., Merrill, B.D., DeFelice, B.C., Sanchez, J.M., Higginbottom, S.K., Guthrie, L., Fall, L.A., Dodd, D., Fischbach, M.A., Sonnenburg, J. L., 2021. A metabolomics pipeline for the mechanistic interrogation of the gut microbiome. Nature 595, 415–420.

Hanske, L., Loh, G., Sczesny, S., Blaut, M., Braune, A., 2010. Recovery and metabolism of xanthohumol in germ-free and human microbiota-associated rats. Mol. Nutr. Food Res. 54, 1405–1413.

Hao, W., Kwek, E., He, Z., Zhu, H., Liu, J., Zhao, Y., Ma, K.Y., He, W.-S., Chen, Z.-Y., 2020. Ursolic acid alleviates hypercholesterolemia and modulates the gut microbiota in hamsters. Food Funct 11, 6091–6103.

Hao, Z., Wang, W., Guo, R., Liu, H., 2019. Faecalibacterium prausnitzii (ATCC 27766) has preventive and therapeutic effects on chronic unpredictable mild stress-induced depression-like and anxiety-like behavior in rats. Psychoneuroendocrinology 104, 132–142.

Hattori, M., Shu, Y.-Z., El-Sedawy, A.I., Namba, T., Kobashi, K., Tomimori, T., 1988. Metabolism of Homoorientin by Human Intestinal Bacteria. J. Nat. Prod. 51, 874–878.
He, S., Li, X., Li, C., Deng, H., Shao, Y., Yuan, L., 2019. Isoorientin attenuates benzo[*a*]pyrene-induced colonic injury and gut microbiota disorders in mice. Food Res. Int. 126, 108599.
Heinlein, A., Metzger, M., Walles, H., Buettner, A., 2014. Transport of hop aroma compounds across Caco-2 monolayers. Food Funct 5, 2719–2730.
Heinritz, S.N., Mosenthin, R., Weiss, E., 2013. Use of pigs as a potential model for research into dietary modulation of the human gut microbiota. Nutr. Res. Rev. 26, 191–209.
Hirschfeld, R.M., 2000. History and evolution of the monoamine hypothesis of depression. J. Clin. Psychiatry 61 (6), 4–6. Suppl.
Huang, D., Zhang, L., Yang, J., Luo, Y., Cui, T., Du, T., Jiang, X., 2019. Evaluation on monoamine neurotransmitters changes in depression rats given with sertraline, meloxicam or/and caffeic acid. Genes Dis 6, 8343052.
Hong, Z., Piao, M., 2018. Effect of Quercetin Monoglycosides on Oxidative Stress and Gut Microbiota Diversity in Mice with Dextran Sodium Sulphate-Induced Colitis. Biomed Res. Int. 2018, 8343052.
Huang, J., Chen, L., Xue, B., Liu, Q., Ou, S., Wang, Y., Peng, X., 2016. Different Flavonoids Can Shape Unique Gut Microbiota Profile *In Vitro*. J. Food Sci. 81, H2273–H2279.
Ishisaka, M., Kakefuda, K., Yamauchi, M., Tsuruma, K., Shimazawa, M., Tsuruta, A., Hara, H., 2011. Luteolin Shows an Antidepressant-Like Effect via Suppressing Endoplasmic Reticulum Stress. Biol. Pharm. Bull. 34, 1481–1486.
Jacobs, B.P., Bent, S., Tice, J.A., Blackwell, T., Cummings, S.R., 2005. An Internet-Based Randomized, Placebo-Controlled Trial of Kava and Valerian for Anxiety and Insomnia. Medicine (Baltimore) 84, 197–207.
James, S.L., et al., 2018. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392, 1789-1858.
James, K.R., Gomes, T., Elmentaite, R., Kumar, N., Gulliver, E.L., King, H.W., Stares, M. D., Bareham, B.R., Ferdinand, J.R., Petrova, V.N., Polański, K., Forster, S.C., Jarvis, L.B., Suchanek, O., Howlett, S., James, L.K., Jones, J.L., Meyer, K.B., Clatworthy, M.R., Saeb-Parsy, K., Lawley, T.D., Teichmann, S.A., 2020. Distinct microbial and immune niches of the human colon. Nat. Immunol. 21, 343–353.
Jiang, H., Ling, Z., Zhang, Y., Mao, H., Ma, Z., Yin, Y., Wang, W., Tang, W., Tan, Z., Shi, J., Li, L., Ruan, B., 2015. Altered fecal microbiota composition in patients with major depressive disorder. Brain. Behav. Immun. 48, 186–194.
Kamuhabwa, A.R., Augustijns, P., De Witte, P.A., 1999. In vitro transport and uptake of protohypericin and hypericin in the Caco-2 model. Int. J. Pharm. 188, 81–86.
Karan, N.B., 2019. Influence of lavender oil inhalation on vital signs and anxiety: a randomized clinical trial. Physiol. Behav. 211, 112676.
Kazemi, A., Noorbala, A.A., Azam, K., Eskandari, M.H., Djafarian, K., 2019. Effect of probiotic and prebiotic vs placebo on psychological outcomes in patients with major depressive disorder: a randomized clinical trial. Clin. Nutr. 38, 522–528.
Kelly, J.R., Borre, Y., O' Brien, C., Patterson, E., El Aidy, S., Deane, J., Kennedy, P.J., Beers, S., Scott, K., Moloney, G., Hoban, A.E., Scott, L., Fitzgerald, P., Ross, P., Stanton, C., Clarke, G., Cryan, J.F., Dinan, T.G., 2016. Transferring the blues: depression-associated gut microbiota induces neurobehavioural changes in the rat. J. Psychiatr. Res. 82, 109–118.
Kim, H.J., Yim, S.H., Han, F., Kang, B.Y., Choi, H.J., Jung, D.W., Williams, D.R., Gustafson, K.R., Kennelly, E.J., Lee, I.S., 2019. Biotransformed metabolites of the hop prenylflavanone isoxanthohumol. Molecules 24, 394.
Kirsch, I., 2019. Placebo Effect in the Treatment of Depression and Anxiety. Front. Psychiatry 10, 407.
Koen, N., Stein, D.J., 2011. Pharmacotherapy of anxiety disorders: a critical review. Dialogues Clin. Neurosci. 13, 423–437.
Kosakowska, O., Bączek, K., Przybył, J.L., Pióro-Jabrucka, E., Czupa, W., Synowiec, A., Gniewosz, M., Costa, R., Mondello, L., Węglarz, Z., 2018. Antioxidant and Antibacterial Activity of Roseroot (Rhodiola rosea L.) Dry Extracts. Molecules 23, 1767.
Kristensen, N.B., Bryrup, T., Allin, K.H., Nielsen, T., Hansen, T.H., Pedersen, O., 2016. Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. Genome Med 8, 52.
Kuypers, K.P.C., 2019. Psychedelic medicine: the biology underlying the persisting psychedelic effects. Med. Hypotheses 125, 21–24.
Kyriakoudi, A., OCallaghan, Y.C., Galvin, K., Tsimidou, M.Z., OBrien, N.M., 2015. Cellular Transport and Bioactivity of a Major Saffron Apocarotenoid, Picrocrocin (4-(β- d -Glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde). J. Agric. Food Chem. 63, 8662–8668.
Kyriakoudi, A., Tsimidou, M.Z., O'Callaghan, Y.C., Galvin, K., O'Brien, N.M., 2013. Changes in total and individual crocetin esters upon in vitro gastrointestinal digestion of saffron aqueous extracts. J. Agric. Food Chem. 61, 5318–5327.
Kyrou, I., Christou, A., Panagiotakos, D., Stefanaki, C., Skenderi, K., Katsana, K., Tsigos, C., 2017. Effects of a hops (Humulus lupulus L.) dry extract supplement on self-reported depression, anxiety and stress levels in apparently healthy young adults: a randomized, placebo-controlled, double-blind, crossover pilot study. Hormones 16, 171–180.
Lautenschläger, M., Sendker, J., Hüwel, S., Galla, H.J., Brandt, S., Düfer, M., Riehemann, K., Hensel, A., 2015. Intestinal formation of trans-crocetin from saffron extract (Crocus sativus L.) and in vitro permeation through intestinal and blood brain barrier. Phytomedicine 22, 36–44.
Leach, M.J., Page, A.T., 2015. Herbal medicine for insomnia: a systematic review and meta-analysis. Sleep Med. Rev. 24, 1–12.
Legette, L., Karnpracha, C., Reed, R.L., Choi, J., Bobe, G., Christensen, J.M., Rodriguez-Proteau, R., Purnell, J.Q., Stevens, J.F., 2014. Human pharmacokinetics of xanthohumol, an antihyperglycemic flavonoid from hops. Mol. Nutr. Food Res. 58, 248–255.
Lekomtseva, Y., Zhukova, I., Wacker, A., 2017. Rhodiola rosea in Subjects with Prolonged or Chronic Fatigue Symptoms: results of an Open-Label Clinical Trial. Complement. Med. Res. 24, 46–52.
Leslie, D.L., Kozma, L., Martin, A., Landeros, A., Katsovich, L., King, R.A., Leckman, J.F., 2008. Neuropsychiatric Disorders Associated With Streptococcal Infection: a Case-Control Study Among Privately Insured Children. J. Am. Acad. Child Adolesc. Psychiatry 47, 1166–1172.
Li, H., Xi, Y., Xin, X., Tian, H., Hu, Y., 2020. Salidroside improves high-fat diet-induced non-alcoholic steatohepatitis by regulating the gut microbiota–bile acid–farnesoid X receptor axis. Biomed. Pharmacother. 124, 109915.
Leyrolle, Q., Cserjesi, R., D.G.H., Mulders, M., Zamariola, G., Hiel, S., Gianfrancesco, M. A., Portheault, D., Amadieu, C., Bindels, L.B., Leclercq, S., Rodriguez, J., Neyrinck, A.M., Cani, P.D., Lanthier, N., Trefois, P., Bindelle, J., Paquot, N., Cnop, M., Thissen, J.-P., Klein, O., Luminet, O., Delzenne, N.M., 2021. Prebiotic effect on mood in obese patients is determined by the initial gut microbiota composition: a randomized, controlled trial. Brain. Behav. Immun. 94, 289-298.
Li, R., Wang, X., Qin, T., Qu, R., Ma, S., 2016. Apigenin ameliorates chronic mild stress-induced depressive behavior by inhibiting interleukin-1β production and NLRP3 inflammasome activation in the rat brain. Behav. Brain Res. 296, 318–325.
Li, R., Zhao, D., Qu, R., Fu, Q., Ma, S., 2015. The effects of apigenin on lipopolysaccharide-induced depressive-like behavior in mice. Neurosci. Lett. 594, 17–22.
Liang, S., Wu, X., Hu, X., Wang, T., Jin, F., 2018. Recognizing Depression from the Microbiota–Gut–Brain Axis. Int. J. Mol. Sci. 19, 1592.
Liang, Y., Cui, L., Gao, J., Zhu, M., Zhang, Y., Zhang, H.-L., 2021. Gut Microbial Metabolites in Parkinson's Disease: implications of Mitochondrial Dysfunction in the Pathogenesis and Treatment. Mol. Neurobiol. 58, 3745–3758.
Lin, P., Ding, B., Feng, C., Yin, S., Zhang, T., Qi, X., Lv, H., Guo, X., Dong, K., Zhu, Y., Li, Q., 2017. Prevotella and Klebsiella proportions in fecal microbial communities are potential characteristic parameters for patients with major depressive disorder. J. Affect. Disord. 207, 300–304.
Lin, R., Piao, M., Song, Y., 2019. Dietary Quercetin Increases Colonic Microbial Diversity and Attenuates Colitis Severity in Citrobacter rodentium-Infected Mice. Front. Microbiol. 10, 1092.
Liu, R.T., Rowan-Nash, A.D., Sheehan, A.E., Walsh, R.F.L., Sanzari, C.M., Korry, B.J., Belenky, P., 2020. Reductions in anti-inflammatory gut bacteria are associated with depression in a sample of young adults. Brain. Behav. Immun. 88, 308–324.
Liu, Y., Zhang, L., Wang, X.X., Wang, Z., Zhang, J., Jiang, R., Wang, X.X., Wang, K., Liu, Z., Xia, Z., Xu, Z., Nie, Y., Lv, X., Wu, X., Zhu, H., Duan, L., 2016. Similar Fecal Microbiota Signatures in Patients With Diarrhea-Predominant Irritable Bowel Syndrome and Patients With Depression. Clin. Gastroenterol. Hepatol. 14, 1602–1611.e5.
Liu, W., Zhou, Y., Qin, Y., Yu, L., Li, R., Chen, Y., Xu, Y., 2020. Quercetin Intervention Alleviates Offspring's Oxidative Stress, Inflammation, and Tight Junction Damage in the Colon Induced by Maternal Fine Particulate Matter (PM2.5) Exposure through the Reduction of Bacteroides. Nutrients 12, 3095.
Liu, R.T., Walsh, R.F.L., Sheehan, A.E., 2019. Prebiotics and probiotics for depression and anxiety: a systematic review and meta-analysis of controlled clinical trials. Neurosci. Biobehav. Rev. 102, 13–23.
Llaneza, P., García-Portilla, M.P., Llaneza-Suárez, D., Armott, B., Pérez-López, F.R., 2012. Depressive disorders and the menopause transition. Maturitas 71, 120–130.
Logan, I., Miranda, C., Lowry, M., Maier, C., Stevens, J., Gombart, A., 2019. Antiproliferative and Cytotoxic Activity of Xanthohumol and Its Non-Estrogenic Derivatives in Colon and Hepatocellular Carcinoma Cell Lines. Int. J. Mol. Sci. 20, 1203.
Logan, I.E., Shulzhenko, N., Sharpton, T.J., Bobe, G., Liu, K., Nuss, S., Jones, M.L., Miranda, C.L., Vasquez-Perez, S., Pennington, J.M., Leonard, S.W., Choi, J., Wu, W., Gurung, M., Kim, J.P., Lowry, M.B., Morgun, A., Maier, C.S., Stevens, J.F., Gombart, A.F., 2021. Xanthohumol Requires the Intestinal Microbiota to Improve Glucose Metabolism in Diet-Induced Obese Mice. Mol. Nutr. Food Res. 2100389, 1–13.
Lopresti, A.L., Smith, S.J., Hood, S.D., Drummond, P.D., 2019. Efficacy of a standardised saffron extract (affron®) as an add-on to antidepressant medication for the treatment of persistent depressive symptoms in adults: a randomised, double-blind, placebo-controlled study. J. Psychopharmacol. 33, 1415–1427.
Lozupone, C., Lladser, M.E., Knights, D., Stombaugh, J., Knight, R., 2011. UniFrac: an effective distance metric for microbial community comparison. ISME J 5, 169–172.
Luppino, F.S., de Wit, L.M., Bouvy, P.F., Stijnen, T., Cuijpers, P., Penninx, B.W.J.H., Zitman, F.G., 2010. Overweight, Obesity, and Depression: a systematic review and meta-analysis of longitudinal studies. Arch. Gen. Psychiatry 67, 220–229.
Madureira, A.R., Campos, D.A., Fernandes, J.C., Marques, C., Gullón, B., Rodríguez-alcalá, L.M., Calhau, C., Maria, A., Manuela, M., Reis, F., 2016. Safety profile of solid lipid nanoparticles loaded with rosmarinic acid for oral use : in vitro and animal approaches. Int. J. Nanomedicine 11, 3621–3640.
Maes, M., Kubera, M., Leunis, J.-C., Berk, M., 2012. Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut. J. Affect. Disord. 141, 55–62.
Mahmood, T., Silverstone, T., 2001. Serotonin and bipolar disorder. J. Affect. Disord. 66, 1–11.
Maier-Salamon, A., Trauner, G., Hiltscher, R., Reznicek, G., Kopp, B., Thalhammer, T., Jäger, W., 2009. Hepatic metabolism and biliary excretion of valerenic acid in isolated perfused rat livers: role of Mrp2 (Abcc2). J. Pharm. Sci. 98, 3839–3849.

Mao, J.J., Xie, S.X., Zee, J., Soeller, I., Li, Q.S., Rockwell, K., Amsterdam, J.D., 2015. Rhodiola rosea versus sertraline for major depressive disorder: a randomized placebo-controlled trial. Phytomedicine 22, 394–399.
Mao, Y., Zhang, Xinrong, Zhang, Xiaodong, Lu, G., 2007. Development of an HPLC method for the determination of salidroside in beagle dog plasma after administration of salidroside injection: application to a pharmacokinetics study. J. Sep. Sci. 30, 3218-322.
Martinez, S.E., Lakowski, T.M., Davies, N.M., 2014. Enantiospecific analysis of 8-Prenylnaringenin in biological fluids by liquid-chromatography-electrospray ionization mass spectrometry: application to preclinical pharmacokinetic investigations. Chirality 26, 419–426.
Martínez-Damas, M.G., Genis-Mendoza, A.D., Pérez-de la Cruz, V., Canela-Tellez, G.D., Jiménez-Estrada, I., Sanchez, J.H.N., Ramos-Chávez, L.A., García, S., Ramírez-Ramírez, M., Coral-Vázquez, R.M., 2021. Epicatechin treatment generates resilience to chronic mild stress-induced depression in a murine model. Physiol. Behav. 238, 113466.
Martínez-Hernández, G.B., Jiménez-Ferrer, E., Román-Ramos, R., Zamilpa, A., González-Cortazar, M., León-Rivera, I., Vargas-Villa, G., Herrera-Ruiz, M., 2021. A mixture of quercetin 4′-O-rhamnoside and isoquercitrin from Tilia americana var. mexicana and its biotransformation products with antidepressant activity in mice. J. Ethnopharmacol. 267, 113619.
Mathews, J.M., Etheridge, A.S., Valentine, J.L., Black, S.R., Coleman, D.P., Patel, P., So, J., Burka, L.T., 2005. Pharmacokinetics and disposition of the kavalactone kawain: interaction with kava extract and kavalactones in vivo and in vitro. Drug Metab. Dispos. 33, 1555–1563.
Matthias, A., Blanchfield, J.T., Penman, K.G., Bone, K.M., Toth, I., Lehmann, R.P., 2007. Permeability studies of Kavalactones using a Caco-2 cell monolayer model. J. Clin. Pharm. Ther. 32, 233–239.
McGaughey, K.D., Yilmaz-Swenson, T., Elsayed, N.M., Cruz, D.A., Rodriguiz, R.M., Kritzer, M.D., Peterchev, A.V., Roach, J., Wetsel, W.C., Williamson, D.E., 2019. Relative abundance of Akkermansia spp. and other bacterial phylotypes correlates with anxiety- and depressive-like behavior following social defeat in mice. Sci. Rep. 9, 3281.
Million, M., Maraninchi, M., Henry, M., Armougom, F., Richet, H., Carrieri, P., Valero, R., Raccah, D., Vialettes, B., Raoult, D., 2012. Obesity-associated gut microbiota is enriched in Lactobacillus reuteri and depleted in Bifidobacterium animalis and Methanobrevibacter smithii. Int. J. Obes. 36, 817–825.
Miranda, C.L., Johnson, L.A., de Montgolfier, O., Elias, V.D., Ullrich, L.S., Hay, J.J., Paraiso, I.L., Choi, J., Reed, R.L., Revel, J.S., Kioussi, C., Bobe, G., Iwaniec, U.T., Turner, R.T., Katzenellenbogen, B.S., Katzenellenbogen, J.A., Blakemore, P.R., Gombart, A.F., Maier, C.S., Raber, J., Stevens, J.F., 2018. Non-estrogenic Xanthohumol derivatives mitigate insulin resistance and cognitive impairment in high-fat diet-induced obese mice. Sci. Rep 8, 613.
Miroddi, M., Calapai, G., Navarra, M., Minciullo, P.L., Gangemi, S., 2013. Passiflora incarnata L.: ethnopharmacology, clinical application, safety and evaluation of clinical trials. J. Ethnopharmacol. 150, 791–804.
Miyasaka, L.S., Atallah, Á.N., Soares, B., 2007. Passiflora for anxiety disorder. Cochrane Database Syst. Rev. CD004518.
Moghadam, B.H., Bagheri, R., Roozbeh, B., Ashtary-Larky, D., Gaeini, A.A., Dutheil, F., Wong, A., 2021. Impact of saffron (Crocus Sativus Linn) supplementation and resistance training on markers implicated in depression and happiness levels in untrained young males. Physiol. Behav. 233, 113352.
Moratalla-López, Bagur, Lorenzo, Salinas, Alonso, 2019. Bioactivity and bioavailability of the major metabolites of crocus sativus L. Flower. Molecules 24, 2827.
Mosca, A., Leclerc, M., Hugot, J.P., 2016. Gut microbiota diversity and human diseases: should we reintroduce key predators in our ecosystem? Front. Microbiol. 7, 455.
Mosele, J.I., Martín-Peláez, S., Macià, A., Farràs, M., Valls, R.M., Catalán, Ú., Motilva, M. J., 2014. Study of the catabolism of thyme phenols combining in vitro fermentation and human intervention. J. Agric. Food Chem. 62, 10954–10961.
Movafegh, A., Alizadeh, R., Hajimohamadi, F., Esfehani, F., Nejatfar, M., 2008. Preoperative Oral Passiflora Incarnata Reduces Anxiety in Ambulatory Surgery Patients: a Double-Blind, Placebo-Controlled Study. Anesth. Analg. 106, 1728–1732.
Mykletun, A., Jacka, F., Williams, L., Pasco, J., Henry, M., Nicholson, G.C., Kotowicz, M. A., Berk, M., 2010. Prevalence of mood and anxiety disorder in self reported irritable bowel syndrome (IBS). An epidemiological population based study of women. BMC Gastroenterol 10, 88.
Nayfach, S., Shi, Z.J., Seshadri, R., Pollard, K.S., Kyrpides, N.C., 2019. New insights from uncultivated genomes of the global human gut microbiome. Nature 568, 505–510.
Neuhaus, W., Trauner, G., Gruber, D., Oelzant, S., Kieoal, W., Kopp, B., Noe, C.R., 2008. Transport of a GABAA receptor modulator and its derivatives from Valeriana officinalis L s. I. across an in vitro cell culture model of the blood-brain barrier. Planta Med 74, 1338–1344.
Neunlist, M., Van Landeghem, L., Mahé, M.M., Derkinderen, P., des Varannes, S.B., Rolli-Derkinderen, M., 2013. The digestive neuronal–glial–epithelial unit: a new actor in gut health and disease. Nat. Rev. Gastroenterol. Hepatol. 10, 90–100.
Ng, Q.X., Venkatanarayanan, N., Ho, C.Y.X., 2017. Clinical use of Hypericum perforatum (St John's wort) in depression: a meta-analysis. J. Affect. Disord. 210, 211–221.
Ngan, A., Conduit, R., 2011. A double-blind, Placebo-controlled Investigation of the Effects of Passiflora incarnata (Passionflower) Herbal Tea on Subjective Sleep Quality. Phyther. Res. 25, 1153–1159.
Nicolussi, S., Drewe, J., Butterweck, V., Meyer zu Schwabedissen, H.E., 2020. Clinical relevance of St. John's wort drug interactions revisited. Br. J. Pharmacol. 177, 1212–1226.
Nie, J., Zhang, L., Zhao, G., Du, X., 2019. Quercetin reduces atherosclerotic lesions by altering the gut microbiota and reducing atherogenic lipid metabolites. J. Appl. Microbiol. 127, 1824–1834.
Noguchi-Shinohara, M., Ono, K., Hamaguchi, T., Iwasa, K., Nagai, T., Kobayashi, S., Nakamura, H., Yamada, M., 2015. Pharmacokinetics, safety and tolerability of Melissa officinalis extract which contained rosmarinic acid in healthy individuals: a randomized controlled trial. PLoS ONE 10, e0126422.
Nöldner, M., Germer, S., Koch, E., 2011. Pharmacokinetics of linalool and linalyl acetate, the two main constituents of silexan, an essential oil from Lavandula angustifolia flowers, in rats. Planta Med 77, PM44.
Ohara, K., Misaizu, A., Kaneko, Y., Fukuda, T., Miyake, M., Miura, Y., Okamura, H., Yajima, J., Tsuda, A., 2018. β-Eudesmol, an oxygenized sesquiterpene, reduces the increase in saliva 3-Methoxy-4-Hydroxyphenylglycol after the "Trier Social Stress Test" in healthy humans: a randomized, double-blind, placebo-controlled cross-over study. Nutrients 11, 9.
Olennikov, D.N., Chirikova, N.K., Vasilieva, A.G., Fedorov, I.A., 2020. LC-MS profile, gastrointestinal and gut microbiota stability and antioxidant activity of Rhodiola rosea herb metabolites: a comparative study with subterranean organs. Antioxidants 9, 1–29.
Onyszkiewicz, M., Gawrys-Kopczynska, M., Sałagaj, M., Aleksandrowicz, M., Sawicka, A., Koźniewska, E., Samborowska, E., Ufnal, M., 2020. Valeric acid lowers arterial blood pressure in rats. Eur. J. Pharmacol. 877, 173086.
Ooi, S.L., Henderson, P., Pak, S.C., 2018. Kava for Generalized Anxiety Disorder: a Review of Current Evidence. J. Altern. Complement. Med. 24, 770–780.
Ou, J., Huang, J., Zhao, D., Du, B., Wang, M., 2018. Protective effect of rosmarinic acid and carnosic acid against streptozotocin-induced oxidation, glycation, inflammation and microbiota imbalance in diabetic rats. Food Funct 9, 851–860.
Paraiso, I.L., Plagmann, L.S., Yang, L., Zielke, R., Gombart, A.F., Maier, C.S., Sikora, A.E., Blakemore, P.R., Stevens, J.F., 2019. Reductive Metabolism of Xanthohumol and 8-Prenylnaringenin by the Intestinal Bacterium *Eubacterium ramulus*. Mol. Nutr. Food Res. 63, e1800923.
Parkar, S.G., Trower, T.M., Stevenson, D.E., 2013. Fecal microbial metabolism of polyphenols and its effects on human gut microbiota. Anaerobe 23, 12–19.
Pei, Y., Otieno, D., Gu, I., Lee, S.-O., Parks, J.S., Schimmel, K., Kang, H.W., 2021. Effect of quercetin on nonshivering thermogenesis of brown adipose tissue in high-fat diet-induced obese mice. J. Nutr. Biochem. 88, 108532.
Peng, F., Zhang, H., He, X., Song, Z., 2021. Effects of Ursolic Acid on Intestinal Health and Gut Bacteria Antibiotic Resistance in Mice. Front. Physiol. 12, 1–15.
Peng, L., Zhang, Q., Zhang, Y., Yao, Z., Song, P., Wei, L., Zhao, G., Yan, Z., 2020. Effect of tartary buckwheat, rutin, and quercetin on lipid metabolism in rats during high dietary fat intake. Food Sci. Nutr. 8, 199–213.
Peterson, C.T., Sharma, V., Uchitel, S., Denniston, K., Chopra, D., Mills, P.J., Peterson, S. N., 2018. Prebiotic Potential of Herbal Medicines Used in Digestive Health and Disease. J. Altern. Complement. Med. 24, 656–665.
Pilkington, K., Wieland, L.S., 2020. Self-care for anxiety and depression: a comparison of evidence from Cochrane reviews and practice to inform decision-making and priority-setting. BMC Complement. Med. Ther. 20, 247.
Pittler, M.H., Ernst, E., 2003. Kava extract versus placebo for treating anxiety. Cochrane Database Syst. Rev. CD003383.
Pollak, D.D., Rey, C.E., Monje, F.J., 2010. Rodent models in depression research: classical strategies and new directions. Ann. Med. 42, 252–264.
Porras, D., Nistal, E., Martínez-Flórez, S., Pisonero-Vaquero, S., Olcoz, J.L., Jover, R., González-Gallego, J., García-Mediavilla, M.V., Sánchez-Campos, S., 2017. Protective effect of quercetin on high-fat diet-induced non-alcoholic fatty liver disease in mice is mediated by modulating intestinal microbiota imbalance and related gut-liver axis activation. Free Radic. Biol. Med. 102, 188–202.
Possemiers, S., Bolca, S., Grootaert, C., Heyerick, A., Decroos, K., Dhooge, W., De Keukeleire, D., Rabot, S., Verstraete, W., Van de Wiele, T., 2006. The Prenylflavonoid Isoxanthohumol from Hops (Humulus lupulus L.) Is Activated into the Potent Phytoestrogen 8-Prenylnaringenin In Vitro and in the Human Intestine. J. Nutr. 136, 1862–1867.
Powell, N., Walker, M.M., Talley, N.J., 2017. The mucosal immune system: master regulator of bidirectional gut–brain communications. Nat. Rev. Gastroenterol. Hepatol. 14, 143–159.
Power, K.A., Lu, J.T., Monk, J.M., Lepp, D., Wu, W., Zhang, C., Liu, R., Tsao, R., Robinson, L.E., Wood, G.A., Wolyn, D.J., 2016. Purified rutin and rutin-rich asparagus attenuates disease severity and tissue damage following dextran sodium sulfate-induced colitis. Mol. Nutr. Food Res. 60, 2396–2412.
Pusceddu, M.M., Murray, K., Gareau, M.G., 2018. Targeting the Microbiota, From Irritable Bowel Syndrome to Mood Disorders: focus on Probiotics and Prebiotics. Curr. Pathobiol. Rep. 6, 1–13.
Qian, Y., Jiang, S., Zhu, Z., Wang, Q., Su, S., Tao, J., Duan, J., 2017. Simultaneous quantification and semi-quantification of amentoflavone and its metabolites in human intestinal bacteria by liquid chromatography Orbitrap high-resolution mass spectrometry. Biomed. Chromatogr. 31, e3990.
Reichling, J., Weseler, A., Saller, R., 2001. A Current Review of the Antimicrobial Activity of Hypericum perforatum L. Pharmacopsychiatry 34, 116–118.
Rieder, R., Wisniewski, P.J., Alderman, B.L., Campbell, S.C., 2017. Microbes and mental health: a review. Brain. Behav. Immun. 66, 9–17.
Riva, A., Kolimár, D., Spittler, A., Wisgrill, L., Herbold, C.W., Abrankó, L., Berry, D., 2020. Conversion of Rutin, a Prevalent Dietary Flavonol, by the Human Gut Microbiota. Front. Microbiol. 11, 585428.
Rizzello, F., Ricci, C., Scandella, M., Cavazza, E., Giovanardi, E., Valerii, M.C., Campieri, M., Comparone, A., De Fazio, L., Candela, M., Turroni, S., Spisni, E., 2018. Dietary geraniol ameliorates intestinal dysbiosis and relieves symptoms in irritable bowel syndrome patients: a pilot study. BMC Complement. Altern. Med. 18, 338.
Roh, D., Jung, J.H., Yoon, K.H., Lee, C.H., Kang, L.Y., Lee, S., Shin, K., Kim, D.H., 2019. Valerian extract alters functional brain connectivity: a randomized double-blind placebo-controlled trial. Phyther. Res. 33, 939–948.

Romijn, A.R., Rucklidge, J.J., Kuijer, R.G., Frampton, C., 2017. A double-blind, randomized, placebo-controlled trial of *Lactobacillus helveticus* and *Bifidobacterium longum* for the symptoms of depression. Aust. New Zeal. J. Psychiatry 51, 810–821.
Rong, H., Xie, X., Zhao, J., Lai, W., Wang, M., Xu, D., Liu, Y., Guo, Y., Xu, S., Deng, W., Yang, Q., Xiao, L., Zhang, Y., He, F., Wang, S., Liu, T., 2019. Similarly in depression, nuances of gut microbiota: evidences from a shotgun metagenomics sequencing study on major depressive disorder versus bipolar disorder with current major depressive episode patients. J. Psychiatr. Res. 113, 90–99.
Ross, S.M., 2014. Rhodiola rosea (SHR-5), Part 2. Holist. Nurs. Pract. 28, 217–221.
Rothenberg, D.O., Zhang, L., 2019. Mechanisms Underlying the Anti-Depressive Effects of Regular Tea Consumption. Nutrients 11, 1361.
Salehi-Pourmehr, H., Ostadrahimi, A., Ebrahimpour-Mirzarezaei, M., Farshbaf-Khalili, A., 2020. Does aromatherapy with lavender affect physical and psychological symptoms of menopausal women? A systematic review and meta-analysis. Complement. Ther. Clin. Pract. 39, 101150.
Salek, R., Dehghani, M., Mohajeri, S.A., Talaei, A., Fanipakdel, A., Javadinia, S.A., 2021. Amelioration of anxiety, depression, and chemotherapy related toxicity after crocin administration during chemotherapy of breast cancer: a double blind, randomized clinical trial. Phyther. Res. 35, 5143–5153.
Salminen, S., Collado, M.C., Endo, A., Hill, C., Lebeer, S., Quigley, E.M.M., Sanders, M.E., Shamir, R., Swann, J.R., Szajewska, H., Vinderola, G., 2021. The international scientific association of probiotics and prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nat. Rev. Gastroenterol. Hepatol. 18, 649–667.
Salviati, E., Sommella, E., Carrizzo, A., Di Sarno, V., Bertamino, A., Venturini, E., Vecchione, C., Campiglia, P., 2021. Characterization of phase I and phase II metabolites of hop (Humulus lupulus L.) bitter acids: in vitro and in vivo metabolic profiling by UHPLC-Q-Orbitrap. J. Pharm. Biomed. Anal. 201, 114107.
Sampath, C., Haug, K., Thanei, S., Hamburger, M., Derendorf, H., Frye, R., Butterweck, V., 2012. Pharmacokinetics of valerenic acid in rats after intravenous and oral administrations. Planta Med 78, 575–581.
Sanada, K., Nakajima, S., Kurokawa, S., Barceló-Soler, A., Ikuse, D., Hirata, A., Yoshizawa, A., Tomizawa, Y., Salas-Valero, M., Noda, Y., Mimura, M., Iwanami, A., Kishimoto, T., 2020. Gut microbiota and major depressive disorder: a systematic review and meta-analysis. J. Affect. Disord. 266, 1–13.
Sanders, M.E., 2016. Probiotics and microbiota composition. BMC Med 14, 82.
Sarris, J., 2018. Herbal medicines in the treatment of psychiatric disorders: 10-year updated review. Phyther. Res. 32, 1147–1162.
Sarris, J., Byrne, G.J., Bousman, C.A., Cribb, L., Savage, K.M., Holmes, O., Murphy, J., Macdonald, P., Short, A., Nazareth, S., Jennings, E., Thomas, S.R., Ogden, E., Chamoli, S., Scholey, A., Stough, C., 2020. Kava for generalised anxiety disorder: a 16-week double-blind, randomised, placebo-controlled study. Aust. New Zeal. J. Psychiatry 54, 288–297.
Sarris, J., McIntyre, E., Camfield, D.A., 2013. Plant-Based Medicines for Anxiety Disorders, Part 2: a Review of Clinical Studies with Supporting Preclinical Evidence. CNS Drugs 27, 301–319.
Sarris, J., Panossian, A., Schweitzer, I., Stough, C., Scholey, A., 2011. Herbal medicine for depression, anxiety and insomnia: a review of psychopharmacology and clinical evidence. Eur. Neuropsychopharmacol. 21, 841–860.
Sayed, A.M., Morsy, S., Tawfik, G.M., Naveed, S., Minh-Duc, N.T., Hieu, T.H., Ali, Z.A., Shinkar, A., Doheim, M.F., Hashan, M.R., Huy, N.T., 2020. The best route of administration of lavender for anxiety: a systematic review and network meta-analysis. Gen. Hosp. Psychiatry 64, 33–40.
Schulz, H.U., Schürer, M., Bässler, D., Weiser, D., 2005. Investigation of the bioavailability of hypericin, pseudohypericin, hyperforin and the flavonoids quercetin and isorhamnetin following single and multiple oral dosing of a hypericum extract containing tablet. Arzneimittel-Forschung/Drug Res 55, 15–22.
Schulz, V., 2006. Safety of St. John's Wort extract compared to synthetic antidepressants. Phytomedicine 13, 199–204.
Sciascia, Q., Daş, G., Metges, C.C., 2016. Review: the pig as a model for humans: effects of nutritional factors on intestinal function and health. J. Anim. Sci. 94, 441–452.
Seifritz, E., Hatzinger, M., Holsboer-Trachsler, E., 2016. Efficacy of Hypericum extract WS ® 5570 compared with paroxetine in patients with a moderate major depressive episode – a subgroup analysis. Int. J. Psychiatry Clin. Pract. 20, 126–132.
Shakeri, A., Sahebkar, A., Javadi, B., 2016. Melissa officinalis L. – A review of its traditional uses, phytochemistry and pharmacology. J. Ethnopharmacol. 188, 204–228.
Shakya, R., Nepal, M.R., Kang, M.J., Jeong, T.C., 2020. Effects of intestinal microbiota on pharmacokinetics of crocin and crocetin in male sprague-dawley rats. Metabolites 10, 424.
Shammas, R.L., Marks, C.E., Broadwater, G., Le, E., Glener, A.D., Sergesketter, A.R., Cason, R.W., Rezak, K.M., Phillips, B.T., Hollenbeck, S.T., 2021. The Effect of Lavender Oil on Perioperative Pain, Anxiety, Depression, and Sleep after Microvascular Breast Reconstruction: a Prospective, Single-Blinded, Randomized, Controlled Trial. J. Reconstr. Microsurg. 37, 530–540.
Sharkey, K.A., Beck, P.L., McKay, D.M., 2018. Neuroimmunophysiology of the gut: advances and emerging concepts focusing on the epithelium. Nat. Rev. Gastroenterol. Hepatol. 15, 765–784.
Shi, T., Bian, X., Yao, Z., Wang, Y., Gao, W., Guo, C., 2020. Quercetin improves gut dysbiosis in antibiotic-treated mice. Food Funct 11, 8003–8013.
Simpson, C.A., Diaz-Arteche, C., Eliby, D., Schwartz, O.S., Simmons, J.G., Cowan, C.S.M., 2021. The gut microbiota in anxiety and depression – a systematic review. Clin. Psychol. Rev. 83 ,101943.
Slavich, G.M., Irwin, M.R., 2014. From stress to inflammation and major depressive disorder: a social signal transduction theory of depression. Psychol. Bull. 140, 774–815.
Slykerman, R.F., Hood, F., Wickens, K., Thompson, J.M.D., Barthow, C., Murphy, R., Kang, J., Rowden, J., Stone, P., Crane, J., Stanley, T., Abels, P., Purdie, G., Maude, R., Mitchell, E.A., 2017. Effect of Lactobacillus rhamnosus HN001 in Pregnancy on Postpartum Symptoms of Depression and Anxiety: a Randomised Double-blind Placebo-controlled Trial. EBioMedicine 24, 159–165.
Stanley, P.F., Wan, L.F., Karim, R.A., 2020. A Randomized Prospective Placebo-Controlled Study of the Effects of Lavender Aromatherapy on Preoperative Anxiety in Cataract Surgery Patients. J. PeriAnesthesia Nurs. 35, 403–406.
Stoupi, S., Williamson, G., Drynan, J.W., Barron, D., Clifford, M.N., 2010. A comparison of the in vitro biotransformation of (-)-epicatechin and procyanidin B2 by human faecal microbiota. Mol. Nutr. Food Res. 54, 747–759.
Strandwitz, P., Kim, K.H., Terekhova, D., Liu, J.K., Sharma, A., Levering, J., McDonald, D., Dietrich, D., Ramadhar, T.R., Lekbua, A., Mroue, N., Liston, C., Stewart, E.J., Dubin, M.J., Zengler, K., Knight, R., Gilbert, J.A., Clardy, J., Lewis, K., 2019. GABA-modulating bacteria of the human gut microbiota. Nat. Microbiol 4, 396–403.
Štulíková, K., Karabín, M., Nešpor, J., Dostálek, P., 2018. Therapeutic perspectives of 8-prenylnaringenin, a potent phytoestrogen from hops. Molecules 23, 660.
Suez, J., Zmora, N., Segal, E., Elinav, E., 2019. The pros, cons, and many unknowns of probiotics. Nat. Med. 25, 716–729.
Süntar, I., Oyardı, O., Akkol, E.K., Ozçelik, B., 2016. Antimicrobial effect of the extracts from Hypericum perforatum against oral bacteria and biofilm formation. Pharm. Biol. 54, 1065–1070.
Szegedi, A., Kohnen, R., Dienel, A., Kieser, M., 2005. Acute treatment of moderate to severe depression with hypericum extract WS 5570 (St John's wort): randomised controlled double blind non-inferiority trial versus paroxetine. BMJ 330, 503.
Takahashi, K., Nakagawasai, O., Nemoto, W., Odaira, T., Sakuma, W., Onogi, H., Nishijima, H., Furihata, R., Nemoto, Y., Iwasa, H., Tan-No, K., Tadano, T., 2019. Effect of Enterococcus faecalis 2001 on colitis and depressive-like behavior in dextran sulfate sodium-treated mice: involvement of the brain–gut axis. J. Neuroinflammation 16, 201.
Tan, S., Caparros-Martin, J.A., Matthews, V.B., Koch, H., O'Gara, F., Croft, K.D., Ward, N. C., 2018. Isoquercetin and inulin synergistically modulate the gut microbiome to prevent development of the metabolic syndrome in mice fed a high fat diet. Sci. Rep. 8, 10100.
Taylor, A.M., Thompson, S.V., Edwards, C.G., Musaad, S.M.A., Khan, N.A., Holscher, H. D., 2020. Associations among diet, the gastrointestinal microbiota, and negative emotional states in adults. Nutr. Neurosci. 23, 983–992.
Thumann, T.A., Pferschy-Wenzig, E.-M., Moissl-Eichinger, C., Bauer, R., 2019. The role of gut microbiota for the activity of medicinal plants traditionally used in the European Union for gastrointestinal disorders. J. Ethnopharmacol. 245, 112153.
Tian, P., Wang, G., Zhao, J., Zhang, H., Chen, W., 2019. Bifidobacterium with the role of 5-hydroxytryptophan synthesis regulation alleviates the symptom of depression and related microbiota dysbiosis. J. Nutr. Biochem. 66, 43–51.
Tomas-Barberan, F.A., Selma, M.V., Espín, J.C., 2018. Polyphenols' Gut Microbiota Metabolites: bioactives or Biomarkers? J. Agric. Food Chem. 66, 3593–3594.
Tremmel, M., Kiermaier, J., Heilmann, J., 2021. In vitro metabolism of six c-glycosidic flavonoids from passiflora incarnata l. Int. J. Mol. Sci. 22, 6566.
Umigai, N., Murakami, K., Ulit, M.V., Antonio, L.S., Shirotori, M., Morikawa, H., Nakano, T., 2011. The pharmacokinetic profile of crocetin in healthy adult human volunteers after a single oral administration. Phytomedicine 18, 575–578.
Uzun, S., Kozumplik, O., Jakovljević, M., Sedić, B., 2010. Side effects of treatment with benzodiazepines. Psychiatr. Danub. 22, 90–93.
Valles-Colomer, M., Falony, G., Darzi, Y., Tigchelaar, E.F., Wang, J., Tito, R.Y., Schiweck, C., Kurilshikov, A., Joossens, M., Wijmenga, C., Claes, S., Van Oudenhove, L., Zhernakova, A., Vieira-Silva, S., Raes, J., 2019. The neuroactive potential of the human gut microbiota in quality of life and depression. Nat. Microbiol 4, 623–632.
Verjee, S., Kelber, O., Kolb, C., Abdel-Aziz, H., Butterweck, V., 2019. Permeation characteristics of hypericin across Caco-2 monolayers in the presence of single flavonoids, defined flavonoid mixtures or Hypericum extract matrix. J. Pharm. Pharmacol. 71, 58–69.
Vissiennon, C., Nieber, K., Kelber, O., Butterweck, V., 2012. Route of administration determines the anxiolytic activity of the flavonols kaempferol, quercetin and myricetin — are they prodrugs? J. Nutr. Biochem. 23, 733–740.
Wan, S.-Z., Liu, C., Huang, C.-K., Luo, F.-Y., Zhu, X., 2019. Ursolic Acid Improves Intestinal Damage and Bacterial Dysbiosis in Liver Fibrosis Mice. Front. Pharmacol. 10, 1321.
Wan, S., Huang, C., Wang, A., Zhu, X., 2020. Ursolic acid improves the bacterial community mapping of the intestinal tract in liver fibrosis mice. PeerJ 8, e9050.
Wang, L., Sun, Y., Zhao, T., Li, Y., Zhao, X., Zhang, L., Wu, L., Zhang, L., Zhang, T., Wei, G., Altamirano, A., Tong, Y., Yan, Z., 2020. Antidepressant Effects and Mechanisms of the Total Iridoids of Valeriana jatamansi on the Brain-Gut Axis. Planta Med 86, 172–179.
Wang, L., Zhang, Y., Fan, G., Ren, J., Zhang, L., Pan, S., 2019. Effects of orange essential oil on intestinal microflora in mice. J. Sci. Food Agric. 99, 4019–4028.
Wang, P.S., Aguilar-Gaxiola, S., Alonso, J., Angermeyer, M.C., Borges, G., Bromet, E.J., Bruffaerts, R., de Girolamo, G., de Graaf, R., Gureje, O., Haro, J.M., Karam, E.G., Kessler, R.C., Kovess, V., Lane, M.C., Lee, S., Levinson, D., Ono, Y., Petukhova, M., Posada-Villa, J., Seedat, S., Wells, J.E., 2007. Use of mental health services for anxiety, mood, and substance disorders in 17 countries in the WHO world mental health surveys. Lancet 370, 841–850.
Wang, Y., Eans, S.O., Stacy, H.M., Narayanapillai, S.C., Sharma, A., Fujioka, N., Haddad, L., McLaughlin, J., Avery, B.A., Xing, C., 2018. A stable isotope dilution tandem mass spectrometry method of major kavalactones and its applications. PLoS ONE 13, e0197940.

Warren, M.B., Cowen, P.J., Harmer, C.J., 2019. Subchronic treatment with St John's wort produces a positive shift in emotional processing in healthy volunteers. J. Psychopharmacol. 33, 194–201.
Weng, L., Guo, X., Li, Y., Yang, X., Han, Y., 2016. Apigenin reverses depression-like behavior induced by chronic corticosterone treatment in mice. Eur. J. Pharmacol. 774, 50–54.
White, C.M., 2018. The Pharmacology, Pharmacokinetics, Efficacy, and Adverse Events Associated With Kava. J. Clin. Pharmacol. 58, 1396–1405.
WHO Regional Office for South-East Asia, 2017. Self Care For Health.
Williamson, E., 2001. Synergy and other interactions in phytomedicines. Phytomedicine 8, 401–409.
Willis, A.D., 2019. Rarefaction. Alpha Diversity, and Statistics. Front. Microbiol. 10, 2407.
Wilson, I.D., Nicholson, J.K., 2017. Gut microbiome interactions with drug metabolism, efficacy, and toxicity. Transl. Res. 179, 204–222.
Wiste, A.K., Arango, V., Ellis, S.P., Mann, J.J., Underwood, M.D., 2008. Norepinephrine and serotonin imbalance in the locus coeruleus in bipolar disorder. Bipolar Disord 10, 349–359.
World Health Organization, 2017. Depression and Other Common Mental Disorders: Global Health Estimates.
Wu, D.-N., Guan, L., Jiang, Y.-X., Ma, S.-H., Sun, Y.-N., Lei, H.-T., Yang, W.-F., Wang, Q.-F., 2019. Microbiome and metabonomics study of quercetin for the treatment of atherosclerosis. Cardiovasc. Diagn. Ther. 9, 545–560.
Xi, L., Qian, Z., Du, P., Fu, J., 2007. Pharmacokinetic properties of crocin (crocetin digentiobiose ester) following oral administration in rats. Phytomedicine 14, 633–636.
Xiao, Q., Shu, R., Wu, C., Tong, Y., Xiong, Z., Zhou, J., Yu, C., Xie, X., Fu, Z., 2020. Crocin-I alleviates the depression-like behaviors probably via modulating "microbiota-gut-brain" axis in mice exposed to chronic restraint stress. J. Affect. Disord. 276, 476–486.
Xie, J., Song, W., Liang, X., Zhang, Q., Shi, Y., Liu, W., Shi, X., 2020. Protective effect of quercetin on streptozotocin-induced diabetic peripheral neuropathy rats through modulating gut microbiota and reactive oxygen species level. Biomed. Pharmacother. 127, 110147.
Xie, X., Xiao, Q., Xiong, Z., Yu, C., Zhou, J., Fu, Z., 2019. Crocin-I ameliorates the disruption of lipid metabolism and dysbiosis of the gut microbiota induced by chronic corticosterone in mice. Food Funct 10, 6779–6791.
Xie, Z., Lu, H., Yang, S., Zeng, Y., Li, W., Wang, L., Luo, G., Fang, F., Zeng, T., Cheng, W., 2020. Salidroside Attenuates Cognitive Dysfunction in Senescence-Accelerated Mouse Prone 8 (SAMP8) Mice and Modulates Inflammation of the Gut-Brain Axis. Front. Pharmacol. 11, 568423.
Xing, Y.-W., Lei, G.-T., Wu, Q.-H., Jiang, Y., Huang, M.-X., 2019. Procyanidin B2 protects against diet-induced obesity and non-alcoholic fatty liver disease *via* the modulation of the gut microbiota in rabbits. World J. Gastroenterol. 25, 955–966.
Xue, B., Xie, J., Huang, J., Chen, L., Gao, L., Ou, S., Wang, Y., Peng, X., 2016. Plant polyphenols alter a pathway of energy metabolism by inhibiting fecal Bacteroidetes and Firmicutes in vitro. Food Funct 7, 1501–1507.
Yamashita, M., Fukizawa, S., Nonaka, Y., 2020. Hop-derived prenylflavonoid isoxanthohumol suppresses insulin resistance by changing the intestinal microbiota and suppressing chronic inflammation in high fat diet-fed mice. Eur. Rev. Med. Pharmacol. Sci. 1537–1547.
Yang, J., Qian, D., Guo, J., Jiang, S., Shang, E., Duan, J., Xu, J., 2013. Identification of the major metabolites of hyperoside produced by the human intestinal bacteria using the ultra performance liquid chromatography/quadrupole-time-of-flight mass spectrometry. J. Ethnopharmacol. 147, 174–179.
Yang, S., Wang, G., Ma feei, Z., Qin, L., Zhai, Y., Yu, Z., Xue, M., Zhang, Y., Wan, Z., 2020a. Dietary Advanced Glycation End Products–InducedCognitive Impairment in Aged ICR Mice: protective Role of Quercetin. Mol. Nutr. Food Res. 64 e1901019.
Yang, S., Zhang, Y., Li, W., You, B., Yu, J., Huang, X., Yang, R., 2021. Gut Microbiota Composition Affects Procyanidin A2-Attenuated Atherosclerosis in ApoE-/-Mice by Modulating the Bioavailability of Its Microbial Metabolites. J. Agric. Food Chem. 69, 6989–6999.
Yang, S., Zhou, H., Wang, G., Zhong, X., Shen, Q., Zhang, X., Li, R., Chen, L., Zhang, Y., Wan, Z., 2020. Quercetin is protective against short-term dietary advanced glycation end products intake induced cognitive dysfunction in aged ICR mice. J. Food Biochem. 44 e13164.
Yap, W.S., Dolzhenko, A.V., Jalal, Z., Hadi, M.A., Khan, T.M., 2019. Efficacy and safety of lavender essential oil (Silexan) capsules among patients suffering from anxiety disorders: a network meta-analysis. Sci. Rep. 9, 18042.
Yechiam, E., Ben-Eliezer, D., Ashby, N.J.S., Bar-Shaked, M., 2019. The acute effect of Hypericum perforatum on short-term memory in healthy adults. Psychopharmacology (Berl) 236, 613–623.
Yi, L.-T., Li, J.-M., Li, Y.-C., Pan, Y., Xu, Q., Kong, L.-D., 2008. Antidepressant-like behavioral and neurochemical effects of the citrus-associated chemical apigenin. Life Sci 82, 741–751.
Yu, J., Guo, H., Xie, J., Luo, J., Li, Y., Liu, L., Ou, S., Zhang, G., Peng, X., 2019. The Alternate Consumption of Quercetin and Alliin in the Traditional Asian Diet Reshaped Microbiota and Altered Gene Expression of Colonic Epithelial Cells in Rats. J. Food Sci. 84, 678–686.
Yu, S., Liu, L., Wen, T., Liu, Y., Wang, D., He, Y., Liang, Y., Liu, X., Xie, L., Wang, G., Wei, W., 2008. Development and validation of a liquid chromatographic/electrospray ionization mass spectrometric method for the determination of salidroside in rat plasma: application to the pharmacokinetics study. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 861, 10–15.
Yuan, L., Li, X., He, S., Gao, C., Wang, C., Shao, Y., 2018. Effects of Natural Flavonoid Isoorientin on Growth Performance and Gut Microbiota of Mice. J. Agric. Food Chem. 66, 9777–9784.
Yuan, M., Shi, D.-Z., WANG, T.-Y., ZHENG, S.-Q., Liu, L.-J., Sun, Z.-X., Wang, R.-F., Ding, Y., 2016. Transformation of trollioside and isoquercetin by human intestinal flora in vitro. Chin. J. Nat. Med. 14, 220–226.
Yuan, Y., Wu, X., Zhang, X., Hong, Y., Yan, H., 2019. Ameliorative effect of salidroside from Rhodiola Rosea L. on the gut microbiota subject to furan-induced liver injury in a mouse model. Food Chem. Toxicol. 125, 333–340.
Zenger, K., Agnolet, S., Schneider, B., Kraus, B., 2015. Biotransformation of Flavokawains A, B, and C, Chalcones from Kava (Piper methysticum), by Human Liver Microsomes. J. Agric. Food Chem. 63, 6376–6385.
Zhai, Q., Feng, S., Arjan, N., Chen, W., 2019. A next generation probiotic, *Akkermansia muciniphila*. Crit. Rev. Food Sci. Nutr. 59, 3227–3236.
Zhang, M., Hu, Z., Fang, B., Bao, X., Xiang, Z., Wang, H., 2019. Pharmacokinetic study of rosavin in rat plasma with ultra performance LC-MS/MS after intravenous and gavage administration. Bioanalysis 11, 837–845.
Zhang, Q., Yun, Y., An, H., Zhao, W., Ma, T., Wang, Z., Yang, F., 2021. Gut Microbiome Composition Associated With Major Depressive Disorder and Sleep Quality. Front. Psychiatry 12, 645045.
Zhang, L., Liu, C., Yuan, M., 2020. Eriodictyol produces antidepressant-like effects and ameliorates cognitive impairments induced by chronic stress. Neuroreport 31, 1111–1120.
Zhang, T., Lu, S.H., Bi, Q., Liang, L., Wang, Y.F., Yang, X.X., Gu, W., Yu, J., 2017. Volatile Oil from Amomi Fructus Attenuates 5-Fluorouracil-Induced Intestinal Mucositis. Front. Pharmacol. 8, 786.
Zhang, W., Gan, D., Jian, J., Huang, C., Luo, F., Wan, S., Jiang, M., Wan, Y., Wang, A., Li, B., Zhu, X., 2019b. Protective effect of ursolic acid on the intestinal mucosal barrier in a rat model of liver fibrosis. Front. Physiol. 10, 1–11.
Zhang, X., Bu, H., Jiang, Y., Sun, G., Jiang, R., Huang, X., Duan, H., Huang, Z., Wu, Q., 2019. The antidepressant effects of apigenin are associated with the promotion of autophagy via the mTOR/AMPK/ULK1 pathway. Mol. Med. Rep. 20, 2867–2874.
Zhang, Y., Bobe, G., Revel, J.S., Rodrigues, R.R., Sharpton, T.J., Fantacone, M.L., Raslan, K., Miranda, C.L., Lowry, M.B., Blakemore, P.R., Morgun, A., Shulzhenko, N., Maier, C.S., Stevens, J.F., Gombart, A.F., 2020. Improvements in Metabolic Syndrome by Xanthohumol Derivatives Are Linked to Altered Gut Microbiota and Bile Acid Metabolism. Mol. Nutr. Food Res. 64, e1900789.
Zhang, Y., Geng, J., Hong, Y., Jiao, L., Li, S., Sun, R., Xie, Y., Yan, C., Aa, J., Wang, G., 2019. Orally Administered Crocin Protects Against Cerebral Ischemia/Reperfusion Injury Through the Metabolic Transformation of Crocetin by Gut Microbiota. Front. Pharmacol. 10, 440.
Zhang, Y., Li, L., Lin, L., Liu, J., Zhang, Z., Xu, D., Xiang, F., 2013. Pharmacokinetics, tissue distribution, and excretion of salidroside in rats. Planta Med 79, 1429–1433.
Zhang, Z., Shen, P., Lu, X., Li, Y., Liu, J., Liu, B., Fu, Y., Cao, Y., Zhang, N., 2017. In Vivo and In Vitro Study on the Efficacy of Terpinen-4-ol in Dextran Sulfate Sodium-Induced Mice Experimental Colitis. Front. Immunol. 8, 558.
Zheng, P., Zeng, B., Zhou, C., Liu, M., Fang, Z., Xu, X., Zeng, L., Chen, J., Fan, S., Du, X., Zhang, X., Yang, D., Yang, Y., Meng, H., Li, W., Melgiri, N.D., Licinio, J., Wei, H., Xie, P., 2016. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. Mol. Psychiatry 21, 786–796.
Zheng, S., Geng, D., Liu, S.S., Wang, Q., Liu, S.S., Wang, R., 2019. A newly isolated human intestinal bacterium strain capable of deglycosylating flavone C-glycosides and its functional properties. Microb. Cell Fact. 18, 94.
Zorić, Z., Markić, J., Pedisić, S., Bučevic-Popović, V., Generalic-Mekinić, I., Grebenar, K., Kulišić-Bilušic, T., 2016. Stability of rosmarinic acid in aqueous extracts from different lamiaceae species after in vitro digestion with human gastrointestinal enzymes. Food Technol. Biotechnol. 54, 97–102.
Zou, L., Harkey, M.R., Henderson, G.L., 2005. Synthesis, in vitro reactivity, and identification of 6-phenyl-3-hexen-2- one in human urine after kava-kava (Piper methysticum) ingestion. Planta Med 71, 142–146.

Supplementary materials to:

Publication No. 1

Phytotherapy of mood disorders in the light of microbiota-gut-brain axis.

Maciej Korczak [a], Maciej Pilecki [b], Sebastian Granica [a], Aleksandra Gorczyńska [a], Karolina Aleksandra Pawłowska [a], Jakub Patryk Piwowarski [a]

[a] Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland

[b] Department of Psychiatry, Collegium Medicum, Jagiellonian University, Cracow, Poland

Phytomedicine. 2023 Mar

111:154642

Phytotherapy of mood disorders in the light of microbiota-gut-brain axis.

Maciej Korczak[1], Maciej Pilecki[2], Sebastian Granica[1], Aleksandra Gorczyńska[1], Karolina A. Pawłowska[1], Jakub P. Piwowarski[1*]

[1]Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland

[2]Department of Psychiatry, Collegium Medicum, Jagiellonian University, Cracow

*Corresponding author: Jakub P. Piwowarski, ul. Banacha 1, 02-097 Warsaw, Poland, tel/fax +48 22 5720985, jpiwowarski@wum.edu.pl

Table S1. Antimicrobial properties

Plant	Type of extract/ standardization/ isolated principle	Test model	Antimicrobial effect (MIC)		Dose	Ref.
Hypericum perforatum			**A.**	**B.**		
	A. 96% ethanolic extract of aerial parts of St. John's wort **B.** Aqueous extract of aerial parts of St. John's wort	*L. plantarum (G+,C)*	32 µg/ml	8 µg/ml	bacterial suspension incubated with tested extract concentration ranging from 0.78125 to 100 µg/mL	(Süntar et al., 2016)
		E. faecalis (G+,O)	32 µg/ml	16 µg/ml		
		S. mutans (G+,P)	64 µg/ml	32 µg/ml		
			A.	**B.**		
	A. 30% ethanolic extract of St. John's wort obtained at 170W of microwave power **B.** 50% ethanolic extract of St. John's wort waste powder extracted in the dark at room temperature for 72h	*S. aureus* (G+,O)	10 000 µg/mL	10 000 µg/mL	microbial suspension incubated with tested extract concentration ranging from 16 to 20 000 µg/mL	(Milutinović et al., 2021)
		L. monocytogenes (G+,P)	2500 µg/mL	2500 µg/mL		
		E. faecalis (G+,O)	5000 µg/mL	5000 µg/mL		
		E. coli (G-,O)	(-)	(-)		
		P. aeruginosa (G-,O)	5000 µg/mL	5000 µg/mL		
		L. rhamnosus ATCC 7469 (G+,C)	(-)	20 000 µg/mL		
		L. rhamnosus GG (G+,C)	(-)	10 000 µg/mL		
		L. plantarum (G+,C)	(-)	(-)		
		C. albicans (O)	(-)	(-)		

	Essential oil (obtained with hydrodistillation in 100°C in Clevenger-type apparatus)	*S. aureus* (G+,O)	5000 µg/mL	microbial suspension incubated with oil sample in acetone at concentrations ranging from 10 to 0.001 mg/mL	Maggi et al., 2010)
		E. faecalis (G+,O)	5000 µg/mL		
		B. subtilis (G+,C)	155 µg/mL		
		E. coli (G-,O)	5000 µg/mL		
		C. albicans (O)	310 µg/mL		
		B. cereus (G+,P)	250 µg/mL	microbial suspension incubated with oil sample at concentrations ranging from 16 to 500 µg/mL	Pirbalouti et al., 2014)
		L.monocytogenes (G+,P)	250 µg/mL		
		P. aeruginosa (G-,O)	250 µg/mL		
		S. typhimurium (G-,P)	500 µg/mL		
	70% ethanolic extract of St. John's wort	*A. odontolyticus* (G+, O)	37.5 µg/mL	bacterial suspension incubated with 300, 150, 75 and 37.5 µg/ml of active extracts	(Tambur et al., 2020)
		S. mitis (G+,O)	(-)		
		S. sanguinis (G+, O)	37.5 µg/mL		
		L. acidophilus (G+,C)	(-)		
		S. mutans (G+,P)	300 µg/mL		
		E. corrodens (G-,O)	37.5 µg/mL		
		F. nucleatum (G-,P)	37.5 µg/mL		
	Aqueous infusion from St. John's Wort	*S. aureus (G+,O)*	1.3-2.5[1] µg/mL	microbial suspension	(Weseler & Saller, 2001)
		MRSA (G+,P)	1.3 µg/mL		

(aqueous extract obtained with boiling water)	*S. epidermidis* (G+,P)	2.5 μg/mL[1]	incubated with St. John's Wort aqueous infusions
	E. faecalis (G+,O)	5.0 μg/mL	
	E. faecium (G+,O)	5.0 μg/mL	
	E. durans (G+,C)	5.0-10.0 μg/mL[1]	
	L.monocytogenes (G+,P)	1.3 μg/mL	
	S. pyogenes (G+,O)	7.5 μg/mL[1]	
	K. aerogenes (G-,O)	(-)	
	P. aeruginosa (G-,O)	(-)	
	E. coli (G-,O)	(-)	
	S. choleraesuis (G-,P)	(-)	
	K. pneumoniae (G-,O)	30.0 μg/mL	
	C. freundii (G-,O)	(-)	
	S. flexneri (G-,P)	(-)	
	C. albicans (O)	(-)	
Hyperforin	*S. aureus* (G+,O)	0.1 μg/mL	microbial suspension incubated with acetonic extract of hyperforin at concentrations
	S. lutea (G+,O)	0.1 μg/mL	
	B. subtilis (G+,C)	0.2 μg/mL	
	S. faecalis (G+,O)	1.0 μg/mL	
	E. coli (G-,O)	400 μg/mL	

		P. vulgaris (G-,O)	400 µg/mL	ranging from 0.1 to 400 µg/mL	
		C. albicans (O)	400 µg/mL		
Valeriana officinalis	Essential oil (hydrodistillation of valerian root at 100°C)	*B. subtilis* (G+,C)	62.5 µg/mL	microbial suspension incubated with oil sample in acetone at concentrations ranging from 0.2 to 4.0 mg/mL	(Wang et al., 2010)
		S. aureus (G+,O)	200 µg/mL		
		S. haemolyticus (G+,O)	62.5 µg/mL		
		E. coli (G-,O)	200 µg/mL		
		S. typhimurium (G-,P)	200 µg/mL		
		C. albicans (O)	200 µg/mL		
Lavandula officinalis	EO (obtained with hydrodistillation of flowering tops)	*B. subtilis* (G+,C)	4.0 µg/mL	bacterial suspension incubated with essential oil solution at concentrations ranging from 0.02 to 15.0 µg/mL	(Sokovicǵ et al., 2010)
		S. epidermidis (G+,P)	4.0 µg/mL		
		S. aureus (G+,O)	5.0 µg/mL		
		S. enteritidis (G-,P)	5.0 µg/mL		
		S. typhimurium (G-,P)	5.0 µg/mL		
		E. coli (G-,O)	6.0 µg/mL		
		E. cloacae (G-,O)	6.0 µg/mL		
		P. mirabilis (G-,P)	7.0 µg/mL		
		P. aeruginosa (G-,O)	7.0 µg/mL		
		L.monocytogenes (G+,P)	5.5 µg/mL		
	EO	*(MRSA)* (G+,P)	2.00 mg/mL		

	(MGRSA) (G+,P)		2.00 mg/mL		microbial suspension incubated with essential oil solution at concentrations ranging from 0.0625 to 8 mg/mL	(Rapper et al., 2013)
	S. aureus (G+,O)		2.00 mg/mL			
	S. epidermidis (G+,P)		2.00 mg/mL			
	Vancomycin-resistant E. faecalis (G+,P)		2.00 mg/mL			
	E. faecalis (G+,O)		2.00 mg/mL			
	K. pneumoniae (G-,O)		1.50mg/mL			
	P. aeruginosa (G-,O)		2.00 mg/mL			
	C. tropicalis (O)		0.75 mg/mL			
	C. albicans (O)		3.00 mg/mL			
		A.	**B.**	**C.**		
A. EO (obtained with hydrodistillation) **B.** Linalool **C.** Linalyl acetate	*B. cereus* (G+,P)	3.0 mg/mL	2.5 mg/mL	4.0 mg/mL	microbial suspension incubated with: **A.** essential oil solution at concentrations ranging from 0.05 to 50.0 mg/mL **B.** linalool concentrations ranging from 0.023 to 0.75 mg/L	(Bla et al., 2018)
	E. faecalis (G+,O)	2.0 mg/mL	2.5 mg/mL	5.0 mg/mL		
	L. monocytogenes (G+,P)	1.0 mg/mL	0.75 mg/mL	5.0 mg/mL		
	S. aureus (G+,O)	2.0 mg/mL	1.5 mg/mL	2.5 mg/mL		
	S. pyogenes (G+,O)	1.0 mg/mL	0.75 mg/mL	2.5 mg/mL		
	E. coli (G-,O)	1.0 mg/mL	0.5 mg/mL	5.0 mg/mL		
	K. oxytoca (G-,O)	2.5 mg/mL	1.5 mg/mL	25.0 mg/mL		

		K. pneumoniae (G-,O)	1.5 mg/mL	1.5 mg/mL	25.0 mg/mL	**C.** linalyl acetate concentrations ranging from 0.039 to 1.25 mg/L	
		P. mirabilis (G-,P)	2.0 mg/mL	1.5 mg/mL	1.5 mg/mL		
		P. aeruginosa (G-,O)	0.5 mg/mL	0.5 mg/mL	5.0 mg/mL		
		S. enteritidis (G-,P)	1.5 mg/mL	0.75 mg/mL	20.0 mg/mL		
		Y. enterocolitica (G-,P)	2.0 mg/mL	1.5 mg/mL	10.0 mg/mL		
		C. albicans (O)	0.75 mg/mL	0.75 mg/mL	15.0 mg/mL		
		C. glabrata (O)	0.5 mg/mL	0.5 mg/mL	1.5 mg/mL		
		C. tropicalis (O)	0.5 mg/mL	0.5 mg/mL	0.75 mg/mL		
		H. anomala (O)	0.75 mg/mL	0.75 mg/mL	15.0 mg/mL		
		S. capitata (O)	1.5 mg/mL	1.5 mg/mL	20.0 mg/mL		
Piper methysticum	Aqueous extract from leaves	*S. aureus (G+,O)*		0 mm*		filter disk saturated with a 100 µg/mL of the extract placed on medium agar plate with the test bacteria	(Locher et al., 1995)
		S. pyogenes (G+,O)		0 mm*			
		E. coli (G-,O)		0 mm*			
		P. aeruginosa (G-,O)		0 mm*			
	Hydroalcoholic rhizome extract	*S. aureus (G+,O)*		1 µg/mL		bacterial suspension incubated with extract	(Pereira et al., 2015)

		E. coli (G-,O)		125 µg/mL			concentration ranging from 15.6 µg/mL to 2 mg/mL	
Humulus lupulus			**A.**	**B.**	**C.**	**D.**		
	A. Commercial CO2 hops extract (containing 47% of α-bitter acids and 27% of β-bitter acids) **B.** α-bitter acids **C.** β-bitter acids **D.** Xanthohumol	*S. aureus (G+,O)*	7.5 µg/mL	30 µg/mL	0.5 µg/mL	4 µg/mL	The microbial suspension incubated with hop fractions' solutions at concentrations ranging from 0.1-1000 mg/L	(Bogdanova et al., 2018)
		E. faecalis (G+,O)	60 µg/mL	60 µg/mL	15 µg/mL	7.5 µg/mL		
		E. coli (G-,O)	(-)	(-)	(-)	(-)		
		P. aeruginosa (G-,O)	(-)	(-)	(-)	(-)		
		MRSA (G+,P)	7.5 µg/mL	60 µg/mL	1 µg/mL	4 µg/mL		
		vancomycin-resistant E. faecium VanA (G+,P)	30 µg/mL	60 µg/mL	15 µg/mL	7.5 µg/mL		
		fluoroquinolone-resistant S. haemolyticus FQR (G+)	15 µg/mL	30 µg/mL	1 µg/mL	7.5 µg/mL		
		fluoroquinolone-resistant E. coli (G-)	(-)	(-)	(-)	(-)		
		fluoroquinolone-resistant P. aeruginosa (G-)	(-)	(-)	(-)	(-)		
		C. albicans (O)	250 µg/mL	250 µg/mL	500 µg/mL	60 µg/mL		
		C. tropicalis (O)	1000 µg/mL	1000 µg/mL	(-)	30 µg/mL		

	C. parapsilosis (O)	500 µg/mL	500 µg/mL	1000 µg/mL	7.5 µg/mL		
Hydro-ethanolic crude extracts of hops' cones	*E. faecalis* (G+,O)		39 µg/mL			The microbial suspension incubated with hop cones crude extract at concentrations ranging from 3.9-1250 mg/L	(Bocquet et al., 2019)
	Enterococcus sp. (G+,O)		156 µg/mL				
	S. aureus 8146 (G+,O)		39 µg/mL				
	S. aureus 8147 (G+,O)		39 µg/mL				
	S. epidermidis 5001 (G+,P)		39 µg/mL				
	S. epidermidis 10282 (G+,P)		98 µg/mL				
	S. lugdunensis (G+,P)		156 µg/mL				
	S. agalactiae T25-7 (G+,O)		39 µg/mL				
	S. agalactiae T53C2 (G+,O)		78 µg/mL				
	S. dysgalactiae (G+,O)		39 µg/mL				
	E. cloacae 11050 (G-,O)		(-)				
	E. cloacae 11051 (G-,O)		(-)				
	E. cloacae 11053 (G-,O)		(-)				
	E. coli 8138(G-,O)		(-)				
	E. coli 8157(G-,O)		(-)				

		E. coli ATCC 25922 (G-,O)	(-)		
		K. pneumoniae 11016 (G-,O)	(-)		
		K. pneumoniae 11017 (G-,O)	(-)		
		P. stuartii (G-,O)	(-)		
		P. aeruginosa 8131 (G-,O)	(-)		
		P. aeruginosa ATCC 27583 (G-,O)	(-)		
		S. marcescens 11056 (G-,P)	(-)		
		S. marcescens 11056 (G-,P)	(-)		
		C. albicans 13203 (O)	156 µg/mL		
		C. albicans ATCC 10231 (O)	<39 µg/mL		
Melissa officinalis	Essential oil obtained with water distillation	*S. aureus (G+,O)*	3 µL/mL	microbial suspension incubated with essential oil samples at concentrations ranging from 0.1 to 50 µL/mL	(Abdellatif et al., 2014)
		B. subtilis (G+,C)	2 µL/mL		
		L. monocytogenes (G+,P)	2 µL/mL		
		P. aeruginosa (G-,O)	2 µL/mL		
		E. coli (G-,O)	2 µL/mL		
		K. pneumoniae (G-,O)	3 µL/mL		
		S. enterica (G-,P)	5 µL/mL		

	C. albicans (O)	3 µL/mL			
	S. cerevisiae (O)	2 µL/mL			
Essential oil obtained with water distillation at 100°C	*S. aureus (G+,O)*	0.12 mg/mL		bacterial suspension incubated with essential oil samples at concentrations ranging from 0.062 to 4.000 mg/mL	(Ehsani et al., 2017)
	L.monocytogenes (G+,P)	1 mg/mL			
	S. typhimurium (G-,P)	1 mg/mL			
	E. coli (G-,O)	2 mg/mL			
		A.	**B.**		
Essential oil obtained with hydrodistillation **A.** 20% EO solution in n-hexane **B.** 50% EO solution in n-hexane	*B. subtilis* (G+,C)	29.6 mm*	28.2 mm*	microbial suspension incubated with tested essential oil samples (20 and 50% solutions in n-hexane)	(Mimica-Dukic et al., 2004)
	S. lutea (G+,O)	27.0 mm*	24.6 mm*		
	S. aureus (G+,O)	24.0 mm*	19.4 mm*		
	S. epidermidis (G+,P)	18.2 mm*	26.6 mm*		
	P. aeruginosa ATCC 27853 (G-,O)	13.4 mm*	14.8 mm*		
	P. aeruginosa IPH-MR (G-,O)	16.6 mm*	0.0 mm*		
	E. coli ATCC 35218 (G-,O)	14.8 mm*	17.2 mm*		
	E. coli ATCC 25922 (G-,O)	30.2 mm*	39.8 mm*		
	E. coli IPH-MR (G-,O)	18.2 mm*	18.6 mm*		
	S. enteritidis (G-,P)	15.2 mm*	16.2 mm*		

		S. typhi (G-,P)	19.8 mm*		24.4 mm*		
		S. sonnei (G-,P)	37.4 mm*		38.4 mm*		
			A.	**B.**	**C.**		
Rhodiola rosea	**A.** 60% ethanolic extract obtained at 50°C **B.** Aqueous extract obtained at 50°C **C.** Powdered raw material	*S. epidermidis* (G+,P)	2 mg/mL	1 mg/mL	1 mg/mL	microbial suspension incubated with tested extracts at concentrations ranging from 0.5–64 mg/mL	(Kosakowska et al., 2018)
		S. aureus ATCC 25923 (G+,O)	1 mg/mL	4 mg/mL	4 mg/mL		
		S. aureus A 529 (G+,O)	2 mg/mL	8 mg/mL	8 mg/mL		
		B. cereus 15(G+,P)	4 mg/mL	16 mg/mL	16 mg/mL		
		B. cereus ATCC 11778 (G+,P)	4 mg/mL	16 mg/mL	32 mg/mL		
		B. subtilis (G+,C)	2 mg/mL	16 mg/mL	32 mg/mL		
		L.monocytogenes (G+,P)	4 mg/mL	8 mg/mL	32 mg/mL		
		K. pneumoniae (G-,O)	1 mg/mL	1 mg/mL	2 mg/mL		
		Y. enterocolitica (G-)	4 mg/mL	4 mg/mL	4 mg/mL		
		P. mirabilis (G-,P)	4 mg/mL	8 mg/mL	8 mg/mL		
		S. sonnei (G-,P)	4 mg/mL	8 mg/mL	8 mg/mL		
		S. ser. Enteritidis ATCC 13076 (G-,P)	8 mg/mL	8 mg/mL	16 mg/mL		
		S. ser. Enteritidis 322/11 (G-,P)	16 mg/mL	16 mg/mL	32 mg/mL		

		Salmonella ser. Typhimurium (G-,P)	16 mg/mL	16 mg/mL	32 mg/mL		
		P. aeruginosa (G-,O)	8 mg/mL	16 mg/mL	32 mg/mL		
		K. aerogenes (G-,O)	32 mg/mL	32 mg/mL	32 mg/mL		
		E. coli (G-,O)	32 mg/mL	32 mg/mL	64 mg/mL		
Crocus sativus	Methanolic extract of saffron flowers	*S. aureus (G+,O)*		75 µg/mL		microbial suspension incubated with tested extract	(Jastaniah, 2014)
		B. subtilis (G+C)		75 µg/mL			
		P. aeruginosa (G-,O)		50 µg/mL			
		S. typhi (G-,P)		75 µg/mL			
		S. dysentiriae (G-,P)		75 µg/mL			
		E. coli (G-,O)		75 µg/mL			

Abbreviations:
(G+)- Gram-positive bacteria, (G-)- Gram-negative bacteria, C- commensal microorganism in gut microbiota, P- pathogenic microorganism in gut microbiota. O- opportunistic pathogen in gut microbiota, EO- essential oil, MRSA - Methicillin-resistant *S. aureus*, MGRSA- Methicillin-gentamicin resistant *S. aureus* , (-) depicts positive growth of microorganisms even in the highest tested concentrations of tested extract was applied
[1]The antimicrobial effect was varying for particular microbial strains/ tested plant solutions; the outcome is therefore expressed as a range of values, * effect expressed as inhibition zone of microorganism growth in millimeters [mm]

References

Abdellatif, F., Boudjella, H., Zitouni, A., & Hassani, A. (2014). Chemical composition and antimicrobial activity of the essential oil from leaves of Algerian Melissa officinalis L. *EXCLI Journal*, *13*, 772–781.

Bla, B., Yang, W., Wang, Y., Li, C., Kindl, M., & Pepeljnjak, S. (2018). *Industrial Crops & Products Chemical composition , antimicrobial and antioxidant activities of essential oils of Lavandula × intermedia ' Budrovka ' and L . angustifolia cultivated in Croatia*. *123*(February), 173–182.

Bocquet, L., Sahpaz, S., Bonneau, N., Beaufay, C., Mahieux, S., Samaillie, J., Roumy, V., Jacquin, J., Bordage, S., Hennebelle, T., Chai, F., Quetin-Leclercq, J., Neut, C., Rivière, C., 2019. Phenolic Compounds from Humulus lupulus as Natural Antimicrobial Products: New Weapons in the Fight against Methicillin Resistant Staphylococcus aureus, Leishmania mexicana and Trypanosoma brucei Strains. Molecules 24, 1024.

Bogdanova, K., Kolar, M., Langova, K., Dusek, M., Mikyska, A., Bostikova, V., Bostik, P., & Olsovska, J. (2018). Inhibitory effect of hop fractions against gram-positive multi-resistant bacteria. A pilot study. *Biomedical Papers*, *164*(4), 276–283.

Ehsani, A., Alizadeh, O., Hashemi, M., Afshari, A., & Aminzare, M. (2017). Phytochemical, antioxidant and antibacterial properties of Melissa officinalis and Dracocephalum moldavica essential oils. *Veterinary Research Forum : An International Quarterly Journal*, *8*(3), 223–229.

Jastaniah, S. D. (2014). *The antimicrobial activity of some plant extracts, commonly used by Saudi people, against multidrug resistant bacteria*. *11*(8), 78–84.

Kosakowska, O., Bączek, K., Przybył, J. L., Pióro-Jabrucka, E., Czupa, W., Synowiec, A., Gniewosz, M., Costa, R., Mondello, L., & Węglarz, Z. (2018). Antioxidant and antibacterial activity of roseroot (Rhodiola rosea L.) dry extracts. *Molecules*, *23*(7), 1–14.

Locher, C. P., Burch, M. T., Mower, H. F., Berestecky, J., Davis, H., Van Poel, B., Lasure, A., Berghe, D. A. V., & Vlietinck, A. J. (1995). Anti-microbial activity and anti-complement activity of extracts obtained from selected Hawaiian medicinal plants. *Journal of Ethnopharmacology*, *49*(1), 23–32.

Maggi, F., Cecchini, C., Cresci, A., Coman, M. M., Tirillini, B., Sagratini, G., Papa, F., & Vittori, S. (2010). Chemical composition and antimicrobial activity of the essential oils from several Hypericum taxa (Guttiferae) growing in central Italy (Appennino Umbro-Marchigiano). *Chemistry and Biodiversity*, *7*(2), 447–466.

Milutinović, M., Dimitrijević-Branković, S., & Rajilić-Stojanović, M. (2021). Plant Extracts Rich in Polyphenols as Potent Modulators in the Growth of Probiotic and Pathogenic Intestinal Microorganisms. *Frontiers in Nutrition*, *8*.

Mimica-Dukic, N., Bozin, B., Sokovic, M., & Simin, N. (2004). Antimicrobial and Antioxidant Activities of Melissa officinalis L. (Lamiaceae) Essential Oil. *Journal of Agricultural and Food Chemistry*, *52*(9), 2485–2489.

Ożarowski, M., Kędzia, B., Kania-Dobrowolska, M., Baraniak, J., Gryszczyńska, A., Opala, B., Pietrowiak, A., Bogacz, A., Mikołajczak, P. Ł., Thiem, B., & Hołderna-Kędzia, E. (2018). Porównanie aktywności wyciągów z liści Passiflora alata, P. caerulea i P. incarnata wobec wybranych drobnoustrojów klinicznych. *Postępy Fitoterapii*, *19*(2), 75–80.

Pereira, J., Vieira, L., Teofilo, K., Figueiredo, F. J. B., & Dias-Souza, M. V. (2015). Antimicrobial Potential of Passiflora alata and Piper methysticum hydroalcoholic

extracts, Phytotherapics of Anxiolytic-like Activity. *Journal of Applied Pharmaceutical Sciences*, *2*(August), 31–33.

Pirbalouti, A. G., Fatahi-vanani, M., Craker, L., & Shirmardi, H. (2014). *Chemical composition and bioactivity of essential oils of*. *0209*.

Rapper, S. De, Kamatou, G., Viljoen, A., & Vuuren, S. Van. (2013). *The In Vitro Antimicrobial Activity of Lavandula angustifolia Essential Oil in Combination with Other Aroma-Therapeutic Oils*. *2013*.

Sokovicǵ, M., Glamočlija, J., Marin, P. D., Brkić, D., & Van Griensven, L. J. L. D. (2010). Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. *Molecules*, *15*(11), 7532–7546.

Süntar, I., Oyardi, O., Akkol, E. K., & Ozçelik, B. (2016). Antimicrobial effect of the extracts from Hypericum perforatum against oral bacteria and biofilm formation. *Pharmaceutical Biology*, *54*(6), 1065–1070.

Tambur, Z., Miljković-Selimović, B., Opačić, D., Aleksić, E., Ivančajić, L., Jovičić, B., & Vuković, B. (2020). Inhibitory effects of different medicinal plants on the growth of some oral microbiome members. *Medycyna Weterynaryjna*, *76*(8), 476–479.

Wang, J., Zhao, J., Liu, H., Zhou, L., Liu, Z., Wang, J., Han, J., Yu, Z., & Yang, F. (2010). Chemical analysis and biological activity of the essential oils of two valerianaceous species from China: Nardostachys chinensis and valeriana officinalis. *Molecules*, *15*(9), 6411–6422.

Weseler, A. R., & Saller, R. (2001). *A Current Review of the Antimicrobial Activity of Hypericum perforatum L . A Current Review of the Antimicrobial Activity of Hypericum perforatum L . June 2014*.

Zara, S., Petretto, G. L., Mannu, A., Zara, G., Budroni, M., Mannazzu, I., Multineddu, C., Pintore, G., & Fancello, F. (2021). *Antimicrobial Activity and Chemical Characterization of a Non-Polar Extract of Saffron Stamens in Food Matrix*.

Phytotherapy of mood disorders in the light of microbiota-gut-brain axis.

Maciej Korczak[1], Maciej Pilecki[2], Sebastian Granica[1], Aleksandra Gorczyńska[1], Karolina A. Pawłowska[1], Jakub P. Piwowarski[1*]

[1]Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland

[2]Department of Psychiatry, Collegium Medicum, Jagiellonian University, Cracow

*Corresponding author: Jakub P. Piwowarski, ul. Banacha 1, 02-097 Warsaw, Poland, tel/fax +48 22 5720985, jpiwowarski@wum.edu.pl

Table S2. Meta-analyses

Species	Model	No of trials included	Efficacy	Ref
V. officinalis	Meta-analyses on anxiolytic/anti-depressant efficacy of *V. officinalis* were not found.			
L. officinalis	Efficacy of aromatherapy on physical and psychological symptoms in menopausal women; all RCTs	16 (2005-2019), divided into four meta-analyses	Aromatherapy with lavender essential oil alone and in mixtures improves menopausal symptoms HP>C	(Salehi-Pourmehr et al., 2020)
	Anxiolytic efficacy of various lavender administration routes (oral administration, aromatherapy, massage), subjects were of mixed medical backgrounds; all RCTs	32 (1994-2017)	Aromatherapy and massage in short-term treatment of the anxiety HP>C Oral intake of lavender oil for long-term treatment of the anxiety HP>C	(Sayed et al., 2020)
	Anxiolytic efficacy of lavender essential oil; mixed routes of administration; subjects were of different medical backgrounds; all RCTs	22 (2000-2018)	Self-reported anxiety ↓ HP>C Diastolic BP ↓/ (-) HP≥C Systolic BP ↓/ (-) HP≥C HR ↓ HP>C Salivary cortisol ↓ HP>>C Salivary chromogranin A ↓ HP>>C	(Kang et al., 2019)
	Anxiolytic efficacy of orally administered essential oil; some studies included combination therapy; all RCTs	5 (2009-2016)	Anxiety (HAM-A) ↓ 160 mg HP/day > 80 mg HP/day = 20 mg paroxetine; (-) 0.5 mg lorazepam	(Yap et al., 2019)
	Anxiolytic efficacy of lavender essential oil; mixed routes of administration; subjects were of	37 (1998-2018), divided into seven meta-analyses	Most of trials reported a conclusion favourable for use of HP, oral administration of	(Donelli et al., 2019)

	different medical backgrounds; all RCTs		standardized essential oil have the most promising efficacy	
	Anxiolytic efficacy of orally administered essential oil in treatment of participants with diagnosed anxiety; all RCTs	5 (2009-2016)	Anxiety a) HP vs placebo HP>C b) HP vs lorazepam HP=C	(Generoso et al., 2017)
P. methysticum	Anxiolytic efficacy of orally administered kava monopreparations; participants with diagnosed GAD; all placebo-controlled RCTs	3 (2002-2013)	Anxiety (HAM-A) - inconclusive results	(Ooi et al., 2018)
	Anxiolytic efficacy of orally administered, standardized, acetonic kava extract WS 1490; participants with non-psychotic anxiety disorders; all placebo-controlled RCTs	6 (1991-2004)	Anxiety (HAM-A) ↓ HP (150-300 mg of extract/day) >C	(Witte et al., 2005)
	Anxiolytic efficacy of orally administered kava monopreparations, mostly with WS 1490 extract; mostly participants with diagnosed anxiety; all placebo-controlled RCTs	7 (1991-2004)	Anxiety (HAM-A) ↓ HP (105-280 mg of kavalactones/day) >C	(Pittler and Ernst, 2003)
	Anxiolytic efficacy of orally administered kava extract WS 1490; participants with diagnosed GAD; all placebo-controlled RCTs	3 (1991-1997)	Anxiety (HAM-A) ↓ HP (210 mg of kavapyrone/day) >C	(Pittler and Ernst, 2000)

P. incarnata/ pallida	Anxiolytic efficacy of orally administered *Passiflora* preparations; participants with diagnosed GAD or neurosis/anxiety; all RCTs, controlled with beznodiazepines or placebo	2 (1993, 2001)	The data acquired in the included studies was inconclusive.	(Miyasaka et al., 2007)
H. lupulus	Meta-analyses on anxiolytic/anti-depressant efficacy of *H. lupulus* were not found.			
M. officinalis	Anxiolytic efficacy of orally administered *M. officinalis* preparations; subjects were of different medical backgrounds; trials included applied diverse diagnostic questionnaires and herbal preparations; all placebo-controlled RCTs	5 included in meta-analysis on anxiety (2018-2020); 3 included in meta-analysis on depression (2018-2019)	Acute anxiety ↓ HP>C Chronic anxiety HP=C Acute depression ↓ HP>C Chronic depression ↓ HP>C (one trial)	(Ghazizadeh et al., 2021)
R. rosea	Meta-analyses on anxiolytic/anti-depressant efficacy of *R. rosea* were not found			
C. sativus	Efficacy of orally administered *C. sativus* monotherapy in treatment of mild to moderate depression; placebo or SSRIs used as controls; all RCTs	12 (2005-2018)	Depression (both response and remission, corresponding to score reductions by 50% and 75%, respectively) (a) HP (30 or 50 mg/day) vs placebo - HAM-D ↓ HP>C - BDI ↓ HP>C (b) HP (30 mg/day) vs SSRIs - HAM-D ↓ HP=C	(Dai et al., 2020)
	Efficacy of orally administered *C. sativus* preparations and	21 (2005-2019)	Depression - BDI ↓ P>C - HAM-D (-) (↓ P>C in studies on	(Ghaderi et al., 2020)

	active chemical substances; subjects were of different medical backgrounds; some studies included combination therapy with SSRIs; all placebo-controlled RCTs		non-depressed or <50 y.o. subjects, ≥8 weeks interventions) Anxiety - BAI ↓ P>C - HAM-A (-) Sleep quality (PSQI) ↓ P>C CRP levels (-)	
	Efficacy of orally administered *C. sativus* monotherapy in treatment of mild to moderate depression; placebo or SSRIs used as controls; all RCTs	9 (2004-2018)	Depression (a) HP (30-100 mg/day) vs placebo (HAM-D, BDI) ↓ HP>C (b) HP (30 mg/day) vs SSRIs (HAM-D) ↓ HP=C	(Tóth et al., 2019)
	Efficacy of orally administered *C. sativus* monotherapy in treatment of mild to moderate depression; placebo or SSRIs used as controls; all RCTs	5 (2004-2008)	Depression (a) HP (30 mg/day) vs placebo (HAM-D) ↓ HP>C (b) HP (30 mg/day) vs SSRIs (HAM-D) ↓ HP=C	(Hausenblas et al., 2013)
H. perforatum	Efficacy of different complementary and alternative medicine therapies in treatment of moderate to severe depression; all SSRI-controlled RCTs	12 trials on *H. perforatum (*1999-2006)	Depression (HAM-D) ↓ HP (300-1800 mg/day)=C	(Asher et al., 2017)
	Efficacy of orally administered *H. perforatum* preparations and extracts in monotherapy of mild to moderate depression; SSRI-controlled	27 (1999-2014)	Depression (HAM-D) - response HP=C - remission HP=C - baseline change ↓HP=C	(Ng et al., 2017)

	Efficacy of orally administered *H. perforatum* preparations, extracts, and active chemical substances in monotherapy of mild to severe depression; placebo or anti-depressants (SSRI/TCA) used as controls; all RCTs	18 (1994-2010)	Depression (a) P vs placebo - response P>C - remission P=C (b) P vs anti-depressants - response P=C - remission P=C	(Apaydin et al., 2016)
	Efficacy of orally administered *H. perforatum* preparations, in monotherapy of mild to severe depression; placebo and/or SSRIs used as controls; all RCTs	13 (1999-2007)	Depression (HAM-D, HP vs SSRIs) - response HP=C - remission HP=C - baseline change ↓HP=C	(Rahimi et al., 2009)
	Efficacy of orally administered *H. perforatum* extract WS 5570, in monotherapy of depression; placebo or anti-depressants (SSRI/TCA) used as controls; all RCTs	3 (2002-2006)	Depression ↓ HP (600-1200 mg/day)=C	(Kasper et al., 2007)
	Efficacy of orally administered *H. perforatum* extracts in monotherapy of depression; placebo and/or anti-depressants (SSRI/TCA/TeCA) used as controls; all RCTs	37 (1979-2002)	Depression (a) HP (200-1500 mg/day) vs placebo ↓ HP>C (b) HP (300-1800 mg/day) vs anti-depressants ↓ HP=C	(Linde et al., 2005)
	Efficacy of orally administered *H. perforatum* extracts WS 5570 or WS	3 (1998-2001)	Particularly large advantages for HP (900 mg/day) were observed	(S. and A., 2002)

	5572 in monotherapy of depression (DSM-IV); focused on selective effect on particular signs and symptoms of depression; all placebo-controlled RCTs		for: depressed mood, retardation, anxiety, general somatic symptoms, and genital symptoms.	
	Efficacy of orally administered *H. perforatum* extracts in monotherapy of depression; placebo and/or anti-depressants (SSRI/TCA/TeCA) used as controls; all RCTs	22 (1989-2001)	Depression (a) HP (200-1200 mg/day) vs placebo ↓ HP>C (b) HP (375-1800 mg/day) vs anti-depressants ↓ HP=C	(Whiskey et al., 2001)

Influence on the parameter: (-) no influence, ↓ decrease; comparison of herbal preparation and control: HP=C difference not found or statistically insignificant; HP>C/HP<C statistically significant difference in the parameter (more effective HP/C).

Abbreviations:

BAI - Beck Anxiety Inventory; **BDI** - Beck Depression Inventory; **BP** - blood pressure; **C** - control; **CRP** - C-reactive protein; **DER** - drug extract ratio; **DSM** - Diagnostic and Statistical Manual of Mental Disorders; **GAD** - Generalized Anxiety Disorder; **HAM-A** - The Hamilton Anxiety Rating Scale; **HAM-D** - Hamilton Depression Rating Scale; **HP** - herbal preparation; **HR** - heart rate; **ISI** - Insomnia Severity Index; **PSQI** - Pittsburg Sleep Quality Inventory; **RCT** - randomized controlled trial; **SSRI** - selective serotonin reuptake inhibitor; **TCA** - tricyclic anti-depressant; **TeCA** - tetracyclic anti-depressant.

References:

Apaydin, E.A., Maher, A.R., Shanman, R., Booth, M.S., Miles, J.N. V., Sorbero, M.E., Hempel, S., 2016. A systematic review of St. John's wort for major depressive disorder. Syst. Rev. 5, 148.

Asher, G.N., Gartlehner, G., Gaynes, B.N., Amick, H.R., Forneris, C., Morgan, L.C., Coker-Schwimmer, E., Boland, E., Lux, L.J., Gaylord, S., Bann, C., Pierl, C.B., Lohr, K.N., 2017. Comparative Benefits and Harms of Complementary and Alternative Medicine Therapies for Initial Treatment of Major Depressive Disorder: Systematic Review and Meta-Analysis. J. Altern. Complement. Med. 23, 907–919.

Dai, L., Chen, L., Wang, W., 2020. Safety and Efficacy of Saffron (Crocus sativus L.) for Treating Mild to Moderate Depression. J. Nerv. Ment. Dis. 208, 269–276.

Donelli, D., Antonelli, M., Bellinazzi, C., Gensini, G.F., Firenzuoli, F., 2019. Effects of lavender on anxiety: A systematic review and meta-analysis. Phytomedicine 65, 153099.

Generoso, M.B., Soares, A., Taiar, I.T., Cordeiro, Q., Shiozawa, P., 2017. Lavender Oil Preparation (Silexan) for Treating Anxiety. J. Clin. Psychopharmacol. 37, 115–117.

Ghaderi, A., Asbaghi, O., Reiner, Ž., Kolahdooz, F., Amirani, E., Mirzaei, H., Banafshe, H.R., Maleki Dana, P., Asemi, Z., 2020. The effects of saffron (Crocus sativus L.) on mental health parameters and C-reactive protein: A meta-analysis of randomized clinical trials. Complement. Ther. Med. 48, 102250.

Ghazizadeh, J., Sadigh-Eteghad, S., Marx, W., Fakhari, A., Hamedeyazdan, S., Torbati, M., Taheri-Tarighi, S., Araj-khodaei, M., Mirghafourvand, M., 2021. The effects of lemon balm (Melissa officinalis L.) on depression and anxiety in clinical trials: A systematic review and meta-analysis. Phyther. Res. ptr.7252.

Hausenblas, H.A., Saha, D., Dubyak, P.J., Anton, S.D., 2013. Saffron (Crocus sativus L.) and major depressive disorder: a meta-analysis of randomized clinical trials. J. Integr. Med. 11, 377–383.

Kang, H.-J., Nam, E.S., Lee, Y., Kim, M., 2019. How Strong is the Evidence for the Anxiolytic Efficacy of Lavender?: Systematic Review and Meta-analysis of Randomized Controlled Trials. Asian Nurs. Res. (Korean. Soc. Nurs. Sci). 13, 295–305.

Kasper, S., Gastpar, M., Müller, W.E., Volz, H.P., Dienel, A., Kieser, M., Möller, H.J., 2007. Efficacy of St. John's wort extract WS® 5570 in acute treatment of mild depression. Eur. Arch. Psychiatry Clin. Neurosci. 258, 59–63.

Linde, K., Mulrow, C., Berner, M., Egger, M., 2005. St John's Wort for depression, in: Linde, Klaus (Ed.), Cochrane Database of Systematic Reviews. John Wiley & Sons, Ltd, Chichester, UK.

Miyasaka, L.S., Atallah, Á.N., Soares, B., 2007. Passiflora for anxiety disorder. Cochrane Database Syst. Rev.

Ng, Q.X., Venkatanarayanan, N., Ho, C.Y.X., 2017. Clinical use of Hypericum perforatum (St John's wort) in depression: A meta-analysis. J. Affect. Disord. 210, 211–221.

Ooi, S.L., Henderson, P., Pak, S.C., 2018. Kava for Generalized Anxiety Disorder: A Review of Current Evidence. J. Altern. Complement. Med. 24, 770–780.

Pittler, M.H., Ernst, E., 2003. Kava extract versus placebo for treating anxiety. Cochrane Database Syst. Rev.

Pittler, M.H., Ernst, E., 2000. Efficacy of Kava Extract for Treating Anxiety:

Systematic Review and Meta-Analysis. J. Clin. Psychopharmacol. 20, 84–89.
Rahimi, R., Nikfar, S., Abdollahi, M., 2009. Efficacy and tolerability of Hypericum perforatum in major depressive disorder in comparison with selective serotonin reuptake inhibitors: A meta-analysis. Prog. Neuro-Psychopharmacology Biol. Psychiatry 33, 118–127.
S., K., A., D., 2002. Cluster analysis of symptoms during antidepressant treatment with Hypericum extract in mildly to moderately depressed out-patients. A meta-analysis of data from three randomized, placebo-controlled trials. Psychopharmacology (Berl). 164, 301–308.
Salehi-Pourmehr, H., Ostadrahimi, A., Ebrahimpour-Mirzarezaei, M., Farshbaf-Khalili, A., 2020. Does aromatherapy with lavender affect physical and psychological symptoms of menopausal women? A systematic review and meta-analysis. Complement. Ther. Clin. Pract. 39, 101150.
Sayed, A.M., Morsy, S., Tawfik, G.M., Naveed, S., Minh-Duc, N.T., Hieu, T.H., Ali, Z.A., Shinkar, A., Doheim, M.F., Hashan, M.R., Huy, N.T., 2020. The best route of administration of lavender for anxiety: a systematic review and network meta-analysis. Gen. Hosp. Psychiatry 64, 33–40.
Tóth, B., Hegyi, P., Lantos, T., Szakács, Z., Kerémi, B., Varga, G., Tenk, J., Pétervári, E., Balaskó, M., Rumbus, Z., Rakonczay, Z., Bálint, E., Kiss, T., Csupor, D., 2019. The Efficacy of Saffron in the Treatment of Mild to Moderate Depression: A Meta-analysis. Planta Med. 85, 24–31.
Whiskey, E., Werneke, U., Taylor, D., 2001. A systematic review and meta-analysis of Hypericum perforatum in depression: a comprehensive clinical review. Int. Clin. Psychopharmacol. 16, 239–252.
Witte, S., Loew, D., Gaus, W., 2005. Meta-analysis of the efficacy of the acetonic kava-kava extract WS®1490 in patients with non-psychotic anxiety disorders. Phyther. Res. 19, 183–188.
Yap, W.S., Dolzhenko, A. V., Jalal, Z., Hadi, M.A., Khan, T.M., 2019. Efficacy and safety of lavender essential oil (Silexan) capsules among patients suffering from anxiety disorders: A network meta-analysis. Sci. Rep. 9, 18042.

Phytotherapy of mood disorders in the light of microbiota-gut-brain axis.

Maciej Korczak[1], Maciej Pilecki[2], Sebastian Granica[1], Aleksandra Gorczyńska[1], Karolina A. Pawłowska[1], Jakub P. Piwowarski[1*]

[1]Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland

[2]Department of Psychiatry, Collegium Medicum, Jagiellonian University, Cracow

*Corresponding author: Jakub P. Piwowarski, ul. Banacha 1, 02-097 Warsaw, Poland, tel/fax +48 22 5720985, jpiwowarski@wum.edu.pl

Table S3. Clinical trials

Species	Type of preparation	Study model	Effects on anxiety & depression	Other effects investigated	Dose/duration	Controls	Ref.
V. officinalis 2021-2002	Unspecified extract, plant material authenticated	Anxiety about tooth extraction (DAS); RCT, double-blind, split-mouth, cross-over	Anxiety (DAS) ↓ HP<C	Oxygen saturation (-) ∆ HR HP<C Systolic BP ↓ HP<C Diastolic BP ↓ HP<C Respiratory rate ↓ HP<C Sedation (RSS) ↑ HP<C Anterograde amnesia (self-assessment) ↑ HP<C Participants' preference HP>C	100 mg of HP or C 1 h before the procedures; two procedures in total (*p.o.*)	Midazolam 15 mg	(Farah et al., 2019)
	Ethanolic (70%) extract (0.8% valerenic acids)	Mild anxiety, depression, or insomnia symptoms (VAS), without current episodes of depression or anxiety disorder; RCT, double-blind, parallel	Psychiatric assessments (HAM-A, HAM-D, BDI, BAI) (-)	Psychiatric assessments (PSQI, WHO-5, BEPSI, SOFAS, BFI, SDS) (-) Frontal EEG: - alpha coherence ↑ HP>C - theta coherence ↓ HP>C	300 mg/day; 4 weeks (*p.o.*)	Placebo	(Roh et al., 2019)
	Commercial powdered valerian root (valerenic acid content >0.05%)	HIV-positive candidates for starting antiretroviral therapy containing efavirenz; RCT, double-blind, parallel	Anxiety (HAM-A) ↓ HP>C Depression (HAM-D) (-)	Positive and Negative Syndrome (Schizophrenia) (PANSS) (-) Suicide ideations (PANSI) (-) Sleep quality (PSQI) HP>C	530 mg powdered valerian root/day; 1 hour before sleep; 4 weeks (*p.o.*)	Placebo	(Ahmadi et al., 2017)

	Unspecified preparation	Anxiety (VAS) in women undergoing hysterosalpingography; RCT, double-blind, parallel	Anxiety (VAS) ↓ HP>C		1500 mg before the procedure (*p.o.*)	Placebo	(Gharib et al., 2015)
	Valerian extract (unspecified method, dose and DER), each capsule standardized for 3.2 mg valerenic acids, 1% valerenic acid in extract	Subjects reporting problems with sleep and anxiety (ISI, STAI-state); RCT, double-blind, parallel, 3-arm (study also researched kava)	Anxiety (STAI-state) (-)	Sleep disturbation (ISI) ↓ HP=C	2 capsules/day; 1 hour before sleep; 4 weeks (*p.o.*)	Placebo	(Jacobs et al., 2005)
	Valepotriates mixture containing 80% dihydrovaltrate, 15% valtrate and 5% acevaltrate	Participants with diagnosed GAD (DSM III-R); parallel, double-blind, flexible-dose	Anxiety (HAM-A): Somatic symptoms ↓ C, V, P Psychic symptoms ↓ V, P; (-) C Anxiety (STAI) - trait ↓ V; (-) P, C - state ↓ (-) V, P, C		Preparation of varied doses of valepotriates (P; 50, or 100, or 150 mg/day); 4 weeks (*p.o.*)	Varied doses of diazepam (V; 2.5, or 5, or 7.5 mg/day); placebo (C)	(Andreatini et al., 2002)
L. officinalis (2020-2018)	Commercially available essential oil	Participants undergoing microvascular breast reconstruction in the breast cancer treatment; RCT, parallel , single-blind	HADS anxiety and depression (-)		Pre-, intra-, and postoperative aromatherapy with essential oil	Placebo	(Shammas et al., 2021)
	Powdered plant (unspecified part) standardized for a content of phenolic	Participants with mild to moderate depression (DSM-V); RCT, parallel, double-blind, 3-arm	Depression (HAM-D) ↓ HP=C		1 g/day; 8 weeks (*p.o.*)	Fluoxetine 10 mg/day	(Araj-Khodaei et al., 2020)

compounds and flavonoids						
Infusion (prepared by attendees) of portionated plant material	Participants >60 y.o. with mild to moderate depression (BDI-II); RCT, single-blind, parallel	Depression (BDI) ↓ HP>C Anxiety (STAI): - state ↓ HP>C - trait ↓ HP>C		2 g/day; 2 weeks (*p.o.*)	Non-placebo	(Araj-Khodaei et al., 2020)
Essential oil (21.33% of ceineole)	Participants qualified for open-heart surgery; RCT, parallel, double-center, 4-arm (study also researched rose essential oil)	Anxiety (STAI-state) (-)	Surgical site pain (VAS) ↓ HP; (-) C	Aromatherapy inhalation with 0.2 ml of essential oil before the procedure	Placebo, non-placebo	(Babatabar Darzi et al., 2020)
Commercially available 10% essential oil	Participants qualified for bone marrow biopsy; RCT, double-blind, parallel	Anxiety (VAS) HP<C (evaluation took place after the procedure only)		Aromatherapy inhalation with three drops of essential oil before the procedure	Placebo	(Abbaszadeh et al., 2020)
Steam-distilled essential oil	Participants qualified for cataract surgery; RCT, parallel	Anxiety (STAI-state) ↓ HP>C		Aromatherapy inhalation with essential oil before the procedure (twenty drops in the vaporizer)	Placebo	(Stanley et al., 2020)
Commercially available essential oil (specified lot number), plant material originated from France	Anxiety about tooth extraction (DAQ); RCT, parallel	Anxiety: MDAS ↓ HP=C STAI-state ↓ HP=C	Pain intensity (VAS) (-) Intra-operative respiratory rate ↓ HP > ↑ C	Aromatherapy inhalation with essential oil before the procedure	Non-placebo	(Karan, 2019)
Standardized, powdered lavender flower (36.12% linalool, 26.32%	Postmenopausal women; RCT, parallel, 3-arm (study also	Anxiety (STAI): - state ↓ HP>C		1 g/day; 8 weeks (*p.o.*)	Placebo	(Farshbaf-Khalili et al., 2018)

	linalyl acetate, 7.55% caryophyllene)	researched bitter orange)	- trait ↓ HP>C				
*P. methysticum (*2020)	Comprehensively standardized pharmaceutical-grade aqueous extract of kava derived from the dried roots of a 'noble' Borogu cultivar sourced from Vanuatu	Participants diagnosed with GAD (DSM-V); RCT, double-blind, parallel, multicenter	Anxiety (HAM-A) ↓ HP<C Depression (MADRS), anxiety (BAI) (-)	Worry (PSWQ) ↓ HP=C Psychological distress (K-10), sexual functioning (ASEX), insomnia (ISI) (-)	240 mg of kavalactones/day; 16 weeks (*p.o.*)	Placebo	(Sarris et al., 2020)
*P. incarnata / pallida (*2021-2008)	Unspecified preparation of *P. incarnata*	Anxiety about tooth extraction (MDAS); RCT, triple-blind, 4-arm, parallel (study also researched *Erythrina mulungu*)	Anxiety (MDAS) HP=V<C	∆ oxygen saturation, ∆ HR, ∆ systolic and diastolic BP HP=V=C	500 mg of HP or V 0.5 h before the procedures; two procedures in total (*p.o.*)	Midazolam 15 mg (V), placebo (C)	(da Cunha et al., 2021)
	Aqueous standardized extract (commercially available)	Participants without previous experience with anaestesia and surgery, scheduled for surgical procedure under spinal anesthesia; RCT, double-blind, parallel	Anxiety (STAI state and trait) HP (-), ↑C	∆ Sedation (OOA/S) HP=C Psychomotor functions (-)	700 mg/5 ml 0.5 h before the procedure (*p.o.*)	Placebo	(Aslanargun et al., 2012)
	Infusion (prepared by attendees) of portionated leaves, stems, seeds and flowers	Healthy volunteers with minor sleep problems; RCT, double-blind, cross-over	Anxiety (STAI-state) (-)	Polysomnography HP=C Sleep quality ↑ HP>C	2 g/day before sleep; 1 week; 1 week wash-out (*p.o.*)	Placebo (parsley infusion)	(Ngan and Conduit, 2011)
	Commercially available preparation of *P.incarnata* containing 1.01 mg benzoflavone per	Participants scheduled for surgical procedure under general anaesthesia; RCT, double-blind, parallel	Anxiety (NRS) ↓ HP> C	Sedation ↑ HP=C Psychomotor functions HP=C	500 mg, 1.5 h before the procedure (*p.o.*)	Placebo	(Movafegh et al., 2008)

	tablet (500 mg of HP)						
*H. lupulus (*2019-2012)	β-eudesmol (a portion of 950 ng/190 ml water)	Healthy volunteers subjected to TSST; RCT, double-blind, cross-over	Anxiety (STAI-JYZ): - state ↑ P=C - trait (-)	Salivary marker of sympathetic nerve activity (MHPG) ↑ P<C Salivary cortisol ↑ P=C Salivary chromogranin A (-) P; ↑ C Stress (DSSQ-3) ↑ P=C	Single application of a portion of preparation (P) or placebo; 2 week wash-out (*p.o.*)	Placebo	(Ohara et al., 2018)
	Commercially available hops extract (dietary supplement)	Volunteers with mild depression, anxiety and stress (DASS-21); RCT, double-blind, cross-over	DASS-21 scores: - depression ↓ HP>C - anxiety ↓ HP>C - stress ↓ HP>C	Morning cortisol plasma (-)	400 mg/day; 4 weeks; 2 weeks wash-out (*p.o.*)	Placebo	(Kyrou et al., 2017)
	Powdered inflorescence containing 100 µg phytoestrogen per 500 mg of plant material	Pre- and postmenopausal participants (Greene Scale); RCT, double-blind, parallel, multicenter	Mental symptoms (Greene scale) - anxiety ↓ HP>C - depression ↓ HP>C	Greene Scale total score ↓ HP>C	500 mg/day; 90 days (*p.o.*)	Placebo	(Aghamiri et al., 2016)
	Commercially available alcohol-free beer	Healthy volunteers experiencing stress at workplace (ERI)	Anxiety (STAI) - state ↓ HP - trait (-)	Sleep quality ↑ HP	330 ml/day; 2 weeks (*p.o.*)	Baseline comparison	(Franco et al., 2012)
*M. officinalis (*2020)	Powdered leaves standardized for a content of phenolic compounds and flavonoids	Participants with mild to moderate depression (DSM-V); RCT, double-blind, 3-arm, parallel	Depression (HAM-D) ↓ HP=C		1 g/day; 8 weeks (*p.o.*)	Fluoxetine 10 mg/day	(Araj-Khodaei et al., 2020)

R. rosea *(*2020-2007)	Unspecified preparation	Participants with mild to moderate major depressive disorder (DSM-IV); RCT, double-blind, 3-arm, parallel	Depression: - HAM-D and BDI scores ↓ HP>C (inconclusive dose-dependent effect) - CGI/C score improvement HP>C		HP: 0 mg, 300 mg, or 600 mg/day; 12 weeks (*p.o.*); all received the same, not specified dose of sertraline	Placebo	(Gao et al., 2020)
	Dry ethanolic *R. rosea* extract WS® 1375 (DER 1.5-5:1)	Subjects with prolonged or chronic fatigue symptoms; un-controlled, open label study	Depression (BDI) ↓ HP	Fatigue (MFI-20, NAS) ↓ HP Everyday life disruption (SDS) ↓ HP Executive function impairment (NCT) ↓ HP Sleep (PSQI) ↓ HP Stress (PSQ-R) ↓ HP CGI score improvement HP	400 mg/day; 8 weeks (*p.o.*)	Baseline comparison	(Lekomtseva et al., 2017)
	Dry ethanolic *R. rosea* extract WS® 1375 (DER 1.5-5:1)	Volunteers with mild anxiety; RCT, parallel	Anxiety (STAI) ↓ HP>C	Stress (PSS) ↓ HP>C	400 mg/day; 2 weeks (*p.o.*)	Non-placebo	(Cropley et al., 2015)
	SHR-5 powdered extract of *R.rosea* root (standardized to a content of rosavin 3.07%/rhodioloside 1.95%)	Participants with mild to moderate depression (DSM-IV); RCT, double-blind, 3-arm, parallel	Depression - HAM-D score ↓ HP=V=C - BDI score ↓ HP=V=C		Flexible dose depending on the individual results: 340-1360 mg/day; 12 weeks (*p.o.*)	Sertraline 50 mg (V), placebo (C)	(Mao et al., 2015)
	SHR-5 powdered ethanolic (70% w/w) extract of *R.rosea* root (DER 4:1; standardized to	Participants with mild to moderate depression (DSM-IV); RCT, double-blind, 3-arm, parallel	Depression - HAM-D score ↓ HP (A)=HP (B)>C		(A) 340 or (B) 680 mg/day; 6 weeks (*p.o.*)	Placebo	(Ross, 2014)

	a minimum of 3.0% rosavins and 0.8% to 1.0% salidrosides)		- Self esteem score: HP (B)>HP (A)>C - BDI score ↓ HP (A)=HP (B)>C				
	R. rosea extract WSW 1375 (DER 1.5-5:1)	Subjects experiencing life-stress symptoms; un-controlled, open label, multicenter study	Mood impairment (MDMQ) ↓ HP	Fatigue (MFI-20) ↓ HP Everyday life disruption (SDS) ↓ HP Executive function impairment (NCT) ↓ HP Stress: - PSQ ↓ HP - NAS ↓ HP CGI score improvement HP	400 mg/day; 4 weeks (*p.o.*)	Baseline comparison	(Edwards et al., 2012)
	R. rosea extract, standardized (details inconclusive)	Participants diagnosed with GAD (DSM-IV); un-controlled, open label study	Anxiety (HAM-A) ↓ HP Depression (HAM-D) ↓ HP Self-rated anxiety (4DSQ subscale) ↓ HP	CGI score improvement - inconclusive	340 mg/day; 10 weeks (*p.o.*)	Baseline comparison	(Bystritsky et al., 2008)
	SHR-5 powdered ethanolic (70% w/w) extract of *R.rosea* root	Participants with mild to moderate depression (DSM-IV); RCT, double-blind, 3-arm, parallel	Depression - HAM-D score ↓ HP (A)=HP (B)>C - BDI score ↓ HP (B)>HP (A)>C		(A) 340 or (B) 680 mg/day; 6 weeks (*p.o.*)	Placebo	(Darbinyan et al., 2007)
*C. sativus (*2021-2019)	Crocin	Breast cancer participants; RCT, double-blind, parallel	Depression (BDI) ↓ P, ↑ C Anxiety (BAI) ↓ P, ↑ C		30 mg/day; 4 months (*p.o.*)	Placebo	(Salek et al., 2021)

	Saffron	Healthy volunteers subjected to resistance training; RCT, double-blind, parallel	Blood levels of markers implicated in depression: - AEA, 2-AG, dopamine, β-endorphin ↑ HP>C; (-) C - serotonin ↑ HP>C - tryptophane (-)	Happiness assessment (VAS) ↑ HP>C	150 mg after each training; 4 per week; 6 weeks (*p.o.*)	Placebo	(Moghadam et al., 2021)
	Unspecified preparation	Participants ≥ 60 y.o. with depression (DSM-V); RCT, double-blind, parallel	Depression (HAM-D) ↓ HP=C		60 mg/day; 6 weeks (*p.o.*)	Sertraline 100 mg/day	(Ahmadpanah et al., 2019)
	Unspecified preparation	Overweight women with mild to moderate depression (DSM-V); RCT, double-blind, parallel	Depression (BDI) ↓ HP>C	Food craving questionnaire, appetite (VAS), food abstinence (VAS) (-)	30 mg/day; 12 weeks (*p.o.*)	Placebo	(Akhondzadeh et al., 2020)
	Extract derived from the stigmas of *C. sativus,* and standardised to contain > 3.5% safranal and crocin isomers	Participants in the course of anti-depressant therapy experiencing mild to moderate symptoms; RCT, double-blind, parallel	Depression (MADRS) ↓ HP>C Self-rated depression (MADRS-S) ↓ HP=C	Health survey (SF-36) (-)	14 mg/day; 8 weks (*p.o.*)	Placebo	(Lopresti et al., 2019)
*H. perforatum (*2019-2016)	Unspecified preparation	Postmenopausal participants with mild to moderate depression; RCT, double-blind, parallel	Depression (HAM-D) ↓ HP>C	Menopausal symptoms (Kupperman index) ↓ HP>C Hot flashes frequency ↓ HP>C Hot flashes intensity ↓ HP>C	0.99 mg/day; 8 weeks (*p.o.*)	Placebo	(Eatemadnia et al., 2019)
	H. perforatum extract LI 160,	Healthy participants subjected to emotional	Depression (BDI) (-)	Ability to experience pleasure (SHAPS) (-)	900 mg/day; 6 days (*p.o.*)	Placebo	(Warren et al., 2019)

	standardised to contain 0.3% hypericin and 3-5% hyperforin	processing tests; RCT, double-blind, parallel	Self-rated emotions (VAS): happy, sad, hostile, alert, anxious and calm (-) Emotional processing tests: Recognition of disgusted faces ↓ HP>C Attention to fearful faces ↓ HP>C Memory for positive words ↑ HP>C Overall categorization of emotional facial expressions (-) Overall categorization and recall of emotional words (-) Vigilance to happy faces (-)	Personality traits (EPQ) (-) N-back working memory (-)			
	H. perforatum Ze 117 ethanolic (57.9% *v*/*v*) extract (DER 4-7:1, standardized to	Healthy participants subjected to short-memory and cognitive capacities tests; RCT, double-blind, cross-over	State anxiety (STAI-state, DASS) (-) Positive feelings	Digit span and short memory tests: - low dose - improvement HP>C	Single administration of 250 of 500 mg extract before test; average	Placebo	(Yechiam et al., 2019)

	contain 0.1–0.3% of total hypericins, and <1% hyperforin)		(PANAS) ↑HP>C Negative feelings (PANAS) - inconclusive	- high dose - impairment HP>C Error omission/commission (-) Groton Maze task (-)	wash-out 34.1 days (*p.o.*)		
	Commercially available preparations; (A) monofractionated, (B) multi-fractionated extract; both standardized to contain 0.3% hypericin.	Participants with moderate depression; observational, cross-over, open-label,	Depression (SDS*) - 6 months (-) HP (A), ↓ HP (B) - 12 months ↓ HP (B)>HP (A)		600 mg/day; 3 months; 3 months wash-out (*p.o.*)	Baseline comparison	(Di Pierro et al., 2018)
	H. perforatum WS 5570 methanolic (80% *v/v*) extract (DER 3-7:1, standardized to contain 3–6% hyperforin, 0.1–0.3% hypericin, ≥6% flavonoids, ≥1.5% rutin)	Subgroup analysis of data acquired in previously reported trial (Szegedi et al., 2005) focusing on participants with moderate depression (DSM-IV); RCT, double-blind, parallel	Depression (HAM-D) - response ↓HP>C - remission ↓HP>C		900 mg/day; 6 weeks (*p.o.*)	Paroxetine 20 mg/day	(Seifritz et al., 2016)

Influence on the parameter: (-) no influence, ↓ decrease, ↑ increase, Δ change; comparison of herbal preparation and control: HP=C difference not found or statistically insignificant; HP>C/HP<C statistically significant difference in the parameter (more effective HP/C); non-placebo – control group without intervention

Abbreviations:

2-AG - 2-arachidonoylglycerol; **4DSQ** - Four-Dimensional Symptom Questionnaire; **AEA** - anandamide; **ASEX** - Arizona Sexual Experiences Scale; **BAI** - Beck Anxiety Inventory; **BDI** - Beck Depression Inventory; **BEPSI** - Brief Encounter Psychosocial Instrument; **BFI** - Brief Fatigue Inventory; **BP** - blood pressure; **C** - control; **CGI (*I*C)** - Clinical Global Impression (Change); **DAS** - Corah's Dental Anxiety Scale; **DASS-21** - Depression Anxiety and Stress Scale; **DAQ** - Dental Anxiety Questionnaire; **DER** - drug extract ratio; **DSM** - Diagnostic and Statistical Manual of Mental Disorders; **DSSQ** - Dundee Stress State Questionnaire; **EEG** - electroencephalography; **EPQ** - Eysenck Personality Questionnaire; **ERI** - Siegrist's Effort–Reward Imbalance model; **GAD** -

Generalized Anxiety Disorder; **HADS** - Hospital Anxiety and Depression Scale; **HAM-A** - The Hamilton Anxiety Rating Scale; **HAM-D** - Hamilton Depression Rating Scale; **HIV** - human immunodeficiency virus; **HP** - herbal preparation; **HR** - heart rate; **ISI** - Insomnia Severity Index; **K-10** - Kessler Psychological Distress Scale; **MADRS (-S)** – Montgomery-Asberg Depression Rating Scale (self-rated version); **MDAS** - Corah's Modified Dental Anxiety Scale; **MDMQ** - Multidimensional Mood State Questionnaire; **MFI-20** - Multidimensional Fatigue Inventory 20; **MHPG** - 3-methoxy-4-hydroxyphenylglycol; **NAS** - Numeric Analogue Scales; **NCT** - Numbers Connecting Test; **NRS** - numerical rating scale; **OOA/S** - Observer's Assessment of Alertness/Sedation; **PANAS** - Positive and Negative Affect Schedule; **PANSI** - Positive and Negative Suicide Ideation; **PANSS** - Positive and Negative Syndrome Scale; ***p.o.*** - per os (oral administration); **PSQI** - Pittsburg Sleep Quality Inventory; **PSQ-R** – Recent Perceived Stress Questionnaire; **PSS** - Perceived Stress Scale; **PSWQ** - Penn State Worry Questionnaire; **RCT** - randomized controlled trial; **RSS** - Ramsay Sedation Scale; **SHAPS** - Snaith-Hamilton Pleasure Scale; **STAI (-JYZ)** - State-Trait Anxiety Inventory (Form JYZ); **SDS** - Sheehan Disability Scale; **SDS*** - Zung Self-Rating Depression Scale; **SF-36** - Short Form-36 Health Survey; **SOFAS** - Social and Occupational Functioning Assessment Scale; **TSST** - Trier Social Stress Test; **V** - *verum (*positive control); **VAS** - Visual Analogue Scale; **WHO-5** - 5-item World Health Organization Well-Being Index

References:

Abbaszadeh, R., Tabari, F., Asadpour, A., 2020. The Effect of Lavender Aroma on Anxiety of Patients Having Bone Marrow Biopsy. Asian Pacific J. Cancer Prev. 21, 771–775.

Aghamiri, V., Mirghafourvand, M., Mohammad-Alizadeh-Charandabi, S., Nazemiyeh, H., 2016. The effect of Hop (Humulus lupulus L.) on early menopausal symptoms and hot flashes: A randomized placebo-controlled trial. Complement. Ther. Clin. Pract. 23, 130–135.

Ahmadi, M., Khalili, H., Abbasian, L., Ghaeli, P., 2017. Effect of Valerian in Preventing Neuropsychiatric Adverse Effects of Efavirenz in HIV-Positive Patients: A Pilot Randomized, Placebo-Controlled Clinical Trial. Ann. Pharmacother. 51, 457–464.

Ahmadpanah, M., Ramezanshams, F., Ghaleiha, A., Akhondzadeh, S., Sadeghi Bahmani, D., Brand, S., 2019. Crocus Sativus L. (saffron) versus sertraline on symptoms of depression among older people with major depressive disorders–a double-blind, randomized intervention study. Psychiatry Res. 282, 112613.

Akhondzadeh, S., Mostafavi, S., Keshavarz, S.A., Mohammadi, M.R., Hosseini, S., Eshraghian, M.R., 2020. A placebo controlled randomized clinical trial of Crocus sativus L. (saffron) on depression and food craving among overweight women with mild to moderate depression. J. Clin. Pharm. Ther. 45, 134–143.

Andreatini, R., Sartori, V.A., Seabra, M.L. V., Leite, J.R., 2002. Effect of valepotriates (valerian extract) in generalized anxiety disorder: a randomized placebo-controlled pilot study. Phyther. Res. 16, 650–654.

Araj-Khodaei, M., Noorbala, A.A., Yarani, R., Emadi, F., Emaratkar, E., Faghihzadeh, S., Parsian, Z., Alijaniha, F., Kamalinejad, M., Naseri, M., 2020. A double-blind, randomized pilot study for comparison of Melissa officinalis L. and Lavandula angustifolia Mill. with Fluoxetine for the treatment of depression. BMC Complement. Med. Ther. 20, 207.

Aslanargun, P., Cuvas, O., Dikmen, B., Aslan, E., Yuksel, M.U., 2012. Passiflora incarnata Linneaus as an anxiolytic before spinal anesthesia. J. Anesth. 26, 39–44.

Babatabar Darzi, H., Vahedian-Azimi, A., Ghasemi, S., Ebadi, A., Sathyapalan, T., Sahebkar, A., 2020. The effect of aromatherapy with rose and lavender on anxiety, surgical site pain, and extubation time after open-heart surgery: A double-center randomized controlled trial. Phyther. Res. 34, 2675–2684.

Bystritsky, A., Kerwin, L., Feusner, J.D., 2008. A Pilot Study of Rhodiola rosea (Rhodax ®) for Generalized Anxiety Disorder (GAD). J. Altern. Complement. Med. 14, 175–180.

Cropley, M., Banks, A.P., Boyle, J., 2015. The Effects of Rhodiola rosea L. Extract on Anxiety, Stress, Cognition and Other Mood Symptoms. Phyther. Res. 29, 1934–1939.

da Cunha, R.S., Amorim, K.S., Gercina, A.C., de Oliveira, A.C.A., dos Santos Menezes, L., Groppo, F.C., Souza, L.M.A., 2021. Herbal medicines as anxiolytics prior to third molar surgical extraction. A randomized controlled clinical trial. Clin. Oral Investig. 25, 1579–1586.

Darbinyan, V., Aslanyan, G., Amroyan, E., Gabrielyan, E., Malmström, C., Panossian, A., 2007. Clinical trial of Rhodiola rosea L. extract SHR-5 in the treatment of mild to moderate depression. Nord. J. Psychiatry 61, 343–348.

Di Pierro, F., Risso, P., Settembre, R., 2018. Role in depression of a multi-fractionated versus a conventional Hypericum perforatum extract. Panminerva

Med. 60.
Eatemadnia, A., Ansari, S., Abedi, P., Najar, S., 2019. The effect of Hypericum perforatum on postmenopausal symptoms and depression: A randomized controlled trial. Complement. Ther. Med. 45, 109–113.
Edwards, D., Heufelder, A., Zimmermann, A., 2012. Therapeutic Effects and Safety of Rhodiola rosea Extract WS® 1375 in Subjects with Life-stress Symptoms - Results of an Open-label Study. Phyther. Res. 26, 1220–1225.
Farah, G.J., Ferreira, G.Z., Danieletto-Zanna, C.F., Luppi, C.R., Jacomacci, W.P., 2019. Assessment of Valeriana officinalis l. (Valerian) for Conscious Sedation of Patients During the Extraction of Impacted Mandibular Third Molars: A Randomized, Split-Mouth, Double-Blind, Crossover Study. J. Oral Maxillofac. Surg. 77, 1796.e1-1796.e8.
Farshbaf-Khalili, A., Kamalifard, M., Namadian, M., 2018. Comparison of the effect of lavender and bitter orange on anxiety in postmenopausal women: A triple-blind, randomized, controlled clinical trial. Complement. Ther. Clin. Pract. 31, 132–138.
Franco, L., Sánchez, C., Bravo, R., Rodríguez, A.B., Barriga, C., Romero, E., Cubero, J., 2012. The Sedative Effect of Non-Alcoholic Beer in Healthy Female Nurses. PLoS One 7, e37290.
Gao, L., Wu, C., Liao, Y., Wang, J., 2020. Antidepressants effects of Rhodiola capsule combined with sertraline for major depressive disorder: A randomized double-blind placebo-controlled clinical trial. J. Affect. Disord. 265, 99–103.
Gharib, M., Samani, L.N., Panah, Z.E., Naseri, M., Bahrani, N., Kiani, K., 2015. The Effect of Valeric on Anxiety Severity in Women Undergoing Hysterosalpingography. Glob. J. Health Sci. 7.
Jacobs, B.P., Bent, S., Tice, J.A., Blackwell, T., Cummings, S.R., 2005. An Internet-Based Randomized, Placebo-Controlled Trial of Kava and Valerian for Anxiety and Insomnia. Medicine (Baltimore). 84, 197–207.
Karan, N.B., 2019. Influence of lavender oil inhalation on vital signs and anxiety: A randomized clinical trial. Physiol. Behav. 211, 112676.
Kyrou, I., Christou, A., Panagiotakos, D., Stefanaki, C., Skenderi, K., Katsana, K., Tsigos, C., 2017. Effects of a hops (Humulus lupulus L.) dry extract supplement on self-reported depression, anxiety and stress levels in apparently healthy young adults: a randomized, placebo-controlled, double-blind, crossover pilot study. Hormones.
Lekomtseva, Y., Zhukova, I., Wacker, A., 2017. Rhodiola rosea in Subjects with Prolonged or Chronic Fatigue Symptoms: Results of an Open-Label Clinical Trial. Complement. Med. Res. 24, 46–52.
Lopresti, A.L., Smith, S.J., Hood, S.D., Drummond, P.D., 2019. Efficacy of a standardised saffron extract (affron®) as an add-on to antidepressant medication for the treatment of persistent depressive symptoms in adults: A randomised, double-blind, placebo-controlled study. J. Psychopharmacol. 33, 1415–1427.
Mao, J.J., Xie, S.X., Zee, J., Soeller, I., Li, Q.S., Rockwell, K., Amsterdam, J.D., 2015. Rhodiola rosea versus sertraline for major depressive disorder: A randomized placebo-controlled trial. Phytomedicine 22, 394–399.
Moghadam, B.H., Bagheri, R., Roozbeh, B., Ashtary-Larky, D., Gaeini, A.A., Dutheil, F., Wong, A., 2021. Impact of saffron (Crocus Sativus Linn) supplementation and resistance training on markers implicated in depression and happiness levels in untrained young males. Physiol. Behav. 233, 113352.
Movafegh, A., Alizadeh, R., Hajimohamadi, F., Esfehani, F., Nejatfar, M., 2008. Preoperative Oral Passiflora Incarnata Reduces Anxiety in Ambulatory Surgery

Patients: A Double-Blind, Placebo-Controlled Study. Anesth. Analg. 106, 1728–1732.
Ngan, A., Conduit, R., 2011. A Double-blind, Placebo-controlled Investigation of the Effects of Passiflora incarnata (Passionflower) Herbal Tea on Subjective Sleep Quality. Phyther. Res. 25, 1153–1159.
Ohara, K., Misaizu, A., Kaneko, Y., Fukuda, T., Miyake, M., Miura, Y., Okamura, H., Yajima, J., Tsuda, A., 2018. β-Eudesmol, an Oxygenized Sesquiterpene, Reduces the Increase in Saliva 3-Methoxy-4-Hydroxyphenylglycol After the "Trier Social Stress Test" in Healthy Humans: A Randomized, Double-Blind, Placebo-Controlled Cross-Over Study. Nutrients 11, 9.
Roh, D., Jung, J.H., Yoon, K.H., Lee, C.H., Kang, L.Y., Lee, S., Shin, K., Kim, D.H., 2019. Valerian extract alters functional brain connectivity: A randomized double-blind placebo-controlled trial. Phyther. Res. 33, 939–948.
Ross, S.M., 2014. Rhodiola rosea (SHR-5), Part 2. Holist. Nurs. Pract. 28, 217–221.
Salek, R., Dehghani, M., Mohajeri, S.A., Talaei, A., Fanipakdel, A., Javadinia, S.A., 2021. Amelioration of anxiety, depression, and chemotherapy related toxicity after crocin administration during chemotherapy of breast cancer: A double blind, randomized clinical trial. Phyther. Res. 35, 5143–5153.
Sarris, J., Byrne, G.J., Bousman, C.A., Cribb, L., Savage, K.M., Holmes, O., Murphy, J., Macdonald, P., Short, A., Nazareth, S., Jennings, E., Thomas, S.R., Ogden, E., Chamoli, S., Scholey, A., Stough, C., 2020. Kava for generalised anxiety disorder: A 16-week double-blind, randomised, placebo-controlled study. Aust. New Zeal. J. Psychiatry 54, 288–297.
Seifritz, E., Hatzinger, M., Holsboer-Trachsler, E., 2016. Efficacy of Hypericum extract WS ® 5570 compared with paroxetine in patients with a moderate major depressive episode – a subgroup analysis. Int. J. Psychiatry Clin. Pract. 20, 126–132.
Shammas, R.L., Marks, C.E., Broadwater, G., Le, E., Glener, A.D., Sergesketter, A.R., Cason, R.W., Rezak, K.M., Phillips, B.T., Hollenbeck, S.T., 2021. The Effect of Lavender Oil on Perioperative Pain, Anxiety, Depression, and Sleep after Microvascular Breast Reconstruction: A Prospective, Single-Blinded, Randomized, Controlled Trial. J. Reconstr. Microsurg. 37, 530–540.
Stanley, P.F., Wan, L.F., Karim, R.A., 2020. A Randomized Prospective Placebo-Controlled Study of the Effects of Lavender Aromatherapy on Preoperative Anxiety in Cataract Surgery Patients. J. PeriAnesthesia Nurs. 35, 403–406.
Szegedi, A., Kohnen, R., Dienel, A., Kieser, M., 2005. Acute treatment of moderate to severe depression with hypericum extract WS 5570 (St John's wort): randomised controlled double blind non-inferiority trial versus paroxetine. BMJ 330, 503.
Warren, M.B., Cowen, P.J., Harmer, C.J., 2019. Subchronic treatment with St John's wort produces a positive shift in emotional processing in healthy volunteers. J. Psychopharmacol. 33, 194–201.
Yechiam, E., Ben-Eliezer, D., Ashby, N.J.S., Bar-Shaked, M., 2019. The acute effect of Hypericum perforatum on short-term memory in healthy adults. Psychopharmacology (Berl). 236, 613–623.

Phytotherapy of mood disorders in the light of microbiota-gut-brain axis.

Maciej Korczak[1], Maciej Pilecki[2], Sebastian Granica[1], Aleksandra Gorczyńska[1], Karolina A. Pawłowska[1], Jakub P. Piwowarski[1*]

[1]Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland

[2]Department of Psychiatry, Collegium Medicum, Jagiellonian University, Cracow

*Corresponding author: Jakub P. Piwowarski, ul. Banacha 1, 02-097 Warsaw, Poland, tel/fax +48 22 5720985, jpiwowarski@wum.edu.pl

Tabe S4. Preclinical studies (in vivo)

Plant material	Extract/ comopund administrated	Tested model	Effect	Dose/ concentration	Ref.
Valeriana officinalis	valeric acid	Wistar rats	urine ca. 7.07 μM portal blood 2.19-23.82 μM systemic blood 0.26-7.55 μM brain ca. 0.12 μM liver ca. 1.01 μM kidneys ca. 0.24 μM heart ca 0.52 μM lungs ca. 0.22 μM	0.15-0.60 mmol/kg b.w.	(Onyszkiewicz et al., 2020)
	valerian extract (Sedonium)	healthy human volunteers	Serum 0.1-2.8 μg/ml of valerenic acid	600 mg of extract	(Anderson et al., 2005)
	valerenic acid	Male Spraque Dawley Rats	Plasma 0.23-2.38 μg/ml of valerenic acid	5-20 mg/ kg b.w.	(Sampath et al., 2012)
Lavandula officinalis	linalool/linalyl acetate	healthy volunteers	Plasma linalool 2.2-9.0 ng/ml Linalyl acetate <2.0 ng/ml	160 mg of *Lavandula* essential oil	(Doroshyenko et al., 2013)
	linalool **A** linalyl acetate **B** lavender essential oil **C**	rats	**A**: linalool plasma ca. 33 ng/ml liver ca. 218 ng/ml kidney ca. 541 ng/ml fat ca. 1140 ng/ml brain ca. 43 ng/ml **B**: linalool	100 mg/kg b.w of *Lavandula* essential oil or 28,9 mg/kg b.w. of linalool or 36.8 mg/kg b.w of linalyl acetate	(Nöldner et al., 2011)

			plasma ca. 10 ng/ml Liver ca. 274 ng/ml Kidney ca. 255 ng/ml Fat ca. 244 ng/ml **C**: linalool plasma ca. 77 ng/ml liver ca. 2287 ng/ml kidney ca. 670 ng/ml fat ca. 2085 ng/ml brain ca. 164 ng/ml linlyl acetate: brain ca.31 ng/ml		
	Lavender essential oil	rats	linalool: plasma 77-108 ng/ml, brain 140-164 ng/ml, liver 278-2095 ng/ml, kidneys 670-1547 ng/ml, fat 2085-3958 ng/ml Linalyl acetate: brain 21-48 ng/ml, liver ca. 25 ng/ml, kidneys ca. 12 ng/ml, fat ca. 11 ng/ml	100 mg/kg b.w of *Lavandula* essential oil	(Müller et al., 2015)
	Lavender essential oil	Wistar rats	Linalool in plasma 1.92-2.20 μg/ml	200 mg/kg b.w. of lavender essential oil	(Xu et al., 2021)
Piper methysticum	Kava-kava extract (30% kavalactones)	mice	plasma kavain 72-7833 ng/ml	kavain 50 mg/kg b.w., kava-kava extract 1-24.6 mg/kg b.w	(Ferreira et al., 2020)

	Kava-kava extract	mice	kavain: urine 1775-3019 pg/ml, plasma 9473-18618 pg/ml dihydrokavain: urine 1330-1350 pg/ml, plasma 28765-51037 pg/ml methysticin: plasma 6959-11732 pg/ml dihydromethysticin: urine 688-984 pg/ml plasma 54140-75591 pg/ml desmetoxyyangonin: urine 1629-1972 pg/ml plasma 260-808 pg/ml	1 mg of raw extract	(Wang et al., 2018)
	Kava-kava extract	Rats male F-344	kavain: plasma 0.2-7.2 μg/ml	Kava kava extract 256 mg-1g/kg b.w.	(Mathews et al., 2005)
	Kavain	Rats male F-344	kavain: blood ca. 1240 ng-Eq/g brain ca. 38.8 ng-Eq/g heart ca. 309 ng-Eq/g kidneys ca. 2370 ng-Eq/g liver ca. 3150 ng-Eq/g lung ca. 497 ng-Eq/g muscle ca. 121 ng-Eq/g skin ca. 622 ng-Eq/g	[14C]-kavain 100 mg/kg b.w.	(Mathews et al., 2005)
	Kava-kava extract	Human volunteers	Urine: 6-phenyl-3-hexen-2-one ca. 0.6 μg/ml	10 g of kava-kava extract (1.3 g kavalactones)	(Zou et al., 2005)

Humulus lupulus	Hop standardized extract (ICE-4)	Male C57BL/6 mice	Urine: cohumolone 36.41-58.86 ng/ml α-acids humulone 29.14-115.20 ng/ml adhumolone 36.23-79.83 ng/ml β-acids lupulone 4.54-8.24 ng/ml adlupulone 1.78-2.55 ng/ml plasma: : cohumolone 5.28-13.35 ng/ml α-acids humulone 6.41-18.30 ng/ml adhumolone 4.34-11.03 ng/ml β-acids lupulone 1.41-1.67 ng/ml adlupulone 0.26-0.27 ng/ml	0.4 mg/kg b.w of extract	(Salviati et al., 2021)
	xanthohumol	Human volunteers	Plasma 63-178 μg/ml	20/60/180 mg of compound	(Legette et al., 2014)
	xanthohumol **A** hop extract **B**	Wistar rats	blood **A** xanthohumol 22-168 μg/l orally **B** Hop extract xanthohumol 9.46-14.38 μg/l orally Xanthohumol intraveonously ca. 9061 μg/l	40/100/200 mg/kg b.w orally 10 mg/kg b.w. intravenously	(Nowak et al., 2020)

	Hop extract	female human volunteers	Urine: xanthohumol 23.9-138 nmols isoxanthohumol 29.8-172 nmols 8-prenylnaryngenin 5.1-29.4 nmols 6-prenylnaryngenin 26.2-150 nmols Plasma: xanthohumol 4.4-27.6 ng/ml isoxanthohumol 5.7-37.6 ng/ml 8-prenylnaryngenin 1.4-6.7 ng/ml 6-prenylnaryngenin 1.6-5.2 ng/ml	59.5/119/238 mg	(van Breemen et al., 2014)
	6-/8-prenylnaryngenin	Human volunteers	Urine: 6-prenylnaryngenin 4.2-4.3 mg 8-prenylnaryngenin 6.2-8.1 mg Plasma: 6-prenylnaryngenin 483.4-601.9 nmol/l 8-prenylnaryngenin 2249.8-3417.7 nmol/l	500 mg of 6-/8-prenylnaryngenin	(Calvo-Castro et al., 2018)
	R/S-isoxanthohumol/R/S-8-prenylnaryngenin	CD Sprague-Dawley rats	urine: S-isohanthohumol ca. 9 mg total R-isohanthohumol ca. 11 mg total	50 mg/kg b.w. of pure compound PO	(Martinez and Davies, 2015)

			R-8-prenylnaryngenin ca. 13.2 mg total S-8-prenylnaryngenin ca. 5.3 mg total serum: S-isohanthohumol ca. 0.6 μg/ml R-isohanthohumol ca. 0.8 μg/ml		
	R/S-8-prenylnaryngenin	Rats	urine: S-8-prenylnaryngenin ca. 17 μg R-8-prenylnaryngenin ca. 11 μg serum: S-8-prenylnaryngenin ca. 1 μg/ml R-8-prenylnaryngenin ca. 1 μg/ml	10 mg/kg b.w. of racemic compound	(Martinez et al., 2014)
	Beer with hop extract	Human volunteers	blood: 0.08-0.12 mg/l of trans-iso-humolones	10-40 ml/l of iso-alpha acids	(Rodda et al., 2014)
	xanthohumol/isoxanthohumol/8-prenylnaryngenin	Sprague-Dawley rats	plasma: xanthohumol 0.02-0.15 μg/l isoxanthohumol 3.27-31.1 μg/l 8-prenylnaryngenin 1.6-14.7 μg/l	1.86/5.64/16.9 mg/kg b.w	(Legette et al., 2012)
Mellisa officinalis	*Mellisa officinalis* extract **A** rosmarinic acid **B**	Healthy volunteers	Serum: **A** rosmarinic acid 72.2-142.2 nmol/l **B** rosmarinic acid 72.7-94.9 nmol/l	extract 300-1600 mg rosmarinic acid in the extract 100/250 or 500 mg	(Noguchi-Shinohara et al., 2015)

Rhodiola rosea	rosavin	Sprague–Dawley rats	Plasma: rosavin 105.2-324.3 ng/ml	10/20 mg/kg b.w.	(Zhang et al., 2019)
	rosavin	Sprague–Dawley rats	Plasma: rosavin 63.0-113.8 ng/ml	10/50 mg/kg b.w.	(Chen et al., 2016)
	salidroside	Wistar rats	urine: salidroside 7.2-8.0 mg total plasma salidroside 10-84 ng/l tissues: salidroside 0.2-2.7 ng/g feases: salidroside 0.15-27.7 ng total	7.5-30 mg/kg b.w.	(Zhang et al., 2013)
	salidroside	rats	Plasma: salidroside ca. 3716.7 ng/ml	100 mg/kg b.w.	(Guo et al., 2012)
	salidroside	Sprague–Dawley rats	Plasma: salidroside 1.0-4.3 μg/ml orally salidroside 1.0-27.9 μg/ml intravenously	12 mg/kg b.w.	(Yu et al., 2008)
	salidroside	Beagle dogs	Plasma: salidroside 1.5-96.2 μg/ml intravenously	75 mg/kg b.w. intravenously	(Mao et al., 2007)
	salidroside	Sprague–Dawley rats	Plasma: salidroside 100-6493 ng/ml orally salidroside 100-10000 ng/ml intravenously	5 mg/kg b.w. intravenously 25 mg/kg b.w. orally	(Chang et al., 2007)
Crocus sativus	*Crocus* extract	Sprague–Dawley rats	plasma: trans-4-GG-crocin ca. 49.3 ng/ml	40 mg/kg b.w.	(Girme et al., 2021)

			trans-3-GG-crocin ca. 7.6 ng/ml cis-4-GG-crocin ca. 86.1 ng/ml trans-2-gg-crocin ca. 160.4 ng/ml trans-crocetin ca. 2076.2 ng/ml picrocrocin ca. 2722.9 ng/ml		
	crocin	Sprague–Dawley rats	Plasma: crocin 74.9-304.4 ng/ml crocetin 9732-10262 ng/ml urine: crocin 400-800 ng/ml crocetin 200-1400 ng/ml feces: crocin up to 200000 ng/ml crocetin up to 30000 ng/ml	600 mg.kg b.w.	(Shakya et al., 2020)
	Crocus extract (affron)	human volunteers	Plasma: crocetin and derivatives 0.26-0.39 μg/ml	56 mg/84 mg of extract	(Almodóvar et al., 2020)
	Crocus extract	mice	serum: crocetin 3.4-3.9 μg/ml intravenously 2.8-3.5 μg/ml orally Liver: crocetin 2.5-3.1 μg/ml intravenously	60 mg/kg b.w.	(Christodoulou et al., 2019)

			2.6-2.9 μg/ml orally Kidneys: 2.1-2.5 μg/ml intravenously 2.4-2.5 μg/ml orally		
	crocetin	Human volunteers	Plasma: crocetin 100.9-279.7 ng/ml	7.5/15/22.5 mg	(Umigai et al., 2011)
	crocetin	Human volunteers	Plasma: crocetin 0.09-0.35 μg/ml	16 mg	(Mohammadpour et al., 2013)
	Crocus tea	Human volunteers	Plasma: crocetin 0.1-3.7 μM	200 mg of safran in 150 ml of water	(Chryssanthi et al., 2011)
	crocin	Sprague–Dawley rats	Plasma: crocin ca. 0.8 μg/ml Duodenum: crocin 36.0-37.3 mg total Jejunum: crocin 36.2-36.9 mg total Ileum: crocin 36.3-36.9 mg total Colon: crocin 35.1-35.5 mg total	40 mg/kg b.w.	(Xi et al., 2007)
	crocin-1	rats	Plasma: crocin 4.5-11.2 μg/ml	20/40/80 mg/kg b.w.	(Xi and Qian, 2006)
Hypericum perforatum	hyperforin	Rodents (rats/mice)	Plasma: hyperforin 0.04-1.4 nmol/ml Brain hyperforin 0.01-0.06 nmol/ml	0.1-300 mg/kg b.w.	(Schulz et al., 2005)
	Hypericum extract	Healthy volunteers	Plasma: hyperforin 31.1-246 μg/l hypericin 4.4-29 μg/l	612-1200 mg/day	(Caccia, 2005; Caccia and Gobbi, 2010)

			pseudohypericin 5.2-15 μg/l quercetin 45.6-89.2 μg/l isorhamnetin 8.7-17.1 μg/l		
	Hypericum extract	Rats/mice	Plasma: quercetin 3.3-9.6 nnmol/ml Isorhamnetin 1.1-7.4 nmol/ml 13,II8-biapigenin 0.02-0.06 nmol/ml Brain: quercetin 0.1-1.3 nmol/g Isorhamnetin 0.02-2.02 nmol/g 13,II8-biapigenin <0.01 nmol/g	500-1600 mg/kg b.w.	(Caccia and Gobbi, 2010)
	hypericin	mice	Plasma: hypericin 1.0-100 μM	2/5 mg/kg b.w intravenously	(Fox et al., 2001)
	amentoflavone	mice	Plasma: amentoflavone 0.7-23.9 nmol/ml	3/10/30 mg/kg b.w	(Caccia and Gobbi, 2010)
	12,II8-Biapigenin	mice	Plasma: 12,II8-Biapigenin ca.24 nmol/ml Brain: 12,II8-Biapigenin ca. 0.03 nmol/g	10 mg/kg b.w.	(Caccia and Gobbi, 2010)

References:

Almodóvar, P., Briskey, D., Rao, A., Prodanov, M., Inarejos-García, A.M., 2020. Bioaccessibility and Pharmacokinetics of a Commercial Saffron (Crocus sativus L.) Extract. Evidence-based Complement. Altern. Med. 2020.

Anderson, G.D., Elmer, G.W., Kantor, E.D., Templeton, I.E., Vitiello, M. V., 2005. Pharmacokinetics of valerenic acid after administration of valerian in healthy subjects. Phyther. Res. 19.

Caccia, S., 2005. Antidepressant-Like Components of Hypericum perforatum Extracts: An Overview of Their Pharmacokinetics and Metabolism. Curr. Drug Metab. 6.

Caccia, S., Gobbi, M., 2010. St. Johns Wort Components and the Brain: Uptake, Concentrations Reached and the Mechanisms Underlying Pharmacological Effects. Curr. Drug Metab. 10.

Calvo-Castro, L.A., Burkard, M., Sus, N., Scheubeck, G., Leischner, C., Lauer, U.M., Bosy-Westphal, A., Hund, V., Busch, C., Venturelli, S., Frank, J., 2018. The Oral Bioavailability of 8-Prenylnaringenin from Hops (Humulus Lupulus L.) in Healthy Women and Men is Significantly Higher than that of its Positional Isomer 6-Prenylnaringenin in a Randomized Crossover Trial. Mol. Nutr. Food Res. 62.

Chang, Y.W., Yao, H.T., Hsieh, S.H., Lu, T.J., Yeh, T.K., 2007. Quantitative determination of salidroside in rat plasma by on-line solid-phase extraction integrated with high-performance liquid chromatography/electrospray ionization tandem mass spectrometry. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 857.

Chen, D., Sun, H., Shen, J., Igor, L.P., Zheng, X., Hu, S., Xiang, Z., 2016. A Simple and Rapid UPLC Method for the Determination of Rosavin in Rat Plasma and Its Application to a Pharmacokinetic Study. J. Chromatogr. Sci. 54.

Christodoulou, E., Grafakou, M.E., Skaltsa, E., Kadoglou, N., Kostomitsopoulos, N., Valsami, G., 2019. Preparation, chemical characterization and determination of crocetin's pharmacokinetics after oral and intravenous administration of saffron (Crocus sativus L.) aqueous extract to C57/BL6J mice. J. Pharm. Pharmacol. 71.

Chryssanthi, D.G., Lamari, F.N., Georgakopoulos, C.D., Cordopatis, P., 2011. A new validated SPE-HPLC method for monitoring crocetin in human plasma-Application after saffron tea consumption. J. Pharm. Biomed. Anal. 55.

Doroshyenko, O., Rokitta, D., Zadoyan, G., Klement, S., Schläfke, S., Dienel, A., Gramatté, T., Lück, H., Fuhr, U., 2013. Drug cocktail interaction study on the effect of the orally administered lavender oil preparation silexan on cytochrome p450 enzymes in healthy volunteers. Drug Metab. Dispos. 41.

Ferreira, J.V., Braga, A.V., Machado, R. de R., Michel, D., Pianetti, G.A., El-Aneed, A., César, I.C., 2020. Liquid chromatography-tandem mass spectrometry bioanalytical method for the determination of kavain in mice plasma: Application to a pharmacokinetic study. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1137.

Fox, E., Murphy, R.F., McCully, C.L., Adamson, P.C., 2001. Plasma pharmacokinetics and cerebrospinal fluid penetration of hypericin in nonhuman primates. Cancer Chemother. Pharmacol. 47.

Girme, A., Pawar, S., Ghule, C., Shengule, S., Saste, G., Balasubramaniam, A.K., Deshmukh, A., Hingorani, L., 2021. Bioanalytical method development and validation study of neuroprotective extract of kashmiri saffron using ultra-fast liquid chromatography-tandem mass spectrometry (Uflc-ms/ms): In vivo pharmacokinetics of apocarotenoids

and carotenoids. Molecules 26.
Guo, N., Hu, Z., Fan, X., Zheng, J., Zhang, D., Xu, T., Yu, T., Wang, Y., Li, H., 2012. Simultaneous determination of Salidroside and its aglycone metabolite p-tyrosol in rat plasma by liquid chromatography-tandem mass spectrometry. Molecules 17.
Legette, L., Karnpracha, C., Reed, R.L., Choi, J., Bobe, G., Christensen, J.M., Rodriguez-Proteau, R., Purnell, J.Q., Stevens, J.F., 2014. Human pharmacokinetics of xanthohumol, an antihyperglycemic flavonoid from hops. Mol. Nutr. Food Res. 58.
Legette, L., Ma, L., Reed, R.L., Miranda, C.L., Christensen, J.M., Rodriguez-Proteau, R., Stevens, J.F., 2012. Pharmacokinetics of xanthohumol and metabolites in rats after oral and intravenous administration. Mol. Nutr. Food Res. 56.
Mao, Y., Zhang, Xinrong, Zhang, Xiaodong, Lu, G., 2007. Development of an HPLC method for the determination of salidroside in beagle dog plasma after administration of salidroside injection: Application to a pharmacokinetics study. J. Sep. Sci. 30.
Martinez, S.E., Davies, N.M., 2015. Enantiospecific pharmacokinetics of isoxanthohumol and its metabolite 8-prenylnaringenin in the rat. Mol. Nutr. Food Res. 59.
Martinez, S.E., Lakowski, T.M., Davies, N.M., 2014. Enantiospecific Analysis of 8-Prenylnaringenin in Biological Fluids by Liquid-Chromatography-Electrospray Ionization Mass Spectrometry: Application to Preclinical Pharmacokinetic Investigations. Chirality 26.
Mathews, J.M., Etheridge, A.S., Valentine, J.L., Black, S.R., Coleman, D.P., Patel, P., So, J., Burka, L.T., 2005. Pharmacokinetics and disposition of the kavalactone kawain: Interaction with kava extract and kavalactones in vivo and in vitro. Drug Metab. Dispos. 33.
Mohammadpour, A.H., Ramezani, M., Tavakoli Anaraki, N., Malaekeh-Nikouei, B., Amel Farzad, S., Hosseinzadeh, H., 2013. Development and Validation of HPLC Method for Determination of Crocetin, a constituent of Saffron, in Human Serum Samples. Iran. J. Basic Med. Sci. 16, 47–55.
Müller, W.E., Schuwald, A., Nöldner, M., Siegried, K., Friedland, K., 2015. Pharmakologische Grundlagen der therapeutischen Anwendung von Silexan (Lasea®). Psychopharmakotherapie 3–14.
Noguchi-Shinohara, M., Ono, K., Hamaguchi, T., Iwasa, K., Nagai, T., Kobayashi, S., Nakamura, H., Yamada, M., 2015. Pharmacokinetics, Safety and Tolerability of Melissa officinalis Extract which Contained Rosmarinic Acid in Healthy Individuals: A Randomized Controlled Trial. PLoS One 10, e0126422.
Nöldner, M., Germer, S., Koch, E., 2011. Pharmacokinetics of linalool and linalyl acetate, the two main constituents of silexan, an essential oil from Lavandula angustifolia flowers, in rats. Planta Med. 77.
Nowak, B., Poźniak, B., Popłoński, J., Bobak, Ł., Matuszewska, A., Kwiatkowska, J., Dziewiszek, W., Huszcza, E., Szeląg, A., 2020. Pharmacokinetics of xanthohumol in rats of both sexes after oral and intravenous administration of pure xanthohumol and prenylflavonoid extract. Adv. Clin. Exp. Med. 29.
Onyszkiewicz, M., Gawrys-Kopczynska, M., Sałagaj, M., Aleksandrowicz, M., Sawicka, A., Koźniewska, E., Samborowska, E., Ufnal, M., 2020. Valeric acid lowers arterial blood pressure in rats. Eur. J. Pharmacol. 877.
Rodda, L.N., Gerostamoulos, D., Drummer, O.H., 2014. Pharmacokinetics of iso-α-acids in volunteers following the consumption of beer. J. Anal. Toxicol. 38.
Salviati, E., Sommella, E., Carrizzo, A., Di Sarno, V., Bertamino, A., Venturini, E., Vecchione, C.,

Campiglia, P., 2021. Characterization of phase I and phase II metabolites of hop (Humulus lupulus L.) bitter acids: In vitro and in vivo metabolic profiling by UHPLC-Q-Orbitrap. J. Pharm. Biomed. Anal. 201.

Sampath, C., Haug, K., Thanei, S., Hamburger, M., Derendorf, H., Frye, R., Butterweck, V., 2012. Pharmacokinetics of valerenic acid in rats after intravenous and oral administrations. Planta Med. 78.

Schulz, H.U., Schürer, M., Bässler, D., Weiser, D., 2005. Investigation of the bioavailability of hypericin, pseudohypericin, hyperforin and the flavonoids quercetin and isorhamnetin following single and multiple oral dosing of a hypericum extract containing tablet. Arzneimittel-Forschung/Drug Res. 55.

Shakya, R., Nepal, M.R., Kang, M.J., Jeong, T.C., 2020. Effects of Intestinal Microbiota on Pharmacokinetics of Crocin and Crocetin in Male Sprague-Dawley Rats. Metabolites 10.

Umigai, N., Murakami, K., Ulit, M. V., Antonio, L.S., Shirotori, M., Morikawa, H., Nakano, T., 2011. The pharmacokinetic profile of crocetin in healthy adult human volunteers after a single oral administration. Phytomedicine 18.

van Breemen, R.B., Yuan, Y., Banuvar, S., Shulman, L.P., Qiu, X., Ramos Alvarenga, R.F., Chen, S.N., Dietz, B.M., Bolton, J.L., Pauli, G.F., Krause, E., Viana, M., Nikolic, D., 2014. Pharmacokinetics of prenylated hop phenols in women following oral administration of a standardized extract of hops. Mol. Nutr. Food Res. 58.

Wang, Y., Eans, S.O., Stacy, H.M., Narayanapillai, S.C., Sharma, A., Fujioka, N., Haddad, L., McLaughlin, J., Avery, B.A., Xing, C., 2018. A stable isotope dilution tandem mass spectrometry method of major kavalactones and its applications. PLoS One 13, e0197940.

Xi, L., Qian, Z., 2006. Pharmacological properties of crocetin and crocin (digentiobiosyl ester of crocetin) from saffron. Nat. Prod. Commun.

Xi, L., Qian, Z., Du, P., Fu, J., 2007. Pharmacokinetic properties of crocin (crocetin digentiobiose ester) following oral administration in rats. Phytomedicine 14.

Xu, L., Li, X., Zhang, Y., Ding, M., Sun, B., Su, G., Zhao, Y., 2021. The effects of linalool acupoint application therapy on sleep regulation. RSC Adv. 11.

Yu, S., Liu, L., Wen, T., Liu, Y., Wang, D., He, Y., Liang, Y., Liu, X., Xie, L., Wang, G., Wei, W., 2008. Development and validation of a liquid chromatographic/electrospray ionization mass spectrometric method for the determination of salidroside in rat plasma: Application to the pharmacokinetics study. J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 861.

Zhang, M., Hu, Z., Fang, B., Bao, X., Xiang, Z., Wang, H., 2019. Pharmacokinetic study of rosavin in rat plasma with ultra performance LC-MS/MS after intravenous and gavage administration. Bioanalysis 11.

Zhang, Y., Li, L., Lin, L., Liu, J., Zhang, Z., Xu, D., Xiang, F., 2013. Pharmacokinetics, tissue distribution, and excretion of salidroside in rats. Planta Med. 79.

Zou, L., Harkey, M.R., Henderson, G.L., 2005. Synthesis, in vitro reactivity, and identification of 6-phenyl-3-hexen-2- one in human urine after kava-kava (Piper methysticum) ingestion. Planta Med. 71.

Phytotherapy of mood disorders in the light of microbiota-gut-brain axis.

Maciej Korczak[1], Maciej Pilecki[2], Sebastian Granica[1], Aleksandra Gorczyńska[1], Karolina A. Pawłowska[1], Jakub P. Piwowarski[1*]

[1]Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland

[2]Department of Psychiatry, Collegium Medicum, Jagiellonian University, Cracow

*Corresponding author: Jakub P. Piwowarski, ul. Banacha 1, 02-097 Warsaw, Poland, tel/fax +48 22 5720985, jpiwowarski@wum.edu.pl

Table S5. Preclinical studies (in vitro)

Plant material	**Extract/** compound **used for bioassay**	**Tested model**	**Effect/detected metabolites**	C**oncentration used**	Ref.
Valeriana officinalis	valerenic acid	Wistar and TR rats liver perfusion	glucuronides of valerenic and hydroxyvalerenic acids	20 μM	(Maier-Salamon et al., 2009)
	valerenic acid hydroxyvalerenic acid acetoxyvalerenic acid	Rat C6 glioma cells	all compounds are able to pass through the cell monolayers	100 μM	(Neuhaus et al., 2008)
Piper methysticum	kava-kava extract	Caco-2 cells	Kawalactones able to pass cell monolayers: methysticin dihydromethysticin kawain dihydrokawain dehydromethysticin desmethoxyyngonin yangonin	not given	(Matthias et al., 2007)
	flavokawain A flavokawain B flavokawain C cardamonin	human liver macrosomes	hydroxylated and metoxylated derivatives of investigated compounds including helichrysetin; glucuronides of tested compouds including flavokawain A 2'-O-glucuronide	10-100 μM	(Zenger et al., 2015)
Passiflora incarnata	orientin isoorientin schaftoside	Caco-2 cells	all compounds are able to pass throung the cell monolayers;	10-100 μM	(Tremmel et al., 2021)

	isoschaftoside vitexin isovitexin		detection of hydroxylated and methoxylated deriavtives of some flavonoids conjugates with sulfate and glucuronic acid present for some of compounds		
Humulus lupulus	terpinol geraniol nerol linalool terpin humulol caryolanol	Caco-2	all compounds were able to pass cell monolayer	100 μM	(Heinlein et al., 2014)
	cohumolone n-humulone adhumulone colupulone n-lupulone adlupulone	Caco-2	all compounds were able to pass cell monolayer	50 μM	(Cattoor et al., 2010)
	cohumulone n-humulone adhumulone colupulone n-lupulone adlupulone xanthohumol	Mouse liver microsomes	products of oxidation, desaturation, reduction and glucuronidation of investigated compounds were detected	0.4-3.3 mM	(Salviati et al., 2021)
	R/S-8-prenylnaryngenin	Human liver microsomes	different glucuronides of investigated compound were detected	1-100 μM	(Fang et al., 2019)

	isoxanthohumol	fungus *Rhizopus oryzae* KCTC 6399	different microbioal metabolites including cyclization products	0.1 mg/ml	(Kim et al., 2019)
	xanthohumol 8-prenylnaryngenin	bateria *Eubacterium ramulus* and *Eubacterium limosum*	Detection of several metabolites including *O*-desmethylxanthohumol and *O*-desmethyl-*α,β*-dihydroxanthohumol	50 μM	(Paraiso et al., 2019)
	6-prenylnaryngenin	Pork liver enzymes	glucuronides and sulfates of investigated compoud	154 μM	(Van De Schans et al., 2015)
	cohumulone humulone adhumulone colupulone lupulone adlupulone	rabbit microsomes	several metabolites of investigated compounds detected	20 μM	(Cattoor et al., 2013)
	8-prenylnaryngenin	different fungi	sulphates of investigated compound detected	5 mg/ml	(Bartmańska et al., 2013)
	iso-α-acids dihydroiso-α-acids tetrahydroios-α-acids	Caco-2	all compounds can pass cell monolayers	30-120 μM	(Cattoor et al., 2011)
	xanthohumol	gut microbiota	conversion to isoxanthohumol and 8-prenylnaryngenin	100 μM	(Hanske et al., 2010)
Melissa officinalis	rosmarinic acid	human gastrointestinal enzymes	degradation to caffeic acids and other compounds	not given	(Zorić et al., 2016)
Crocus sativus	picrocrocin crocetin trans-4-GG crocetin	Caco-2	all compounds could pass cell monolayers	8-24 μM	(Kyriakoudi et al., 2015)
	crocin-1 trans-crocetin	Caco-2 BCEC	crocin-1 does not penetrate caco-2 monolayers while	10-1000 μM	(Lautenschläger et al., 2015)

			crocetin passes cell monolayers of Caco-2 and BCEC cells		
	Saffron extract	artificial gastrointestinal fluids	crocins and crocetin are degradated in simulated experiments no metabolites were characterized	7.5/15 mg corcin esters per liter	(Kyriakoudi et al., 2013)
Hypericum perforatum	extract of *Hypericum perforatum*	Caco-2	hypericin, quercetin, hyperoside, isoquercitrin, quercitrin and rutin were able to cross cells monolayers	5-20 μM	(Verjee et al., 2019)
	protohypericin/hypericin	Caco-2	compounds passes cells monolayers in small quantities and accumulate in cells	80-200 μM	(Kamuhabwa et al., 1999)
	amentoflavone	BCEC	Tested compound could pass cell monolayers and was also present in cells	not given	(Gutmann et al., 2002)

References:

Bartmańska, A., Tronina, T., Huszcza, E., 2013. Microbial sulfation of 8-prenylnaringenin. Z. Naturforsch. C. 68, 231–5.

Cattoor, K., Dresel, M., De Bock, L., Boussery, K., Van Bocxlaer, J., Remon, J.P., De Keukeleire, D., Deforce, D., Hofmann, T., Heyerick, A., 2013. Metabolism of hop-derived bitter acids. J. Agric. Food Chem. 61.

Cattoor, K., Remon, J.P., Boussery, K., Van Bocxlaer, J., Bracke, M., De Keukeleire, D., Deforce, D., Heyerick, A., 2011. Bioavailability of hop-derived iso-α-acids and reduced derivatives. Food Funct. 2.

Cattoor, K.O., Bracke, M., Deforce, D., De Keukeleire, D., Heyerick, A., 2010. Transport of hop bitter acids across intestinal Caco-2 cell monolayers. J. Agric. Food Chem. 58.

Fang, J.B., Nikolić, D., Lankin, D.C., Simmler, C., Chen, S.N., Ramos Alvarenga, R.F., Liu, Y., Pauli, G.F., Van Breemen, R.B., 2019. Formation of (2 R)- And (2 S)-8-Prenylnaringenin Glucuronides by Human UDP-Glucuronosyltransferases. J. Agric. Food Chem. 67.

Gutmann, H., Bruggisser, R., Schaffner, W., Bogman, K., Botomino, A., Drewe, J., 2002. Transport of amentoflavone across the blood-brain barrier in vitro. Planta Med. 68.

Hanske, L., Loh, G., Sczesny, S., Blaut, M., Braune, A., 2010. Recovery and metabolism of xanthohumol in germ-free and human microbiota-associated rats. Mol. Nutr. Food Res. 54.

Heinlein, A., Metzger, M., Walles, H., Buettner, A., 2014. Transport of hop aroma compounds across Caco-2 monolayers. Food Funct. 5.

Kamuhabwa, A.R., Augustijns, P., De Witte, P.A., 1999. In vitro transport and uptake of protohypericin and hypericin in the Caco-2 model. Int. J. Pharm. 188.

Kim, H.J., Yim, S.H., Han, F., Kang, B.Y., Choi, H.J., Jung, D.W., Williams, D.R., Gustafson, K.R., Kennelly, E.J., Lee, I.S., 2019. Biotransformed metabolites of the hop prenylflavanone isoxanthohumol. Molecules 24.

Kyriakoudi, A., OCallaghan, Y.C., Galvin, K., Tsimidou, M.Z., OBrien, N.M., 2015. Cellular Transport and Bioactivity of a Major Saffron Apocarotenoid, Picrocrocin (4-(β- d -Glucopyranosyloxy)-2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde). J. Agric. Food Chem. 63.

Kyriakoudi, A., Tsimidou, M.Z., O'Callaghan, Y.C., Galvin, K., O'Brien, N.M., 2013. Changes in total and individual crocetin esters upon in vitro gastrointestinal digestion of saffron aqueous extracts. J. Agric. Food Chem. 61.

Lautenschläger, M., Sendker, J., Hüwel, S., Galla, H.J., Brandt, S., Düfer, M., Riehemann, K., Hensel, A., 2015. Intestinal formation of trans-crocetin from saffron extract (Crocus sativus L.) and in vitro permeation through intestinal and blood brain barrier. Phytomedicine 22.

Maier-Salamon, A., Trauner, G., Hiltscher, R., Reznicek, G., Kopp, B., Thalhammer, T., Jäger, W., 2009. Hepatic metabolism and biliary excretion of valerenic acid in isolated perfused rat livers: Role of Mrp2 (Abcc2). J. Pharm. Sci. 98.

Matthias, A., Blanchfield, J.T., Penman, K.G., Bone, K.M., Toth, I., Lehmann, R.P., 2007. Permeability studies of Kavalactones using a Caco-2 cell monolayer model. J. Clin. Pharm. Ther. 32.

Neuhaus, W., Trauner, G., Gruber, D., Oelzant, S., Kieoal, W., Kopp, B., Noe, C.R., 2008. Transport of a CABAA receptor modulator and its derivatives from Valeriana officinalis L s. l. across an in vitro cell culture model of the blood-brain

barrier. Planta Med. 74.
Paraiso, I.L., Plagmann, L.S., Yang, L., Zielke, R., Gombart, A.F., Maier, C.S., Sikora, A.E., Blakemore, P.R., Stevens, J.F., 2019. Reductive Metabolism of Xanthohumol and 8-Prenylnaringenin by the Intestinal Bacterium Eubacterium ramulus. Mol. Nutr. Food Res. 63.
Salviati, E., Sommella, E., Carrizzo, A., Di Sarno, V., Bertamino, A., Venturini, E., Vecchione, C., Campiglia, P., 2021. Characterization of phase I and phase II metabolites of hop (Humulus lupulus L.) bitter acids: In vitro and in vivo metabolic profiling by UHPLC-Q-Orbitrap. J. Pharm. Biomed. Anal. 201.
Tremmel, M., Kiermaier, J., Heilmann, J., 2021. In vitro metabolism of six c-glycosidic flavonoids from passiflora incarnata l. Int. J. Mol. Sci. 22.
Van De Schans, M.G.M., Bovee, T.F.H., Stoopen, G.M., Lorist, M., Gruppen, H., Vincken, J.P., 2015. Prenylation and Backbone Structure of Flavonoids and Isoflavonoids from Licorice and Hop Influence Their Phase i and II Metabolism. J. Agric. Food Chem. 63.
Verjee, S., Kelber, O., Kolb, C., Abdel-Aziz, H., Butterweck, V., 2019. Permeation characteristics of hypericin across Caco-2 monolayers in the presence of single flavonoids, defined flavonoid mixtures or Hypericum extract matrix. J. Pharm. Pharmacol. 71.
Zenger, K., Agnolet, S., Schneider, B., Kraus, B., 2015. Biotransformation of Flavokawains A, B, and C, Chalcones from Kava (Piper methysticum), by Human Liver Microsomes. J. Agric. Food Chem. 63.
Zorić, Z., Markić, J., Pedisić, S., Bučevic-Popović, V., Generalic-Mekinić, I., Grebenar, K., Kulišić-Bilušic, T., 2016. Stability of rosmarinic acid in aqueous extracts from different lamiaceae species after in vitro digestion with human gastrointestinal enzymes. Food Technol. Biotechnol. 54.

Publication No. 2

Conjugates of urolithin A with NSAIDs, their stability, cytotoxicity, and anti-inflammatory potential.

Maciej Korczak [a], Piotr Roszkowski [b], Sebastian Granica [a], Jakub Patryk Piwowarski [a]

[a] Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland

[b] Laboratory of Natural Products Chemistry, Faculty of Chemistry, University of Warsaw, Warsaw, Poland.

Scientific Reports. 2022 Jul

12(1):11676

Erratum in: *Scientific Reports*. 2022 Nov; 12(1):18503.

scientific reports

Check for updates

OPEN

Conjugates of urolithin A with NSAIDs, their stability, cytotoxicity, and anti-inflammatory potential

Maciej Korczak[1], Piotr Roszkowski[2], Sebastian Granica[1] & Jakub P. Piwowarski[1]✉

Urolithin A (UA, 1), a gut microbiota postbiotic metabolite is attributed to express interesting biological activities indicated by in vitro, in vivo and clinical studies. Due to its strong anti-inflammatory properties it is considered as a promising lead molecule for further drug development, however, its strong phase II metabolism, severely limits its oral application. Therefore, monoesterified UA derivatives with selected NSAIDs: ibuprofen (Mix 3a/3b), mefenamic acid (Mix 4a/4b), diclofenac (Mix 5a/5b) and aspirin (Mix 6a/6b) were designed. Performed array of stability assays indicated Mix 4a/4b as a most suitable candidate for further studies due to its exceptional stability in human plasma. Thus, we evaluated effects of Mix 4a/4b on cell viability as well as the impact on cytokines secretion in THP-1 derived macrophages and compared it to UA. At high concentration (50 µM) Mix 4a/4b expressed a cytotoxic effect, however at concentration of 5 µM it significantly suppressed TNF-α secretion, and significantly increased ani-inflammatory IL-10 secretion at 10 µM without affecting cell viability. This work has led to selection of a novel UA derivatives, which are stable in solutions and in human plasma as well as posess anti-inflammatory activity towards THP-1 macrophages at non-cytotoxic concentrations.

Abbreviations

AhR	Aryl hydrocarbon receptor
API	Active pharmaceutical ingredient
EA	Ellagic acid
ETs	Ellagitannins
IL-6	Interleukin-6
IL-10	Interleukin-10
LPS	Lipopolysaccharide
NSAIDs	Non-steroidal anti-inflammatory drugs
PBMC	Peripheral blood mononuclear cells
TNF-α	Tumor necrosis factor α
UA	Urolithin A
UADs	Urolithin A derivatives
UGT	Uridine 5′-diphospho-glucuronosyltransferase

In recent years, the gut microbiota emerged as a potent modulator of human health, while the abruption of its structure, dysbiosis, is linked with a whole spectrum of diseases, starting from gastrointestinal conditions and ending with mood disorders[1]. Additionally, the usage of probiotics, defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host", has reached considerable popularity, despite the questionable efficacy in many cases[2,3]. The positive influence on human well-being is not restricted to the living microbes, but the postbiotics and bacterial metabolites can also impact human health. Though the definition of postbiotics is a subject of ongoing discussion, The International Scientific Association of Probiotics and Prebiotics (ISAPP) proposed the interpretation of postbiotics as a "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host"[4]. According to this wording,

[1]Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, ul. Banacha 1, 02-097 Warsaw, Poland. [2]Laboratory of Natural Products Chemistry, Faculty of Chemistry, University of Warsaw, Warsaw, Poland. ✉email: jpiwowarski@wum.edu.pl

the purified metabolites should not be considered as postbiotics per se. Nonetheless, these products of bacterial metabolism are continuously studied, given that their biological activity often surpasses maternal compounds. As a perfect example of purified metabolites marked with high biological activity may serve vitamin B_{12}, equol or 8-prenylnaringenin[5,6].

In recent years the gut microbiome-derived metabolites of ellagitannins (ETs) gained a significant attention as candidates for new bioactive molecules[7]. Plants containing high amounts of ellagitannins have been used in traditional medicine for centuries[8–10]. Ellagitannins exhibit several beneficial biological effects in vitro; however, their potential therapeutical use is limited by their low bioavailability. In vivo, after consuming products rich in ellagitannins, they are hydrolysed into hexahydroxydiphenoic acid, which undergoes lactonisation, resulting in the formation of ellagic acid (EA). EA is furtherly metabolised by gut microbiota to urolithins, characterised, contrary to ETs and EA, with good bioavailability[11,12]. Nonetheless, it should be noted that based on the ability to produce urolithins, 3 separate metabotypes can be distinguished in population: metabotype A producing as a final product only urolithin A (UA, compound **1**, Fig. 1), metabotype B producing in addition isourolithin A and urolithin B, and metabotype 0 deprived of appropriate bacteria capable to produce urolithins[13]. Knowing that the administration of ETs does not result in the formation of UA in around 10% of the population (metabotype 0 non-urolithin producers) and UA producing bacterial species are not known yet, the supplementation of ET rich products does not benefit the large group of people[14,15]. The application of UA has been widely studied, for instance, in the context of age-related conditions, muscle function, cancer or inflammation and its health-promoting and anti-inflammatory mechanism of action include induction of mitophagy, suppression of NF-κB signaling pathway and activation of aryl hydrocarbon receptor (AhR) signalling[16].

Despite unambiguously confirmed pharmocological properties of UA, the pharmakokinetic studies revealed significant limitations in its utilisation as an active dietary supplement or active pharmaceutical ingredient for oral use[7]. The phase 2 metabolism occurring in the intestinal wall and the formation of glucuronide and sulfate conjugates significantly narrows the biological activity of maternal compound[17]. In consequence following the ingestion of either ellagitannins or pure UA they are present in bloodstream and tissues in a conjugated form. These limitations highlight the need for alternative strategies to obtain a pharmacologically sufficient concentrations of UA or its biologically active derivatives in human plasma.

Taking the key role of inflammation in the pathogenesis of various chronic diseases and dubious safety profile of currently used medications, there is a special need for novel, safe anti-inflammatory drugs[18,19]. The modification of the chemical structure of natural products is a well-known strategy in novel drugs development. It was presented that 28% of new drugs approved by the US Food and Drug Administration between 1990 and 2008 originated from naturally occurring substances[20].

Researchers investigating the anti-inflammatory properties of naturally occurring compounds can use various in vitro models of immune modulation. THP-1 human leukemia monocytic cells that can be differentiated into THP-1 derived macrophages are commonly used as an in vitro inflammatory response model. THP-1 cells present several benefits comparing to human peripheral blood mononuclear cells (PBMC), with high speed of growth or homogeneous genetic background. On the other hand, small differences with PBMC within the expression of receptors or degree of inflammatory response were observed. Nonetheless, THP-1 monocytes and macrophages are considered as suitable for preclinical studies or screening purposes[21].

Herein we proposed a synthesis of urolithin a derivatives (UADs), with an esterified phenolic hydroxyl group in 3- or 8-position with non-steroidal anti-inflammatory drugs (NSAIDs). Additionally, we performed stability assays of monoesterified mixes of UADs, and for the most stable one tested cytotoxicity and evaluated impact on TNF-α secretion in THP-1 derived macrophages.

Results and discussion

Synthesis of UADs. UA undergoes strong phase II metabolism, being conjugated to glucuronic acid by uridine 5′-diphospho-glucuronosyltransferases (UGTs) enzymes. Knowing that some of the NSAIDs present inhibitory activity towards UGTs, we decided to perform synthesis of esterified UA with ibuprofen, mefenamic acid, diclofenac or acetylsalicylic acid (aspirin)[22,23]. Compounds were chosen as representatives of diverse classes of NSAIDs (propionic acid derivatives, fenamates, acetic acid derivatives and salicylates) and marked with potential different isozyme-specific inhibitory activity toward UGTs[24]. So far there were no studies conducted on synthesis of monoesterified derivatives of UA. Due to the presence of two phenolic groups in UA molecule, initially attempts to conduct esterification resulting in selective obtaining 3-substituted isomer were undertaken. Three-stage strategy was applied to synthesise monoesterified UADs. Firstly, **2** was obtained, and in the next step of synthesis underwent esterification with a particular NSAID. The esterification process was followed by the demethylation of UAD deprived of unblocked –OH groups using $AlCl_3$. Unfortunately, during demethylation, the cleavage of ester bound occurred, resulting in the formation of UA. Regardless of lowering the temperature of a chemical reaction or changing the demethylating agent on BBr_3, the hydrolysis of ester bond (combined with demethylation of UADs in 8-position) repeated. Hence it was decided to synthesise mixtures of monoesterified UADs, with esterified –OH group in 3- or 8-position (Fig. 1). Mixtures of esters with ibuprofen, mefenamic acid, diclofenac and aspirin are referred to as Mix **3a**/**3b**, Mix **4a**/**4b**, Mix **5a**/**5b** and Mix **6a**/**6b**, respectively. In the case of synthesis of ibuprofen and aspirin esters, appropriate acyl chlorides were used, prepared previously according to the literature[25,26]. Mefenamic acid and diclofenac esters were synthesised using DCC/DMAP as a coupling system (Fig. 2).

Hitherto, only the synthesis of etheric derivatives of urolithins was performed[27]. Additionally, obtained compounds were deprived of free hydroxyl groups. In contrast to that study, the goal of our research was to synthesise monosubstituted, esterified UADs. The regioselective synthesis and investigation of monoesterified UADs will

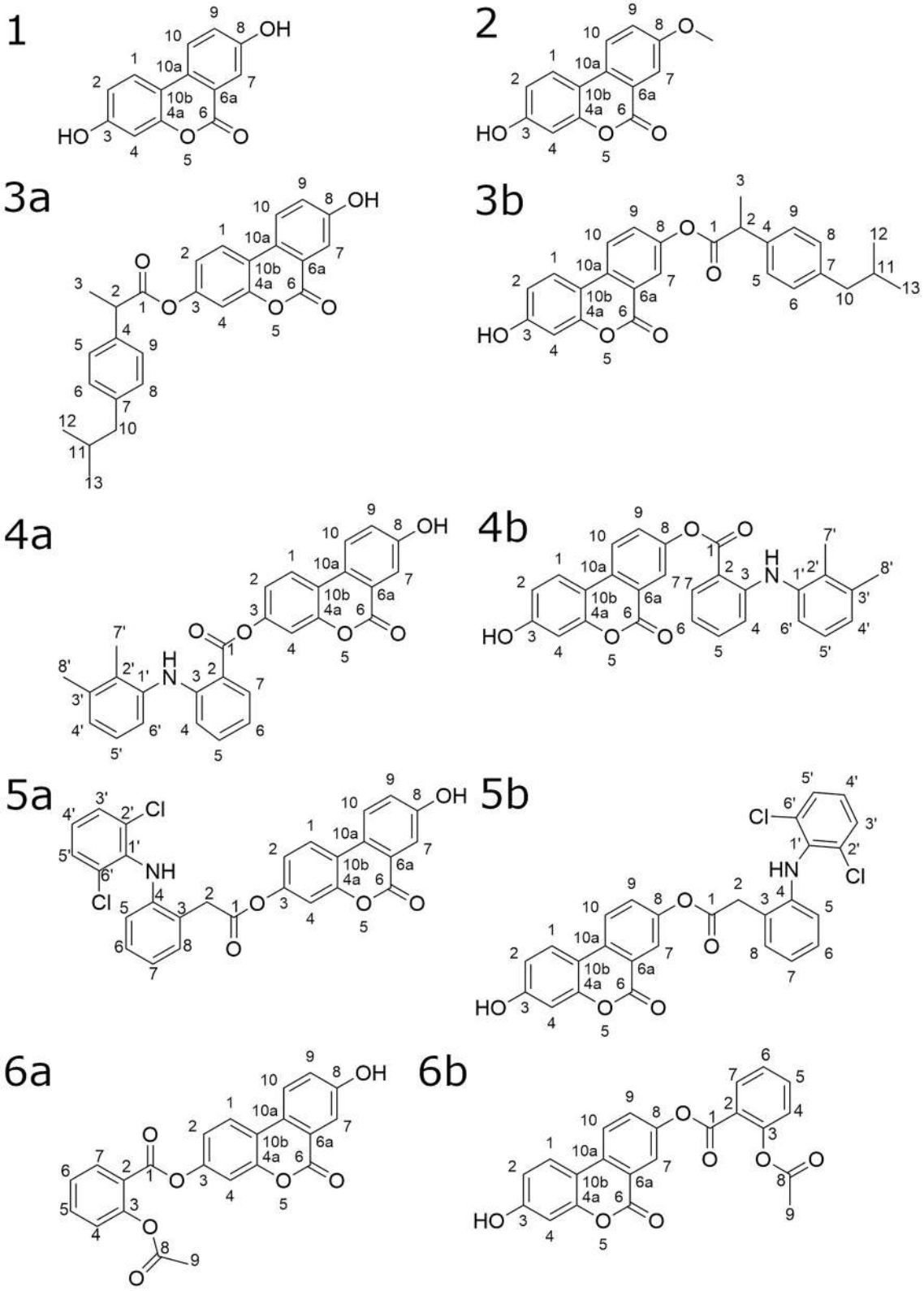


Figure 1. Chemical structure of UA (**1**) and synthesised UADs.

provide more detailed information regarding the structure–activity relationship and may benefit further searching of postbiotic metabolites-derived drugs.

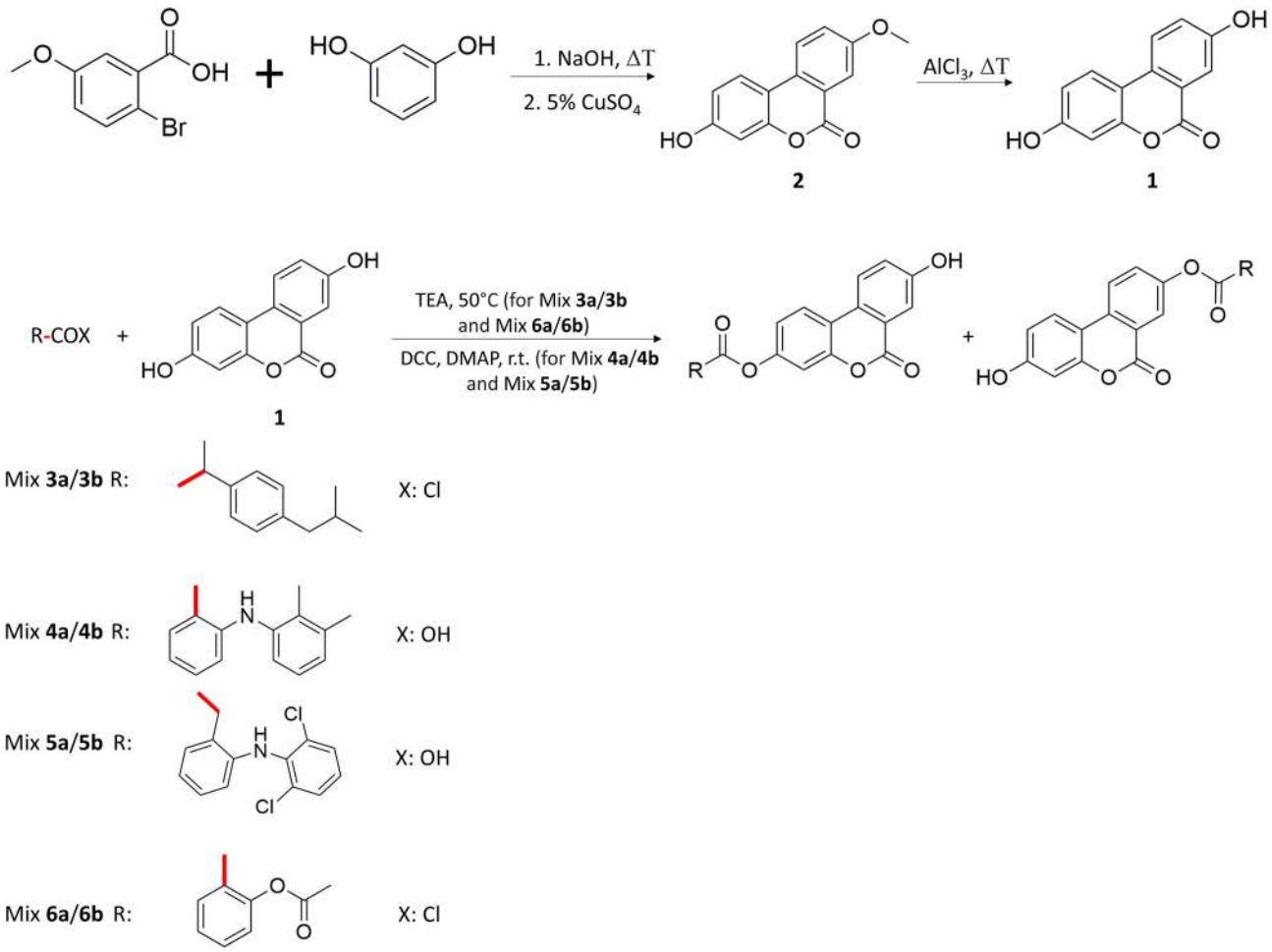


Figure 2. Synthetic strategy used to obtain Mix **3a**/**3b**, Mix **4a**/**4b**, Mix **5a**/**5b** and Mix **6a**/**6b**. Red lines indicate the chemical bonds to the carbonyl group of NSAIDs-based compounds (R-COX) and 5- and 8-substituted UADs.

Characterisation of UADs. After successfully synthesising UADs' mixtures, the identity of obtained compounds was confirmed using the HPLC–MS–DAD method and NMR spectroscopy. All pairs of UADs eluted either as single peaks (Mix **3a**/**3b**, Mix **5a**/**5b**, Mix **6a**/**6b**) or with minimal differences in retention times (Mix **4a**/**4b**) preventing the separation of isomers using C_{18} stationary phases (Fig. S1). The chromatographic strategies to separate the isomeric 3- and 8-substituted monoconjugates were introduced using Phenomenex Kinetex Biphenyl column (150 mm × 2.1 mm, particle size 1.7 µm) and methanol as a mobile phase. No improvement of isomers separation was achieved, what excluded application of preparative HPLC separation using these conditions. However, since the obtained UADs mixtures are similar to the in vivo produced mixture of urolithin A 3- and 8-glucuronides it was decided to conduct further studies on their mixtures. Analogously to our study, the mixture of these phase II metabolites of UA was not separated using the HPLC method[28]. The analysis of mass spectra confirmed the identity of UADs, which gave specific pseudomolecular ions $[M\text{-}H]^-$ at m/z 415, 450, 505 and 389 for Mix **3a**/**3b**, Mix **4a**/**4b**, Mix **5a**/**5b** and Mix **6a**/**6b**, respectively.

Analogously to previously presented urolithin A glucuronides' NMR spectra, the downshifts for H2 and H4 of UADs esterified in 3-position and for H7 and H9 of UADs esterified in 8-position were observed (Fig. 3, Tables 1, 2)[28]. Furthermore, a comparison of the proton signals integration ratio revealed the proportion of isomers in mixtures of UADs. The ratio of 3-esterified UADs to 8-esterified UADs were 1:1.27, 1:1.58, 1:1.89 and 1:1.12 for Mix **3a**/**3b**, Mix **4a**/**4b**, Mix **5a**/**5b** and Mix **6a**/**6b**, respectively.

Stability assays. The high susceptibility to degradation of novel lead compounds may negatively affect the potential usability of promising APIs (Active Pharmaceutical Ingredients). Furthermore, the evaluation of compounds' stability is often not included during in vitro assays. Therefore, the stability assays for Mix **3a**/**3b**, Mix **4a**/**4b**, Mix **5a**/**5b** and Mix **6a**/**6b**, were performed during our study. Results of stability assays are presented in Table 3. Conditions were selected on the basis of previously described stability-examination protocol and possible exposure of UADs on stress conditions during drug formulation, storage, oral or intravenous administration[29].

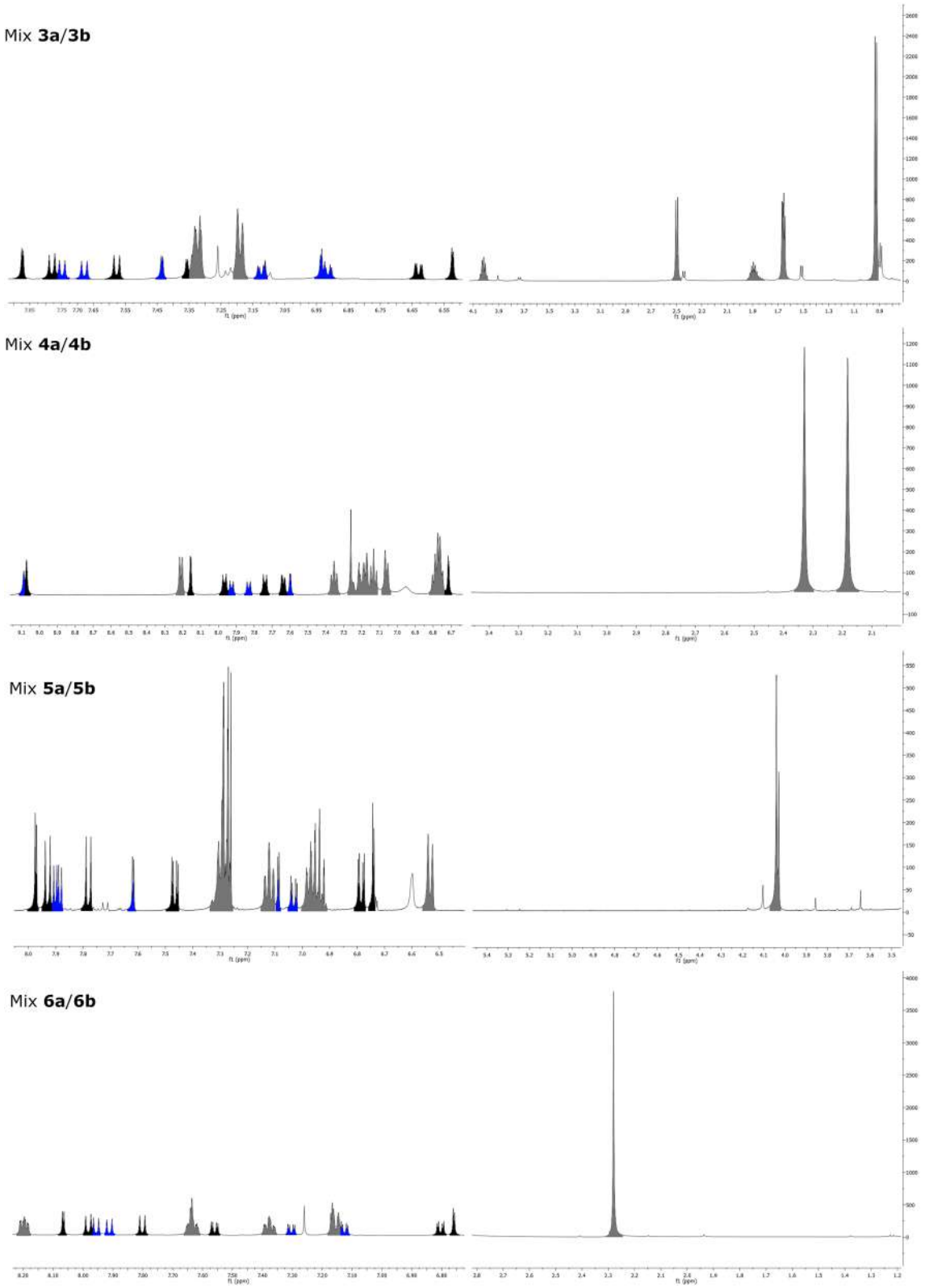


Figure 3. NMR spectra of synthesised UADs. Black: 8-esterified UADs, Blue: 3-esterified UADs, Grey: overlapped signals.

Despite structural similarities in conjugation mode, synthetised UADs differed substantially in terms of their thermal stability in deionised water. Especially, the highest percentage of degradation either at room temperature

	3b	3a	4b	4a
	δ_H	δ_H		
1	7.58 (d, *J* = 8.8 Hz)	7.68 (d, *J* = 8.8 Hz)	7.74 (d, *J* = 9.3 Hz)	7.83 (d, *J* = 9.1 Hz)
2	6.63 (dd, *J* = 8.7, 2.3 Hz)	6.91 (dd, *J* = (8.6, 2.2 Hz)	6.74–6.81 (o)	7.18–7.21 (o)
4	6.52 (d, *J* = 2.3 Hz)	6.94 (d, *J* = 2.0 Hz)	6.71 (d, *J* = 2.1 Hz)	7.21–7.23 (o)
7	7.87 (d, *J* = 2.4 Hz)	7.43 (d, *J* = 2.5 Hz)	8.15 (d, *J* = 2.3 Hz)	7.60 (d, *J* = 2,4 Hz)
9	7.33–7.40 (o)	7.12 (dd, *J* = 8.8, 2.6 Hz)	7.64 (dd, *J* = 7.6, 2.1 Hz)	7.24–7.27 (o)
10	7.78 (d, *J* = 8.8 Hz)	7.75 (d, *J* = 8.6 Hz)	7.97 (d, *J* = 9.3 Hz)	7.93 (d, *J* = 8.7 Hz)
Ibu 2	3.98–4.05 (o)	3.98–4.05 (o)	N/A	N/A
Ibu 3	1.62–1.66 (o)	1.62–1.66 (o)	N/A	N/A
Ibu 5	7.30–7.34 (o)	7.30–7.34 (o)	N/A	N/A
Ibu 6	7.17–7.21 (o)	7.17–7.21 (o)	N/A	N/A
Ibu 8	7.17–7.21 (o)	7.17–7.21 (o)	N/A	N/A
Ibu 9	7.30–7.34 (o)	7.30–7.34 (o)	N/A	N/A
Ibu 10	2.48–2.52 (o)	2.48–2.52 (o)	N/A	N/A
Ibu 11	1.81–1.94 (o)	1.81–1.94 (o)	N/A	N/A
Ibu 12	0.91–0.95 (o)	0.91–0.95 (o)	N/A	N/A
Ibu 13	0.91–0.95 (o)	0.91–0.95 (o)	N/A	N/A
Mef 4	N/A	N/A	6.74–6.81 (o)	6.74–6.81 (o)
Mef 5	N/A	N/A	7.33–7.38 (o)	7.33–7.38 (o)
Mef 6	N/A	N/A	6.74–6.81 (o)	6.74–6.81 (o)
Mef 7	N/A	N/A	8.19–8.23 (o)	8.19–8.23 (o)
Mef 4′	N/A	N/A	7.04–7.08 (o)	7.04–7.08 (o)
Mef 5′	N/A	N/A	7.11–7.16 (o)	7.11–7.16 (o)
Mef 6′	N/A	N/A	7.16–7.20 (o)	7.16–7.20 (o)
Mef 7′	N/A	N/A	2.18 (o)	2.18 (o)
Mef 8′	N/A	N/A	2.33 (o)	2.33 (o)
Mef-NH-	N/A	N/A	9.07 (s)	9.09 (s)

Table 1. Mix **3a**/**3b** and Mix **4a**/**4b** spectroscopic data (500 MHz, $CDCl_3$). Ibu, Atoms of ibuprofen subunit; Mef, Atoms of mefenamic acid subunit; d, Doublet; dd, Doublet of doublets; o, Overlapped signal.

	5b	5a	6b	6a
	δ_H	δ_H		
1	7.78 (d, *J* = 8.8 Hz)	7.89 (d, *J* = 8.8 Hz)	7.80 (d, *J* = 8.8 Hz)	7.91 (d, *J* = 8.8 Hz, 1H)
2	6.79 (dd, *J* = 8.7, 2.4 Hz)	7.03 (dd, *J* = 8.7, 2.3 Hz)	6.80 (dd, *J* = 8.7, 2.4 Hz)	7.13 (dd, *J* = 8.7, 2.3 Hz)
4	6.74 (d, *J* = 2.4 Hz)	7.09 (d, *J* = 2.3 Hz)	6.76 (d, *J* = 2.4 Hz)	7.14–7.18 (o)
7	7.97 (d, *J* = 2.6 Hz)	7.62 (d, *J* = 2.7 Hz)	8.07 (d, *J* = 2.5 Hz)	7.61–7.66 (o)
9	7.46 (dd, *J* = 8.8, 2.6 Hz)	7.26–7.31 (o)	7.56 (dd, *J* = 8.7, 2.5 Hz)	7.30 (dd, *J* = 8.7, 2.7 Hz)
10	7.93 (d, *J* = 8.9 Hz)	7.90 (d, *J* = 8.8 Hz)	7.98 (d, *J* = 8.8 Hz)	7.96 (d, *J* = 8.7 Hz)
Diclo 2	4.02–4.05 (o)	4.02–4.05 (o)	N/A	N/A
Diclo5	7.26–7.31 (o)	7.26–7.31 (o)	N/A	N/A
Diclo 6	6.92–6.99 (o)	6.92–6.99 (o)	N/A	N/A
Diclo 7	6.92–6.99 (o)	6.92–6.99 (o)	N/A	N/A
Diclo 8	6.52–6.55 (o)	6.52–6.55 (o)	N/A	N/A
Diclo 3′	7.26–7.31 (o)	7.26–7.31 (o)	N/A	N/A
Diclo 4′	7.10–7.14 (o)	7.10–7.14 (o)	N/A	N/A
Diclo 5′	7.26–7.31 (o)	7.26–7.31 (o)	N/A	N/A
Asp 4	N/A	N/A	7.14–7.18 (o)	7.14–7.18 (o)
Asp 5	N/A	N/A	7.61–7.66 (o)	7.61–7.66 (o)
Asp 6	N/A	N/A	7.35–7.40 (o)	7.35–7.40 (o)
Asp 7	N/A	N/A	8.18–8.22 (o)	8.18–8.22 (o)
Asp 9	N/A	N/A	2.25–2.30 (o)	2.25–2.30 (o)

Table 2. Mix **5a**/**5b** and Mix **6a**/**6b** spectroscopic data (500 MHz, $CDCl_3$). Diclo, Atoms of ibuprofen subunit; Asp, Atoms of mefenamic acid subunit; d, Doublet; dd, Doublet of doublets; o, Overlapped signal.

	Mix **3a**/**3b**	Mix **4a**/**4b**	Mix **5a**/**5b**	Mix **6a**/**6b**
H_2O 24 h r.t.	27.5 ± 2.6	0.1 ± 0.9	1.1 ± 1.2	16.2 ± 7.5
H_2O 24 h 37 °C	25.5 ± 8.9	15.8 ± 3.0	3.6 ±4.2	0.0 ± 2.4
H_2O 12 h 80 °C	84. 0 ± 0.5	76.1 ± 2.5	97.4 ± 0.1	83.1 ± 1.8
H_2O 24 h –70 °C	25.8 ± 6.6	7.8 ± 7.9	11.9 ± 3.0	17.7 ± 2.9
H_2O Term. cycle	87.5 ± 1.7	33.5 ± 3.2	34. 0 ± 6.1	32.2 ± 5.6
1M HCl 24 h 37 °C	92.8 ± 3.0	49.4 ± 0.6	41.4 ± 4.9	95.1 ± 0.5
1M HCl 12 h 80 °C	98.9 ± 1.9	100.0 ± 0.0	100.0 ± 0.0	98.8 ±0.0
1M NaOH 24 h 37 °C	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
1M NaOH 12 h 80 °C	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
30% H_2O_2 24 h 37 °C	43.1 ± 8.9	53.3 ± 7.2	74.5 ± 14.3	98.6 ± 0.1
30% H_2O_2 12 h 80 °C	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
UV 7 h	46.8 ± 14.4	1.7 ± 0.2	8.7 ± 2.8	18.2 ± 7.4
Plasma 0.5 h 37 °C	79.3 ± 4.3	12.6 ± 8.2	69.8 ± 2.4	100.0 ± 0.0
Plasma 1.5 h 37 °C	96.1 ± 0.6	10.7 ± 13.1	95.9 ± 1.0	100.0 ± 0.0
Plasma 4 h 37 °C	100.0 ± 0.0	18.0 ± 6.1	100.0 ± 0.0	100.0 ± 0.0

Table 3. Percentage of degradation of synthesised UADs. Data express as a mean ± SD.

or at 37 °C after 24 h incubation was observed for Mix **3a**/**3b**, while around a quarter of the native compound diminished. On the other hand, Mix **5a**/**5b** was highly resistant in those conditions with only 1.1 ± 1.2% and 3.6 ± 4.2% degradation, respectively. After 12 h of incubation in 80 °C, all UADs suffered strong degradation ranging from 76.1 ± 2.5% for Mix **4a**/**4b** to 97.4 ± 0.1% for Mix **5a**/**5b**. In negative temperatures, Mix **3a**/**3b** was the most susceptible to degradation, resulting in almost 26% breakdown of the native compound during incubation in – 70 °C for 24 h and 87.5 ± 1.7% breakdown during the cycle of freezing and thawing. Mix **4a**/**4b** and Mix **5a**/**5b** were characterised with the highest tolerance to incubation in – 70 °C (7.8 ± 7.9% and 11,9 ± 3.0% respectively), while the **Mix 3a**/**3b** presented the highest degradation during the cycle of freezing and thawing – 87.5 ± 1.7%.

UADs were tested for their stability in 1 M HCl, and based on received outcomes, they can be divided into two separate groups. Mix **3a**/**3b** and Mix **6a**/**6b** almost completely vanished after incubation for 24 h in 37 °C or 12 h in 80 °C in an acidic environment. Contrarily, Mix **4a**/**4b** and Mix **5a**/**5b** moderately tolerate 24 h incubation in 37 °C with 1 M HCl (49.4 ± 0.6% and 41.4 ± 4.9% degradation, respectively). Nonetheless, Mix **4a**/**4b** and Mix **5a**/**5b** were also undetected after incubation at a higher temperature for 12 h.

In terms of UADs' stability in basic conditions, all native compounds diminished completely or almost completely, no matter of time and temperature of incubation in 1 M NaOH.

UADs' susceptibility to oxidative stress varied widely, and Mix **3a**/**3b** turned out to be the least affected by the incubation in 30% H_2O_2 at 37 °C for 24 h with only 43.1 ± 8.9% degradation. At the other end of the scale, the level of Mix **6a**/**6b** was decreased by almost 99% compared to the control. Unsurprisingly, none of UADs' peaks was detected after incubation at 80 °C for 12 h.

The breakdown of UADs exposed to UV light was tested as well. While Mix **4a**/**4b** was almost unaffected by UV light (1.7 ± 0.2% degradation), Mix **5a**/**5b**, Mix **6a**/**6b**, Mix **3a**/**3b** were partially degraded (8.7 ± 2.8, 18.2 ± 7.4, 46.8 ± 14.4% degradation, respectively).

All UADs except Mix **4a**/**4b** underwent strong degradation in human plasma incubated at 37 °C, resulting in the total absence of UADs' peaks after 4 h. Surprisingly, Mix **4a**/**4b** was marked with good toleration to the human plasma environment with a ratio of degradation of 18.0 ± 6.1% after 4 h.

Cytotoxicity. Taking into account the exceptional stability of Mix **4a**/**4b** in human plasma, this derivatives were chosen for further in vitro assays. The cytotoxicity of Mix **4a**/**4b** on THP-1 derived macrophages was tested using a standard MTT assay performed after administration of UADs, one hour resting and stimulation of cells with LPS for 3 or 24 h. Mix **4a**/**4b** did not exhibit cytotoxic effect up to 10 µM (Fig. 4a,b, Tables S1, S2). However, when 50 µM concentration was used, cell viability significantly declined. Additionally, the impact of UA on THP-1 macrophages viability was assessed, and no cytotoxic effect was observed in the tested concentration range (2–50 µM), similarly to previously obtained results[8,17]. The paradoxical increased viability after 24 h incubation with 5 and 10 µM Mix **4a**/**4b** may result from the dependance of MTT assay on metabolic rate[30]. Furthermore, the cytotoxic effect of 50 µM Mix **4a**/**4b** was independently proved using Neutral Red Uptake (NRU) assay performed 3 and 24 h after LPS stimulation (Fig. 4c,d, Tables S3, S4).

Parallelly, the cytotoxicity of UA and Mix **4a**/**4b** on THP-1 monocytes was investigated using flow cytometry analysis of PI-stained cells with 25 µM parthenolide as a positive control (Fig. 4e, Table S5). As previously described, 25 µM parthenolide decreased THP-1 viability[31]. Similarly to results obtained for THP-1 derived macrophages using MTT and NRU assays, Mix **4a**/**4b** did not influence THP-1 monocytes viability up 10 µM.

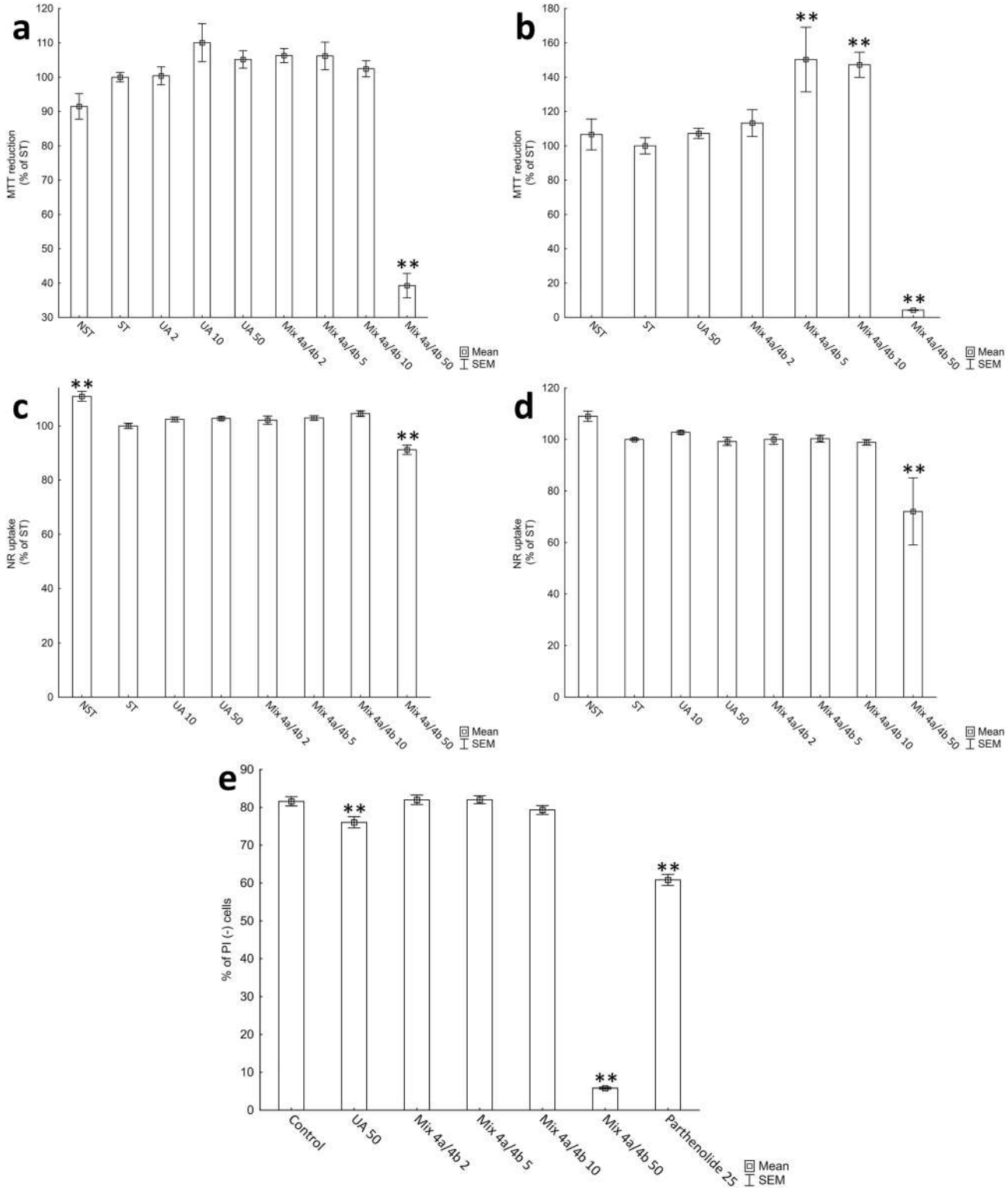


Figure 4. Cytotoxicity of UA and Mix **4a**/**4b**. (**a**) Effects of UA and Mix **4a**/**4b** on THP-1 derived macrophage viability measured using MTT assay after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 3 h. (**b**) Effects of UA and Mix **4a**/**4b** on THP-1 derived macrophage viability measured using MTT assay after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 24 h. (**c**) Effects of UA and Mix **4a**/**4b** on THP-1 derived macrophage viability measured using NRU assay after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 3 h. (**d**) Effects of UA and Mix **4a**/**4b** on THP-1 derived macrophage viability measured using NRU assay after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 24 h (**e**) Effects of UA and Mix **4a**/**4b** on THP-1 monocyte viability using PI flow cytometric assay. Data expressed as a mean ± SEM. Numbers next graph labels indicate tested concentration (in μM). Statistical comparisons were made using the parametric method of one-way ANOVA, followed by the Dunnett's post hoc test. Statistical significance: $^{**}p < 0.01$ versus ST group or Control in PI flow cytometric assay. *NST* Non-stimulated control, *ST* LPS stimulated control.

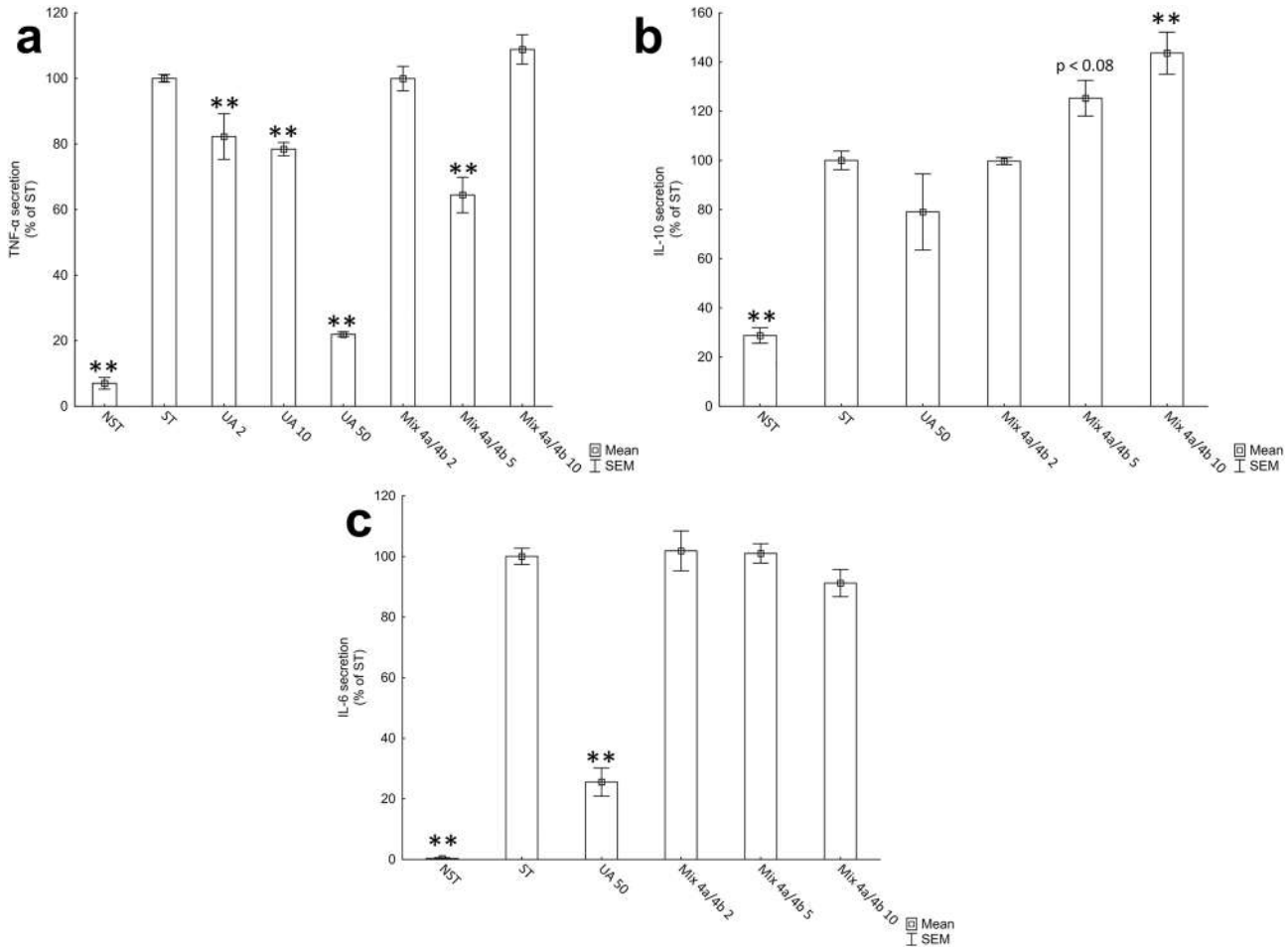


Figure 5. Evaluation of anti-inflammatory activity of UA and Mix **4a**/**4b** using LPS stimulated THP-1 derived macrophages. (**a**) Effects of UA and Mix **4a**/**4b** on TNF-α secretion after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 3 h. (**b**) Effects of UA and Mix **4a**/**4b** on IL-10 secretion after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 24 h. (**c**) Effects of UA and Mix **4a**/**4b** on IL-6 secretion after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 24 h. Data expressed as a mean ± SEM. Numbers next graph labels indicate tested concentration (in μM). Statistical comparisons were made using the parametric method of one-way ANOVA, followed by the Dunnett's post hoc test. Statistical significance:**$p < 0.01$ versus ST group. *NST* Non-stimulated control, *ST* LPS stimulated control.

The drastic drop in cell viability was observed after 24-h incubation with 50 μM Mix **4a**/**4b.** Slight, yet statistically significant decline in monocytes viability after administration of 50 μM UA was observed.

Impact of Mix 4a/4b on cytokines secretion. The TNF-α secretion of LPS-stimulated THP-1 derived macrophages was assessed using ELISA kits. UA was chosen as a control compound based on the well-established inhibitory activity of UA on TNF-α secretion and thoroughly examined the underlying intracellular mechanism of action[16]. As expected, UA exhibited an inhibitory effect on TNF-α secretion in a dose-dependent manner (Fig. 5a, Table S6).

Mix **4a**/**4b** affected TNF-α secretion 3 h after stimulation. While the significant inhibitory effect on TNF-α level was observed already at 5 μM Mix **4a**/**4b**, this impact was not detected using concentration of 10 μM (Fig. 5a, Table S6). The lack of concentration-dependency of determined activity between 5 and 10 μM concentrations (not observed in the case of UA), can arise from the hybrydic properties of the Mix **4a**/**4b**. As described above, UA exhibits an inhibitory effect on TNF-α secretion, affecting the NF-κB signalling pathway in cells. On the other hand, mefenamic acid non-selectively inhibits COX-2, and it is known, that inhibition of this enzyme can increase TNF-α expression in THP-1 macrophages[32,33]. The additional set of experiments using THP-1 derived macrophages confirmed the stimulating effect of mefenamic acid on TNF- α secretion (Fig. S7). Thus, the contrary effect of two Mix **4a**/**4b** subunits might explain the dose-independent manner of action and will be closely evaluated in future research. Moreover, the cytotoxic effect of Mix **4a**/**4b** at 50 μM concentration was presented. After administration of 10 μM Mix **4a**/**4b**, we did not observe the decrease of the viability of THP-1 macrophages. Nevertheless, it was previously demonstrated that despite the not observed decline in THP-1 cells

viability, the induction of apoptotic intracellular signalling pathways, including caspases activity and parallel upregulated production of proinflammatory TNF-α, were detected after administration of several stressors[34,35].

Additionally, the impact of UA and Mix **4a**/**4b** on anti-inflammatory interleukin-10 (IL-10) and interleukin-6 (IL-6) secretion 24 h after stimulation with LPS was tested (Fig. 5b,c, Tables S7, S8). In accordance with previously published results, UA did not significantly affect IL-10 secretion[17]. Interestingly, Mix **4a**/**4b** increased levels of IL-10 comparing to stimulated control in a dose-dependent manner, reaching statistical significance at a concentration of 10 μM. Consistent with hitherto presented results, 50 μM UA inhibited secretion of IL-6[36]. Contrary to this specific UA activity, Mix **4a**/**4b** did not affect IL-6 secretion in THP-1 derived macrophages. While the mefenamic acid alone did not influence IL-10 secretion, it increased IL-6 production in a dose-dependent manner (Fig. S7). These findings suggest distinctive mechanism of action of Mix **4a**/**4b** from that previously determined for UA[37].

Conclusion

In summary, we performed the successful synthesis of mixtures of UADs monoesterified to ibuprofen (Mix **3a**/**3b**), mefenamic acid (Mix **4a**/**4b**), diclofenac (Mix **5a**/**5b**) and aspirin (Mix **6a**/**6b**). Despite similarities in conjugation mode, obtained compounds fundamentally differ in terms of their stability, and contrary to other UADs, Mix **4a**/**4b** well tolerated the human plasma environment. The determined impact of Mix **4a**/**4b** on TNF-α secretion may result either from their cytotoxic effect at high concentration or the opposing activity of Mix **4a**/**4b** subunits at lower concentrations. The influence of Mix **4a**/**4b** on IL-10 secretion and lack of activity towards IL-6 secretion imply mechanism of action, which is different from that previously determined for UA[37]. Presented results create a novel approach in anti-inflammatory drug development, focusing on chemical modification of the structure of postbiotic metabolites. Additional studies should be oriented on the synthesis of monoesterified UADs and close investigation of their intracellular mechanism of action. Further work evaluating UADs bioavailability in vitro, with the special interest in the role of UGTs, are currently in progress.

Methods

Synthetic method. *Synthesis of 3-hydroxy-8-methoxy-6H-benzo[c]chromen-6-one (2).* The synthesis of the first intermediate, **2**, was performed according to the previously described protocol[38]. Briefly, after heating under reflux mixture of resorcinol (1 g; 9.08 mmol, 2.1 eqv), 2-Bromo-5-methoxybenzoic acid (1 g; 4.33 mmol, 1.0 eqv) and NaOH (0.375 g; 9.38 mmol, 2.2 eqv) in water (4.5 mL) for 30 min, a 5% aqueous solution of CuSO4 (1.8 ml; 0.56 mmol, 0.1 eqv) was added dropwise. Afterwards, the solution was heated for additional 10 min. The obtained precipitate was filtered, dried and the identity of **2** was confirmed using the UHPLC-DAD-MS method; 610 mg (58%).

Synthesis of 3,8-dihydroxy-6H-benzo[c]chromen-6-one (UA, 1). The goal of this procedure was to obtain mixtures of UADs with esterified 3- or 8- position. A solution of **2** (0.51 g; 2.11 mmol, 1 eqv) and $AlCl_3$ (1.46 g; 10.95 mmol, 5.2 eqv) in chlorobenzene was refluxed, and the demethylation process was monitored using the UHPLC-DAD-MS method. After complete demethylation of **2**, resulting in the formation of UA, a cooled mixture was added to ice. In the next step, the mixture was extracted with diethyl ether (3 × 125 ml). Subsequently, the organic solvent was evaporated on a rotary evaporator equipped with a vacuum system to reduce the pressure. The identity of obtained UA was confirmed using the UHPLC-DAD-MS method; 196 mg (41%).

Synthesis of mixture of 8-hydroxy-6-oxo-6H-benzo[c]chromen-3-yl 2-(4-isobutylphenyl)propanoate and 3-hydroxy-6-oxo-6H-benzo[c]chromen-8-yl 2-(4-isobutylphenyl)propanoate (Mix 3a/3b). To a magnetically stirred at 50 °C suspension of UA (0.050 g; 0.22 mmol, 1.0 eqv) and triethylamine (0.036 mL; 0.26 mmol, 1.2 eqv) in dioxane (10 mL) solution of ibuprofen chloride (0.049 g; 0.22 mmol, 1.0 eqv) in CH_2Cl_2 (1 mL) was added. After 2 h the reaction mixture was concentrated under reduced pressure. To residue ethyl acetate (30 mL) and water (15 mL) were added and acidified to pH 2–3 with 3% HCl_{aq} solution (2 mL). After separation of the phases the water layer was additionally extracted with ethyl acetate (2 × 20 mL). The combined organic phases were washed with brine (15 mL) and dried over anhydrous Na_2SO_4. After evaporation of the solvent under reduced pressure the product was isolated using column chromatography on silica gel and CH_2Cl_2:MeOH mixture (0–8% MeOH) as an eluent. After purification the product was obtained as an solidifying oil; 40 mg (44%). Mp = 98.3–99.2 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, *J* = 2.4 Hz, 1H), 7.78 (d, *J* = 8.8 Hz, 1H), 7.75 (d, *J* = 8.6 Hz, 1H), 7.68 (d, *J* = 8.8 Hz, 1H), 7.58 (d, *J* = 8.8 Hz, 1H), 7.43 (d, *J* = 2.5 Hz, 1H), 7.40–7.30 (m, 5H), 7.19 (d, *J* = 7.7 Hz, 4H), 7.12 (dd, *J* = 8.8, 2.6 Hz, 1H), 6.94 (d, *J* = 2.0 Hz, 1H), 6.91 (dd, *J* = 8.6, 2.2 Hz, 1H), 6.63 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.52 (d, *J* = 2.3 Hz, 1H), 4.06–3.98 (m, 2H), 2.50 (d, *J* = 7.2 Hz, 4H), 1.94–1.81 (m, 2H), 1.66 (dd, *J* = 7.1, 4.3 Hz, 6H), 0.93 (d, *J* = 6.6 Hz, 12H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.74, 174.72, 161.03, 160.93, 158.40, 156.87, 151.79, 150.85, 150.53, 149.74, 141.41, 141.38, 136.71, 136.59, 133.19, 129.90, 129.87, 129.56, 128.93, 127.34, 127.32, 126.79, 124.06, 123.59, 123.29, 123.07, 122.78, 122.74, 121.55, 120.71, 118.31, 116.26, 114.97, 113.48, 110.95, 110.14, 103.87, 45.45, 45.42, 45.19, 30.33, 30.31, 22.57, 22.55, 18.58, 18.54. MS (m/z) $[M]^+$: 417.

Synthesis of mixture of 8-hydroxy-6-oxo-6H-benzo[c]chromen-3-yl 2-((2,3-dimethylphenyl)amino)benzoate and 3-hydroxy-6-oxo-6H-benzo[c]chromen-8-yl 2-((2,3-dimethylphenyl)amino)benzoate (Mix 4a/4b). To a magnetically stirred at room temperature suspension of UA (0.200 g; 0.88 mmol, 1.0 eqv) in a mixture of dioxane (200 mL) and dichloromethane (100 mL) mefenamic acid (0.212 g; 0.88 mmol, 1.0 eqv), DCC (0.217 g; 1.05 mmol; 1.2 eqv) and DMAP (21.40 mg; 0.18 mmol; 0.2 eqv) were subsequently added. After 24 h the reaction mixture was concentrated under reduced pressure. The residual oil was purified using column chromatography on silica gel and CH_2Cl_2:MeOH mixture (0–8% MeOH) as an eluent. After purification the product was obtained

as pale yellow solid; 105 mg (26%). Mp = 188.0–189.5 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.09 (s, 1H), 9.07 (s, 1H), 8.21 (d, *J* = 6.9 Hz, 2H), 8.15 (d, *J* = 2.3 Hz, 1H), 7.97 (d, *J* = 9.3 Hz, 1H), 7.93 (d, *J* = 8.7 Hz, 1H), 7.83 (d, *J* = 9.1 Hz, 1H), 7.74 (d, *J* = 9.3 Hz, 1H), 7.64 (dd, *J* = 7.6, 2.1 Hz, 1H), 7.60 (d, *J* = 2.4 Hz, 1H), 7.35 (t, *J* = 7.9 Hz, 2H), 7.27–7.24 (m, 1H), 7.23–7.16 (m, 4H) 7.13 (t, *J* = 7.8 Hz, 2H), 7.06 (d, *J* = 7.4 Hz, 2H), 6.81–6.74 (m, 5H), 6.71 (d, *J* = 2.1 Hz, 1H), 2.33 (s, 6H), 2.18 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 167.97 (s, *J* = 5.7 Hz), 167.92, 161.16, 158.52, 156.98, 152.07, 151.05–150.95 (m, *J* = 6.2 Hz), 150.91, 150.86, 150.79, 149.91, 138.54, 138.14, 135.82, 133.24, 133.08 (s, *J* = 10.4 Hz), 132.99, 132.18, 129.56, 127.58 (s, *J* = 4.5 Hz), 127.55, 127.07, 126.23, 124.18, 123.83 (s, *J* = 12.3 Hz), 123.74, 123.50, 123.34, 123.19, 122.89, 121.79, 120.99, 118.97, 116.52 (s, *J* = 19.2 Hz), 116.37, 115.14, 114.07, 113.56, 111.60, 110.45, 108.82 (s, *J* = 13.5 Hz), 108.72, 104.10, 66.06, 33.98, 25.65, 24.98, 20.74, 15.36, 14.21. MS (m/z) $[M]^+$: 452.

Synthesis of mixture of 8-hydroxy-6-oxo-6H-benzo[c]chromen-3-yl 2-(2-((2,6-dichlorophenyl)amino)phenyl) acetate and 3-hydroxy-6-oxo-6H-benzo[c]chromen-8-yl 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (Mix 5a/5b). To a magnetically stirred at room temperature suspension of UA (0.100 g; 0.44 mmol, 1.0 eqv) in a mixture of dioxane (100 mL) and dichloromethane (50 mL) diclofenac (0.260 g; 0.88 mmol, 2.0 eqv), DCC (0.182 g; 0.88 mmol; 2.0 eqv) and DMAP (21.40 mg; 0.18 mmol; 0.4 eqv) were subsequently added. After 24 h the reaction mixture was concentrated under reduced pressure. The residual oil was purified using column chromatography on silica gel and CH_2Cl_2:MeOH mixture (0–8% MeOH) as an eluent. After purification the product was obtained as pale beige solid; 33 mg (14%). Mp = 188.8–191.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.97 (d, *J* = 2.6 Hz, 1H), 7.93 (d, *J* = 8.9 Hz, 1H), 7.90 (d, *J* = 8.8 Hz, 1H), 7.89 (d, *J* = 8.9 Hz, 1H), 7.78 (d, *J* = 8.8 Hz, 1H), 7.62 (d, *J* = 2.7 Hz, 1H), 7.46 (dd, *J* = 8.8, 2.6 Hz, 1H), 7.31–7.26 (m, 7H), 7.13 (dd, *J* = 11.1, 4.4 Hz, 2H), 7.09 (d, *J* = 2.3 Hz, 1H), 7.03 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.99–6.92 (m, 4H), 6.79 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.74 (d, *J* = 2.4 Hz, 1H), 6.53 (d, *J* = 8.0 Hz, 2H), 4.03 (d, *J* = 5.5 Hz, 4H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.51, 170.41, 161.57, 161.32, 159.57, 158.01, 152.16, 150.46, 150.21, 149.31, 142.59, 142.57, 142.48, 142.47, 137.52, 137.46, 133.60, 130.94, 129.42, 129.40, 129.36, 129.34, 128.92, 128.77, 128.34, 126.38, 126.30, 124.60, 124.28, 124.19, 123.86, 123.59, 123.43, 123.40, 123.37, 123.33, 122.76, 122.52, 122.47, 122.32, 122.28, 122.26, 121.60, 120.42, 118.45, 118.21, 116.53, 114.36, 113.43, 110.75, 109.54, 103.49, 49.54, 49.39, 49.37, 49.20, 49.03, 48.86, 48.69, 48.52, 38.35, 38.26. MS (m/z) $[M]^+$: 507.

Synthesis of mixture of 8-hydroxy-6-oxo-6H-benzo[c]chromen-3-yl 2-acetoxybenzoate and 3-hydroxy-6-oxo-6H-benzo[c]chromen-8-yl 2-acetoxybenzoate (Mix 6a/6b). To a magnetically stirred at 50 °C suspension of UA (0.100 g; 0.44 mmol, 1.0 eqv) and triethylamine (0.072 mL; 0.53 mmol, 1.2 eqv) in dioxane (50 mL) solution of aspirin chloride (0.087 g; 0.44 mmol, 1.0 eqv) in CH_2Cl_2 (3 mL) was added. After 3 h the reaction mixture was concentrated under reduced pressure. The residual oil was purified using column chromatography on silica gel and CH_2Cl_2:MeOH mixture (0–2% MeOH) as an eluent. After purification the product was obtained as pale beige solid; 60 mg (35%). Mp = 176.8–178.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.22–8.17 (m, 2H), 8.07 (d, *J* = 2.5 Hz, 1H), 7.98 (d, *J* = 8.8 Hz, 1H), 7.96 (d, *J* = 8.7 Hz, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.80 (d, *J* = 8.8 Hz, 1H), 7.66–7.61 (m, 3H), 7.56 (dd, *J* = 8.7, 2.5 Hz, 1H), 7.40–7.35 (m, 2H), 7.30 (dd, *J* = 8.7, 2.7 Hz, 1H), 7.18–7.14 (m, 3H), 7.13 (dd, *J* = 8.7, 2.3 Hz, 1H), 6.80 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.76 (d, *J* = 2.4 Hz, 1H), 2.28 (s, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.08, 162.79, 162.75, 161.67, 161.41, 159.67, 158.13, 152.31, 151.32, 151.26, 150.56, 150.47, 149.44, 135.10, 135.05, 133.76, 132.23, 129.22, 126.46, 126.41, 126.39, 124.44, 124.16, 124.12, 124.01, 123.74, 123.04, 122.78, 122.74, 122.04, 121.93, 121.75, 120.69, 118.51, 116.73, 114.54, 113.59, 111.08, 109.72, 103.68, 73.38, 68.25, 61.34, 49.69, 49.52, 49.34, 49.17, 49.00, 20.99. MS (m/z) $[M]^+$: 391.

Synthesis of 8-methoxy-6-oxo-6H-benzo[c]chromen-3-yl 2-(4-isobutylphenyl)propanoate (7). To a magnetically stirred at 50 °C solution of **2** (0.030 g; 0.12 mmol, 1.0 eqv) in a mixture of dioxane (10 mL) and dichloromethane (5 mL) triethylamine (0.052 mL; 0.36 mmol, 3.0 eqv) and next solution of ibuprofen chloride (0.058 g; 0.24 mmol, 2.0 eqv) in CH_2Cl_2 (1 mL) were added. After 1 h the reaction mixture was concentrated under reduced pressure. To residue dichloromethane (20 mL) and water (8 mL) were added and acidified to pH 2–3 with 3% HCl_{aq} solution (1 mL). After separation of the phases the water layer was additionally extracted dichloromethane (10 mL). The combined organic phases were washed with water (8 mL) and dried over anhydrous $MgSO_4$. After evaporation of the solvent under reduced pressure the product was isolated using column chromatography on silica gel and CH_2Cl_2 as an eluent. After purification the product was obtained as white solid; 52 mg (98%). Mp = 114.4–115.7 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, *J* = 8.9 Hz, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 7.76 (d, *J* = 2.8 Hz, 1H), 7.36 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 8.2 Hz, 2H), 7.02 (d, *J* = 2.2 Hz, 1H), 6.97 (dd, *J* = 8.7, 2.3 Hz, 1H), 3.95 (q, *J* = 7.1 Hz, 1H), 3.91 (s, 3H), 2.47 (d, *J* = 7.2 Hz, 2H), 1.93–1.83 (m, 1H), 1.61 (d, *J* = 7.2 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.99, 161.12, 160.12, 151.45, 150.86, 141.19, 137.03, 129.77, 127.83, 127.32, 124.56, 123.53, 122.95, 122.08, 118.36, 116.10, 111.38, 110.95, 55.94, 45.41, 45.19, 30.33, 22.55, 18.61. MS (m/z) $[M]^+$: 431.

Synthesis of 8-methoxy-6-oxo-6H-benzo[c]chromen-3-yl 2-((2,3-dimethylphenyl)amino)benzoate (8). To a magnetically stirred at room temperature suspension of **2** (0.050 g; 0.21 mmol, 1.0 eqv) in a mixture of dioxane (20 mL) and dichloromethane (10 mL) mefenamic acid (0.050 g; 0.21 mmol, 1.0 eqv), DCC (0.052 g; 0.25 mmol; 1.2 eqv) and DMAP (5.13 mg; 0.042 mmol; 0.2 eqv) were subsequently added. After 24 h the reaction mixture was concentrated under reduced pressure. The residual oil was purified using column chromatography on silica gel and CH_2Cl_2 as an eluent. After purification the product was obtained as white solid; 50 mg (51%). Mp = 186.7–189.2 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.14 (s, 1H), 8.20 (dd, *J* = 8.1, 1.4 Hz, 1H), 8.04 (d, *J* = 8.7 Hz, 1H), 8.01 (s, 1H), 7.82 (d, *J* = 2.8 Hz, 1H), 7.42 (dd, *J* = 8.8, 2.8 Hz, 1H), 7.34 (ddd, *J* = 8.6, 7.1, 1.4 Hz, 1H), 7.29 (d,

J = 2.3 Hz, 1H), 7.24 (dd, *J* = 8.6, 2.3 Hz, 1H), 7.17 (d, *J* = 7.6 Hz, 1H), 7.12 (t, *J* = 7.7 Hz, 1H), 7.05 (d, *J* = 7.3 Hz, 1H), 6.81 (dd, *J* = 8.6, 0.7 Hz, 1H), 6.76 (ddd, *J* = 8.1, 7.1, 1.1 Hz, 1H), 3.95 (s, 3H), 2.33 (s, 3H), 2.17 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.93, 161.06, 160.03, 151.29, 150.90, 150.53, 138.35, 138.18, 135.33, 132.69, 131.91, 127.76, 127.22, 126.02, 124.48, 123.42 (d, *J* = 7.7 Hz), 123.00, 121.99, 118.84, 116.27, 116.10, 113.86, 111.46, 111.30, 109.01, 55.83, 34.93, 25.46, 24.70, 20.62, 14.02; . MS (m/z) $[M]^+$: 466.

Synthesis of 8-methoxy-6-oxo-6H-benzo[c]chromen-3-yl 2-(2-((2,6-dichlorophenyl)amino)phenyl)acetate (9). To a magnetically stirred at room temperature suspension of **2** (0.050 g; 0.21 mmol, 1.0 eqv) in a mixture of dioxane (20 mL) and dichloromethane (10 mL) diclofenac (0.124 g; 0.42 mmol, 2.0 eqv), DCC (0.087 g; 0.42 mmol; 2.0 eqv) and DMAP (10.30 mg; 0.084 mmol; 0.4 eqv) were subsequently added. After 24 h the reaction mixture was concentrated under reduced pressure. The residual oil was purified using column chromatography on silica gel and CH_2Cl_2 as an eluent. After purification the product was obtained as pale beige solid; 14 mg (13%). Mp = 207.3–208.5 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (d, *J* = 4.9 Hz, 1H), 7.96 (d, *J* = 4.7 Hz, 1H), 7.80 (d, *J* = 2.8 Hz, 1H), 7.40 (dd, *J* = 8.9, 2.8 Hz, 1H), 7.35–7.32 (m, 3H), 7.22–7.16 (m, 1H), 7.17 (d, *J* = 2.1 Hz, 1H), 7.11 (dd, *J* = 8.7, 2.4 Hz, 1H), 6.96–7.07 (m, 2H), 6.61 (d, *J* = 8.0 Hz, 1H), 4.10 (s, 2H), 3.94 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.30, 160.97, 160.09, 150.94, 150.78, 142.73, 137.70, 131.08, 129.49, 128.90, 128.47, 127.63, 124.48, 124.24, 123.57, 123.46, 123.01, 122.43, 122.04, 118.68, 118.30, 116.32, 111.30, 111.00, 55.84, 38.57. MS (m/z) $[M]^+$: 521.

Synthesis of 8-methoxy-6-oxo-6H-benzo[c]chromen-3-yl 2-acetoxybenzoate (10). To a magnetically stirred at 50 °C solution of **2** (0.060 g; 0.25 mmol, 1.0 eqv) in a mixture of dioxane (20 mL) and dichloromethane (10 mL) triethylamine (0.103 mL; 0.75 mmol, 3.0 eqv) and next solution of aspirin chloride (0.099 g; 0.50 mmol, 2.0 eqv) in CH_2Cl_2 (2 mL) were added. After 3 h the reaction mixture was concentrated under reduced pressure. To residue dichloromethane (20 mL) and water (8 mL) were added and acidified to pH 2–3 with 3% HCl_{aq} solution (1 mL). After separation of the phases the water layer was additionally extracted dichloromethane (10 mL). The combined organic phases were washed with water (8 mL) and dried over anhydrous $MgSO_4$. After evaporation of the solvent under reduced pressure the product was isolated using column chromatography on silica gel and CH_2Cl_2 as an eluent. After purification the product was obtained as white solid; 60 mg (60%). Mp = 192.6–193.5 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (dd, *J* = 7.9, 1.7 Hz, 1H), 8.01–7.96 (m, 2H), 7.76 (d, *J* = 2.8 Hz, 1H), 7.64 (ddd, *J* = 8.1, 7.6, 1.7 Hz, 1H), 7.40–7.36 (m, 2H), 7.20 (d, *J* = 2.3 Hz, 1H), 7.16 (ddd, *J* = 8.7, 4.0, 1.7 Hz, 2H), 3.91 (s, 3H), 2.29 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 169.98, 162.66, 161.22, 160.16, 151.31, 150.93, 150.82, 135.06, 132.25, 127.69, 126.38, 124.56, 124.15, 123.58, 123.24, 122.05,121.96, 118.56, 116.43, 111.37, 111.18, 55.86, 49.62, 49.45, 49.28, 21.02. MS (m/z) $[M]^+$: 405.

NMR spectra of UADs with methoxy group in 8 position and esterified group in 3- position (Fig. S2) are presented in Figs. S3–S6.

UHPLC-DAD-MS. Unless otherwise specified, methanol was used to dissolve samples before UHPLC-DAD-MS analysis. The experiments were conducted on a UHPLC-3000 RS system (Dionex, Leipzig, Germany), outfitted with diode array detector coupled with AmaZon SL ion trap mass spectrometer with an ESI interface (Bruker Daltonik GmbH, Bremen, Germany). The injection volume was 10 µL. The compounds were separated using Kinetex XB-C_{18} (Phenomenex, Torrance, CA, 150 mm × 2.1 mm, particle size 1.7 µm) analytical column at temperature 25 °C. The elution was performed in gradient system using mobile phase A (H2O:formic acid (99.9:0.1, *v/v*)), and mobile phase B (acetonitrile:formic acid (99.9:0.1, *v/v*)), starting from with the 35% of mobile phase B, 35–95% for 20 min and additional 5 min with constant, 95% phase B concentration. The flow rate was set to 0.3 ml/min. UV detection of UADs was accomplished at λ = 305 nm. The parameters of ESI source were as follows: nebuliser pressure: 40 psi, drying gas flow rate: 9 L/min, dry gas temperature 300 °C, and capillary voltage: 4.5 kV. The mass spectra were registered by scanning from m/z 70 to 2200. Identification of compounds was based on the determination of their molecular mass, UV–Vis spectra and fragmentation profile.

NMR. The NMR spectra were recorded on a Bruker AVANCE (Bruker, Karlsruhe, Germany) spectrometer operating at 300 or 500 MHz for ^{1}H NMR and at 125 MHz or 75 MHz for ^{13}C NMR. The spectra were measured in $CDCl_3$ and are given as δ values (in ppm) relative to TMS, and coupling constants (J) are reported in Hz.

Stability assays. The stability assays under several stress conditions were performed according to modified, previously described protocol[29]. Detailed information regarding stability assays' conditions are described in Supporting Information. Samples were dissolved in DMSO to obtain 20 mg/ml concentration and added to aliquots to achieve 80 mg/ml concentration at the start of incubation. All experiments were performed in triplicates (n = 3). The degree of degradation was calculated as follows:

$$Degradation(\%) = \left[1 - \left(\frac{Peak\ area\ of\ UAD\ in\ aliqout}{Peak\ area\ of\ UAD\ in\ control}\right)\right] \times 100.$$

THP-1 derived macrophages. THP-1 human monocytic cells (DSMZ, Braunschweig, Germany) were studied between passages 4 and 19. Monocytes were differentiated into THP-1 derived macrophages according to modified, previously described protocol[17]. THP-1 monocytes were seeded in 24-well plates at a density of 400,000 cells per well. Cells were incubated at 37 °C under humidified 5% CO_2 in the culture medium containing RPMI 1640 without phenol red supplemented with 10% FBS and 2 mM glutamine. In order to differenti-

ate THP-1 monocytes into macrophages, cells were treated with 25 ng/mL PMA followed by 48 h incubation, medium change and an additional 24 h resting. Selected UADs were added to THP-1 derived macrophages at concentrations 2–50 μM and left for 1-h resting. Next, cells were treated with 10 ng/ml LPS. Successful differentiation was confirmed by the presence of distinguishing macrophage markers such as: cell adherence, changes in morphology and response to bacterial LPS stimulation and, consequently, TNF-α secretion.

MTT assay. THP-1 monocytes were seeded in 24-well plates at a density of 400,000 cells per well and differentiated into THP-1 derived macrophages according to the method described above. After treatment with the test compounds for 1 h and subsequent stimulation with LPS for 3 or 24 h, cells were washed with warm PBS. Afterwards, a 1 mL of solution of 0.5 mg/mL MTT in culture medium was added, and macrophages were incubated at 37 °C under humidified 5% CO_2 for 30 min. After medium removal, crystals were dissolved in 400 μL DMSO, 200 μL of solution was transferred to 96-well plate and the absorbance of the solution was measured at λ = 570 nm with the correction to 630 nm using a microplate reader. The relative cell viability was shown as the experimental group's ratio to stimulated cells.

Neutral red uptake (NRU) assay. NRU assay was performed according to modified, previously described protocol[39]. 40 mg of neutral red dye in was dissolved in 10 mL distilled water to obtain 4 mg/ mL neutral red stock solution. Subsequently, 140 μL of neutral red stock solution was added to 14 ml of culture medium and sterile filtered in order to obtain 40 μg/mL neutral red medium. THP-1 monocytes were seeded in 24-well plates at a density of 400,000 cells per well and differentiated into THP-1 derived macrophages according to the method described above. After treatment with the test compounds for 1 h and subsequent stimulation with LPS for 3 or 24 h, cells were washed with warm PBS. Afterwards, a 500 μL of warm 40 μg/mL neutral red medium was added and macrophages were incubated at 37 °C under humidified 5% CO_2 for 2 h. After this time cells were washed with PBS and 500 μL of neutral red destain solution was added and the plate was placed in plate shaker for at least 15 min. The absorbance of the solution was measured at λ = 530 nm with the correction to 645 nm using a microplate reader. The relative cell viability was shown as the experimental group's ratio to stimulated cells. The experiment was performed in triplicate (n = 3).

Enzyme-linked immunosorbent assay (ELISA). After treatment with the test compounds for 1 h and subsequent stimulation with LPS for 3 h (for TNF-α secretion measurements) or 24 h (for IL-10 and IL-6 secretion measurements) supernatants were collected. The secretion of cytokines levels was measured by ELISA kits according to the manufacturer's instruction (Human TNF-α, Human IL-10 and Human IL-6 ELISA kits, BD Biosciences, Franklin Lakes, NJ, USA). The relative cytokines levels secretion was shown as the experimental group's ratio to LPS stimulated cells.

Propidium iodide (PI) staining. THP-1 monocytes were seeded in 24-well plates at a density of 400,000. Selected UADs (2–50 μM), control (0.25% DMSO in PBS), UA (50 μM) and parthenolide (25 μM) as a control were added to THP-1 monocytes and left for 24-h incubation at 37 °C under humidified 5% CO_2 in the culture medium containing RPMI 1640 without phenol red supplemented with 10% FBS and 2 mM glutamine. After that time, cell suspensions were transferred to Eppendorf tubes, centrifuged, stained with propidium iodide (PI) and analyzed using FACS (MACSQuant® Analyze, Miltenyi Biotec, Bergisch Gladbach, Germany). The cell viability was reported as the percentage of PI(−) cells.

Statistical analysis. All in vitro experiments were performed in triplicates (n = 3), at least from 3 independent experiments, if not otherwise stated. The significance of obtained differences was determined using one-way ANOVA, and post hoc Dunnett's test was used to compare results with control groups. Tukey's post hoc test was performed to compare differences in inhibitory activity between groups. The p value < 0.05 was considered statistically significant. Statistica 13 software was used to perform all analyses.

Data availability

All data generated or analysed during this study are included in this published article (and its supplementary information files).

Received: 21 March 2022; Accepted: 30 June 2022
Published online: 08 July 2022

References

1. Gomaa, E. Z. Human gut microbiota/microbiome in health and diseases: a review. *Antonie van Leeuwenhoek Int. J. Gen. Mol. Microbiol.* **113**, 2019–2040 (2020).
2. Food and Agricultural Organization of the United Nations and World Health Organization. Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. (World Health Organization, 2001). https://www.fao.org/3/a0512e/a0512e.pdf
3. Suez, J., Zmora, N., Segal, E. & Elinav, E. The pros, cons, and many unknowns of probiotics. *Nat. Med.* **25**, 716–729 (2019).
4. Salminen, S. *et al.* The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nat. Rev. Gastroenterol. Hepatol.* **18**, 649–667 (2021).
5. Marzec, A. & Feleszko, W. Postbiotics—A step beyond pre- and probiotics. 1–17 (2020).
6. Nataraj, B. H., Ali, S. A., Behare, P. V. & Yadav, H. Postbiotics-parabiotics: The new horizons in microbial biotherapy and functional foods. *Microb. Cell Fact.* **19**, 1–22 (2020).

7. García-Villalba, R. *et al.* Urolithins: A comprehensive update on their metabolism, bioactivity, and associated gut microbiota. *Mol. Nutr. Food Res.* **2101019**, 2101019 (2022).
8. Piwowarski, J. P. *et al.* Role of human gut microbiota metabolism in the anti-inflammatory effect of traditionally used ellagitannin-rich plant materials. *J. Ethnopharmacol.* **155**, 801–809 (2014).
9. Piwowarski, J. P., Granica, S. & Kiss, A. K. *Lythrum salicaria* L.—Underestimated medicinal plant from European traditional medicine. A review. *J. Ethnopharmacol.* **170**, 226–250 (2015).
10. Jurenka, J. Therapeutic applications of pomegranate (*Punica granatum* L.): A review. *Altern. Med. Rev.* **13**, 128–144 (2008).
11. Landete, J. M. Ellagitannins, ellagic acid and their derived metabolites: A review about source, metabolism, functions and health. *Food Res. Int.* **44**, 1150–1160 (2011).
12. Marín, L., Miguélez, E. M., Villar, C. J. & Lombó, F. Bioavailability of dietary polyphenols and gut microbiota metabolism: Antimicrobial properties. *BioMed Res. Int.* **2015** (2015).
13. Tomás-Barberán, F. A., García-Villalba, R., González-Sarrías, A., Selma, M. V. & Espín, J. C. Ellagic acid metabolism by human gut microbiota: Consistent observation of three urolithin phenotypes in intervention trials, independent of food source, age, and health status. *J. Agric. Food Chem.* **62**, 6535–6538 (2014).
14. Selma, M. V., Beltrán, D., García-Villalba, R., Espín, J. C. & Tomás-Barberán, F. A. Description of urolithin production capacity from ellagic acid of two human intestinal Gordonibacter species. *Food Funct.* **5**, 1779–1784 (2014).
15. Cortés-Martín, A. *et al.* The gut microbiota urolithin metabotypes revisited: the human metabolism of ellagic acid is mainly determined by aging. *Food Funct.* **9**, 4100–4106 (2018).
16. D'Amico, D. *et al.* Impact of the natural compound urolithin A on health, disease, and aging. *Trends Mol. Med.* **27**, 687–699 (2021).
17. Bobowska, A. *et al.* Comparative studies of urolithins and their phase II metabolites on macrophage and neutrophil functions. *Eur. J. Nutr.* **60**, 1957–1972 (2021).
18. Dinarello, C. A. Anti-inflammatory agents: Present and future. *Cell* **140**, 935–950 (2010).
19. Schjerning, A. M., McGettigan, P. & Gislason, G. Cardiovascular effects and safety of (non-aspirin) NSAIDs. *Nat. Rev. Cardiol.* **17**, 574–584 (2020).
20. Eder, J., Sedrani, R. & Wiesmann, C. The discovery of first-in-class drugs: Origins and evolution. *Nat. Rev. Drug Discov.* **13**, 577–587 (2014).
21. Chanput, W., Mes, J. J. & Wichers, H. J. THP-1 cell line: An in vitro cell model for immune modulation approach. *Int. Immunopharmacol.* **23**, 37–45 (2014).
22. Guellec, C. Le, Lacarelle, B., Point, H., Catalin, J. & Durand, A. Glucuronidation of propofol in microsomal fractions from various tissues and species including humans: Effect of different drugs. *Anesth. Analg.* (1995).
23. Jarrar, Y. B., Kim, D. H., Lee, S. J. & Shin, J. G. Inhibition of 20-hydroxyeicosatetraenoic acid (20-HETE) glucuronidation by non-steroidal anti-inflammatory drugs in human liver microsomes and recombinant UDP-glucuronosyltransferase enzymes. *Prostaglandins Leukot. Essent. Fat. Acids* **153**, 102055 (2020).
24. Joo, J. *et al.* Screening of non-steroidal anti-inflammatory drugs for inhibitory effects on the activities of six UDP-glucuronosyltransferases (UGT1A1, 1A3, 1A4, 1A6, 1A9 and 2B7) using LC-MS/MS. *Biopharm. Drug Dispos.* **36**, 258–264 (2015).
25. Siskou, I. C. *et al.* Design and study of some novel ibuprofen derivatives with potential nootropic and neuroprotective properties. *Bioorg. Med. Chem.* **15**, 951–961 (2007).
26. Kalathil, A. A. *et al.* New formulation of old aspirin for better delivery. *Chem. Commun.* **52**, 140–143 (2016).
27. Tang, L. *et al.* Design, synthesis, and biological evaluation of novel 6h-benzo[c]chromen-6-one derivatives as potential phosphodiesterase ii inhibitors. *Int. J. Mol. Sci.* **22**, 1–12 (2021).
28. Piwowarski, J. P., Stanisławska, I., Granica, S., Stefanska, J. & Kiss, A. K. Phase II conjugates of urolithins isolated from human urine and potential role of β-glucuronidases in their disposition. *Drug Metab. Dispos.* **45**, 657–665 (2017).
29. Bisht, R., Rupenthal, I. D., Sreebhavan, S. & Jaiswal, J. K. Development of a novel stability indicating RP-HPLC method for quantification of Connexin43 mimetic peptide and determination of its degradation kinetics in biological fluids. *J. Pharm. Anal.* **7**, 365–373 (2017).
30. van Tonder, A., Joubert, A. M. & Cromarty, A. D. Limitations of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay when compared to three commonly used cell enumeration assays. *BMC Res. Notes* **8**, 47 (2015).
31. Li, S. *et al.* Parthenolide inhibits LPS-induced inflammatory cytokines through the toll-like receptor 4 signal pathway in THP-1 cells. *Acta Biochim. Biophys. Sin. (Shanghai)* **47**, 368–375 (2015).
32. Voloshyna, I. *et al.* COX-2-dependent and independent effects of COX-2 inhibitors and NSAIDs on proatherogenic changes in human monocytes/macrophages. *J. Investig. Med.* **65**, 694–704 (2017).
33. Pereira, A. C. A. *et al.* Cyclooxygenase (COX)-2 inhibitors reduce toxoplasma gondiiinfection and upregulate the pro-inflammatory immune response in calomys callosusrodents and human monocyte cell line. *Front. Microbiol.* **10**, 1–20 (2019).
34. Hulina-Tomašković, A. *et al.* Effects of extracellular Hsp70 and cigarette smoke on differentiated THP-1 cells and human monocyte-derived macrophages. *Mol. Immunol.* **111**, 53–63 (2019).
35. Qin, Y. *et al.* Graphene quantum dots induce apoptosis, autophagy, and inflammatory response via p38 mitogen-activated protein kinase and nuclear factor-κB mediated signaling pathways in activated THP-1 macrophages. *Toxicology* **327**, 62–76 (2015).
36. Rønning, S. B., Voldvik, V., Bergum, S. K., Aaby, K. & Borge, G. I. A. Ellagic acid and urolithin A modulate the immune response in LPS-stimulated U937 monocytic cells and THP-1 differentiated macrophages. *Food Funct.* **11**, 7946–7959 (2020).
37. Piwowarski, J. P., Kiss, A. K., Granica, S. & Moeslinger, T. Urolithins, gut microbiota-derived metabolites of ellagitannins, inhibit LPS-induced inflammation in RAW 264.7 murine macrophages. *Mol. Nutr. Food Res.* **59**, 2168–2177 (2015).
38. Dobroslawa, B., Kasimsetty, S. G., Khan, S. I. & Daneel, F. Urolithins, intestinal microbial metabolites of pomegranate ellagitannins, exhibit potent antioxidant activity in a cell-based assay. *J. Agric. Food Chem.* **57**, 10181–10186 (2009).
39. Repetto, G., del Peso, A. & Zurita, J. L. Neutral red uptake assay for the estimation of cell viability/ cytotoxicity. *Nat. Protoc.* **3**, 1125–1131 (2008).

Acknowledgements

Project financially supported by Polish National Science Centre research Grant Preludium Bis No. UMO-2019/35/O/NZ7/00619.

Author contributions

Study concept M.K., S.G., J.P.P., Synthesis M.K., P.R.; Characterization of UADs, M.K., J.P.P.; In vitro studies M.K., Supervision, S.G., J.P.P.; Writing manuscript M.K., P.R., J.P.P.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-022-15870-8.

Correspondence and requests for materials should be addressed to J.P.P.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary materials to:

Publication No. 2

Conjugates of urolithin A with NSAIDs, their stability, cytotoxicity, and anti-inflammatory potential.

Maciej Korczak [a], Piotr Roszkowski [b], Sebastian Granica [a], Jakub Patryk Piwowarski [a]

[a] Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland

[b] Laboratory of Natural Products Chemistry, Faculty of Chemistry, University of Warsaw, Warsaw, Poland.

Scientific Reports. 2022 Jul

12(1):11676

Erratum in: *Scientific Reports*. 2022 Nov; 12(1):18503.

Supplementary information

Conjugates of urolithin A with NSAIDs, their stability, cytotoxicity, and anti-inflammatory potential.

Maciej Korczak[1], Piotr Roszkowski[2], Sebastian Granica[1], Jakub P. Piwowarski[1*]

[1]Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Warsaw, Poland

[2]Faculty of Chemistry, Warsaw University, Warsaw, Poland

Stability assay performance

Control experiments. UADs dissolved in DMSO were added to deionised water, mixed thoroughly and, immediately, samples were taken, added to 0.2% formic acid in acetonitrile and subjected to UHPLC-DAD-MS analysis. In experiments evaluating stability in human plasma, UADs dissolved in DMSO were added to plasma, mixed thoroughly and, immediately, samples were taken, added to 0.2% formic acid in acetonitrile, centrifuged at 13000 RPM for 10 minutes and supernatants were subjected to UHPLC-DAD-MS analysis.

Acid hydrolysis. UADs dissolved in DMSO were added to HCl (1M), mixed thoroughly and kept at 37 °C for 24h or 80 °C for 12h. Samples were taken, added to NaOH (1M), mixed with 0.2% formic acid in acetonitrile and subjected to UHPLC-DAD-MS analysis.

Base hydrolysis. UADs dissolved in DMSO were added to NaOH (1M), mixed thoroughly and kept at 37 °C for 24h or 80 °C for 12h. Samples were taken, added to HCl (1M), mixed with 0.2% formic acid in acetonitrile and subjected to UHPLC-DAD-MS analysis.

Thermal degradation. UADs dissolved in DMSO were added to deionised water, mixed thoroughly and kept at room temperature for 24h, 37 °C for 24h, 80 °C for 12h or -70 °C for 24h. Additionally, samples were exposed to a cycle consisting of incubation at -70 °C for 24h followed by 2h thawing in tap water at room temperature, subsequent freezing at -20 °C for 24h and 2h thawing in tap water at room temperature. Samples were taken, mixed with 0.2% formic acid in acetonitrile and subjected to UHPLC-DAD-MS analysis.

UV degradation. ADs dissolved in DMSO were added to deionised water, mixed thoroughly and kept at room temperature for 7h in plastic falcons and exposed to UV-C light (λ=254 nm, Osram G3058/OF lamp, Osram Licht AG, Munich, Germany). Samples were taken, mixed with 0.2% formic acid in acetonitrile and subjected to UHPLC-DAD-MS analysis.

Oxidative degradation. UADs dissolved in DMSO were added to 30% H_2O_2 and kept at 37 °C for 24h or 80 °C for 12h. Samples were taken, mixed with 0.2% formic acid in acetonitrile and subjected to UHPLC-DAD-MS analysis.

Stability in human plasma.

Human plasma was collected from three independent donors and obtained from the Warsaw Blood Donation Center. UADs dissolved in DMSO were added to plasma, mixed thoroughly and kept at 37 °C for 30, 90 and 240 minutes. Samples were taken, added to 0.2% formic acid in acetonitrile, centrifuged at 13000 RPM for 10 minutes and supernatants were subjected to UHPLC-DAD-MS analysis.

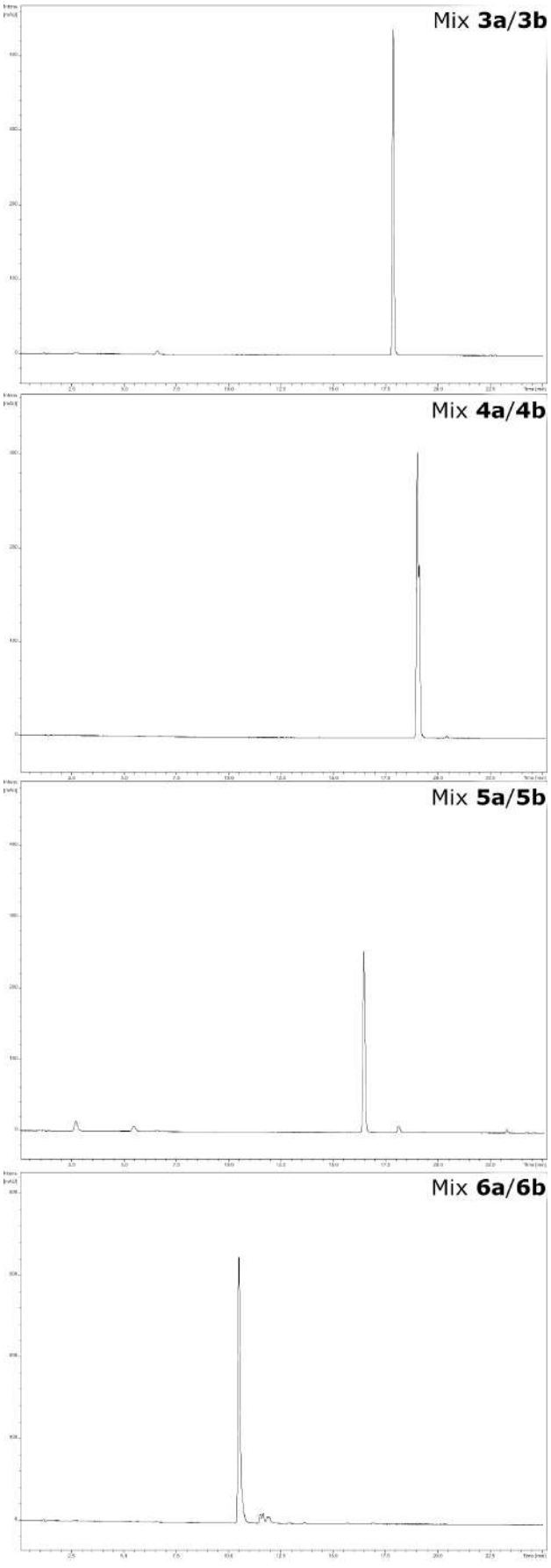


Figure S1. UHPLC profiles of **1** and synthesised UADs (λ= 305 nm)

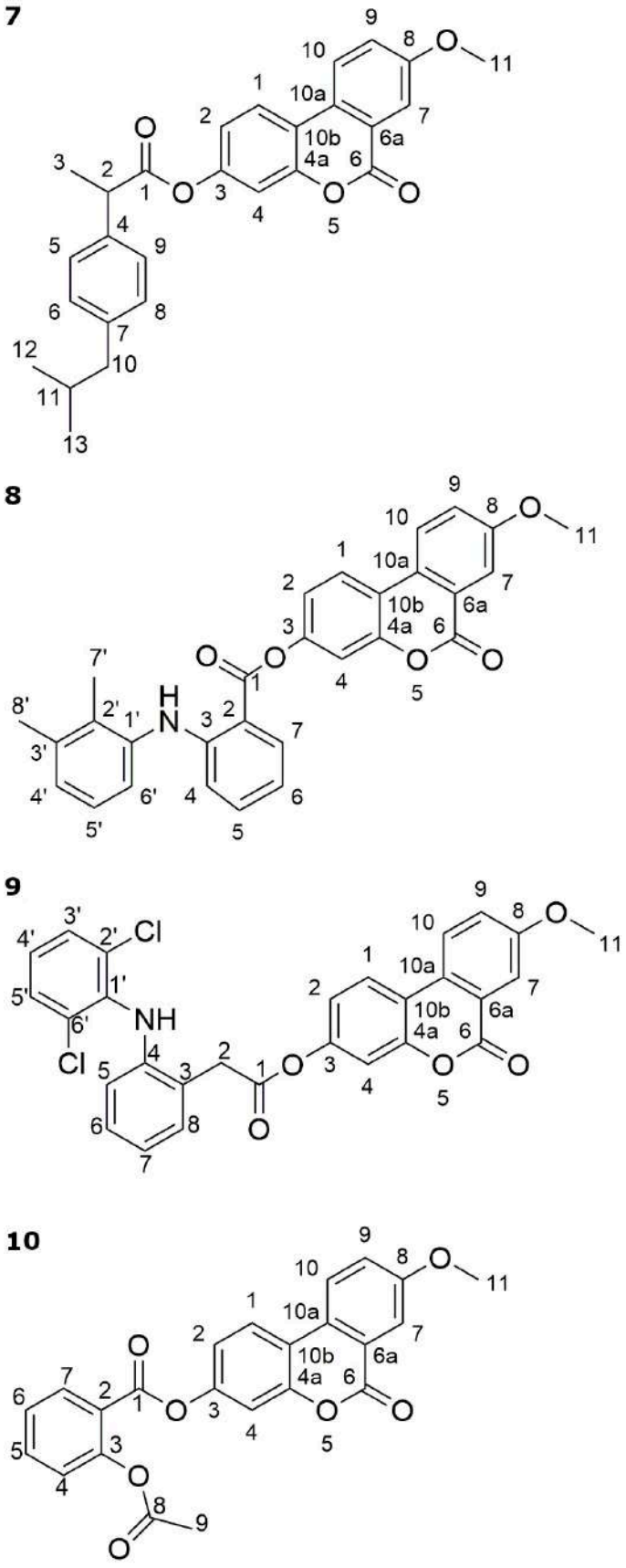


Figure S2 . Chemical structure of 3-NSAID-8-methoxy-UADs

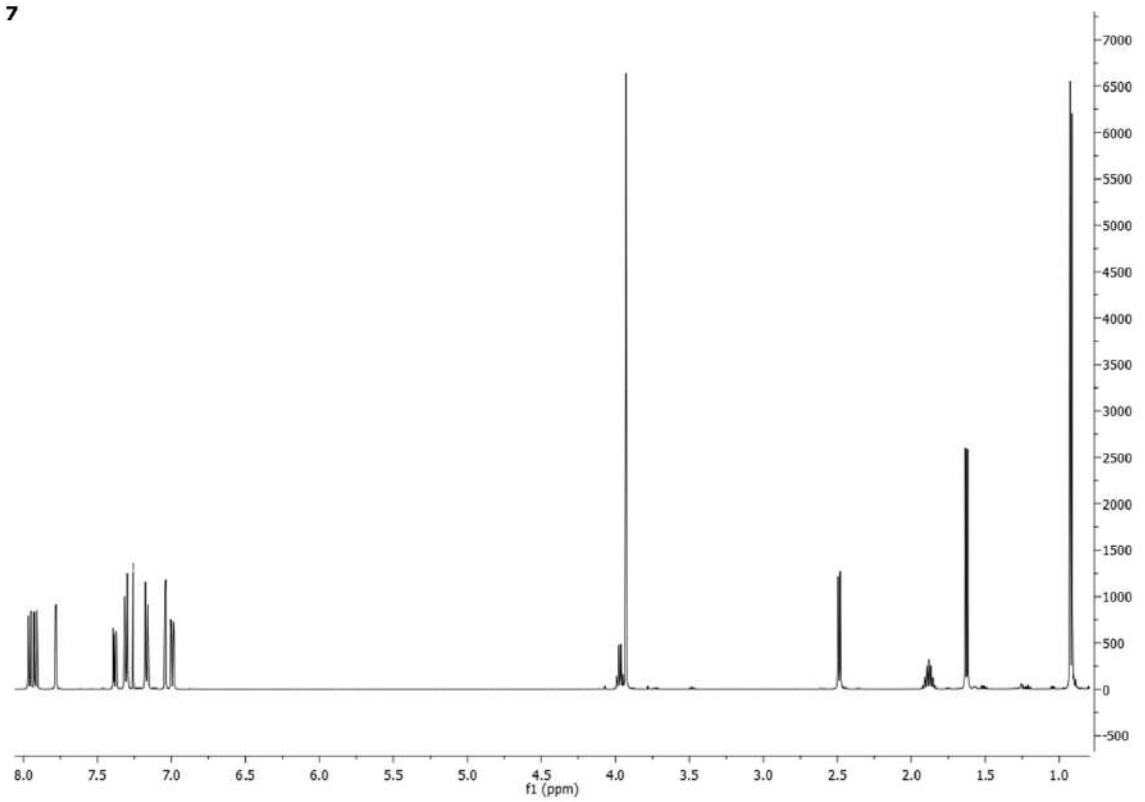


Figure S3. ^{1}H NMR spectrum of **7**

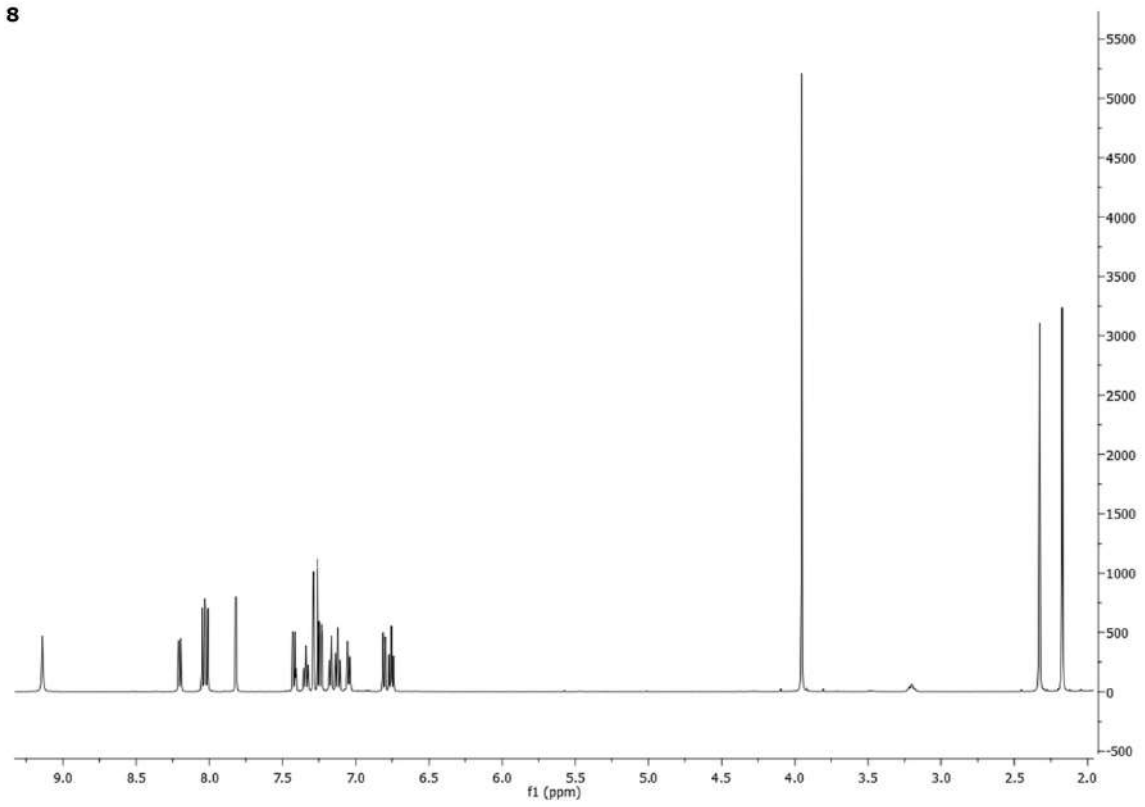


Figure S4. ^{1}H NMR spectrum of **8**

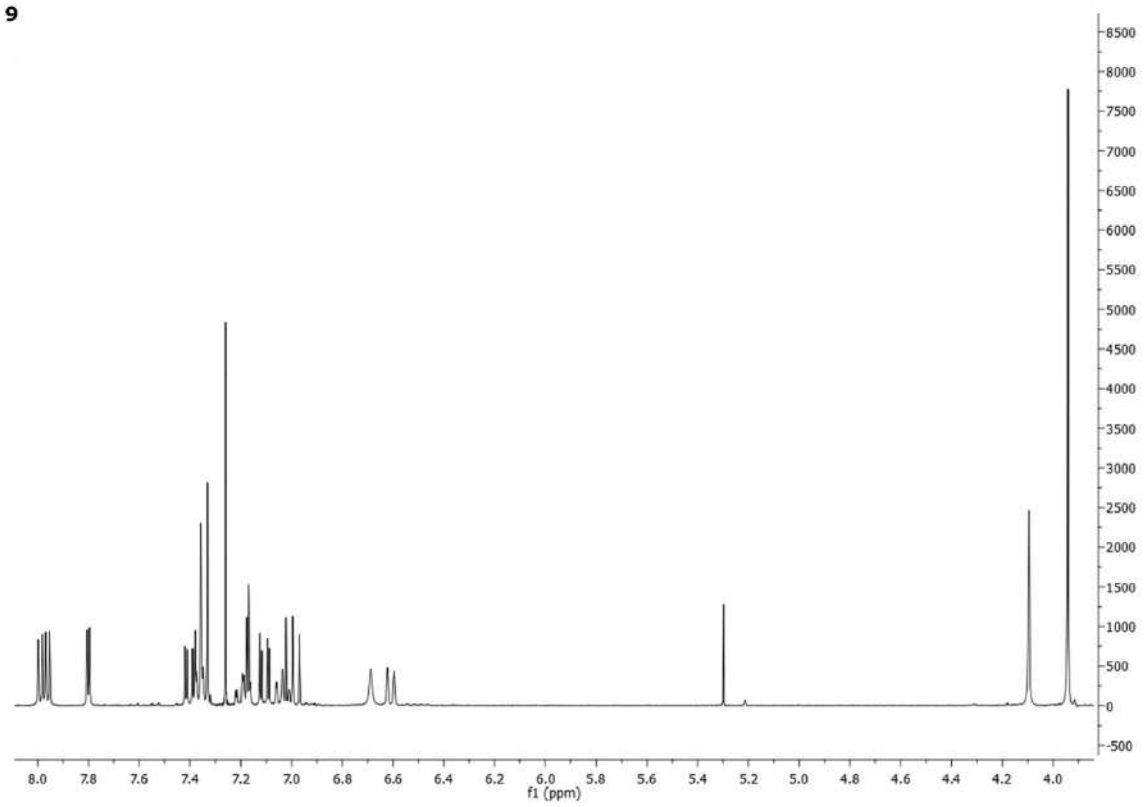


Figure S5. ^{1}H NMR spectrum of **9**

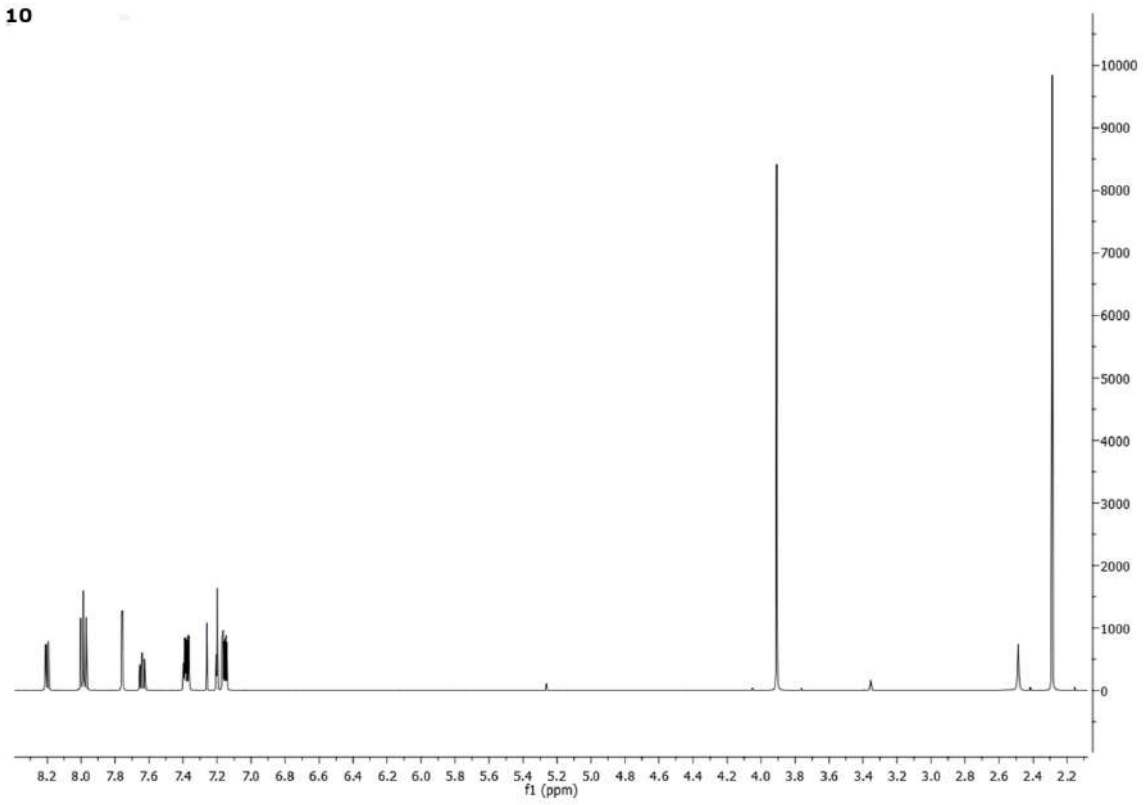


Figure S6. ^{1}H NMR spectrum of **10**

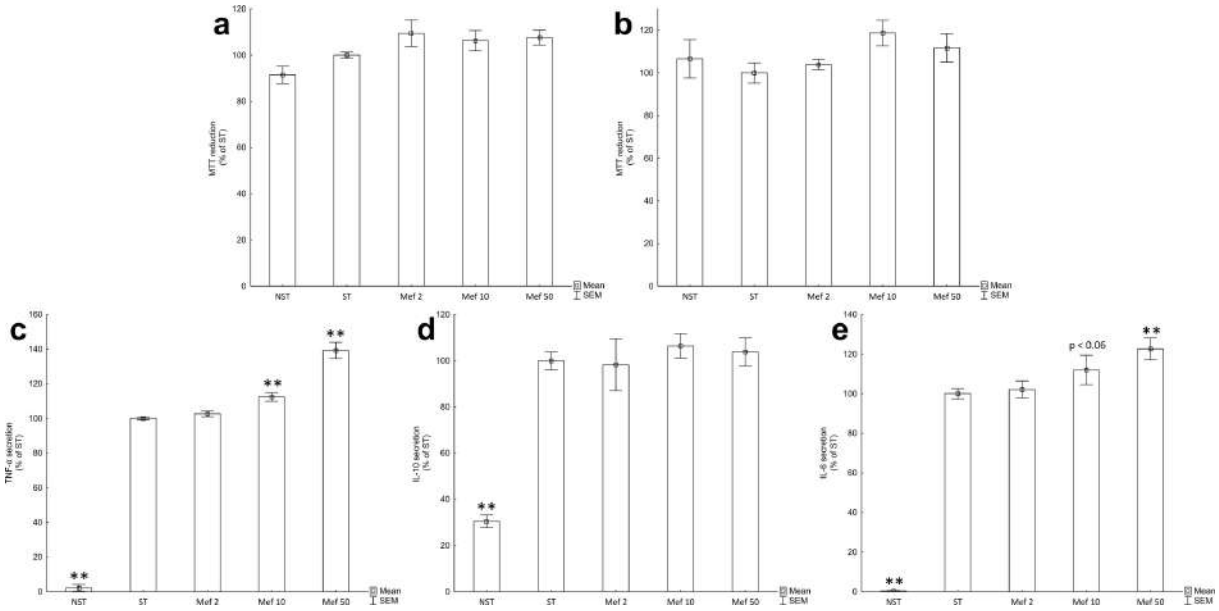


Figure S7. Evaluation of cytotoxic and anti-inflammatory activity of mefenamic acid using LPS stimulated THP-1 derived macrophages. (a) Effects of mefenamic acid on THP-1 derived macrophage viability measured using MTT assay after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 3 h. (b) Effects of mefenamic acid on THP-1 derived macrophage viability measured using MTT assay after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 24 h. (c) Effects of mefenamic acid on TNF-α secretion after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 3 h. (d) Effects of mefenamic acid on IL-10 secretion after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 24 h. (e) Effects of mefenamic acid on IL-6 secretion after treatment with the test compounds for 1 h and subsequent stimulation with LPS for 24 h. Data expressed as a mean±SEM. Numbers next graph labels indicate tested concentration (in μM). Statistical comparisons were made using the parametric method of one-way ANOVA, followed by the Dunnett's post hoc test. Statistical significance:**$p < 0.01$ versus ST group. NST – non-stimulated control, ST – LPS stimulated control, Mef – mefenamic acid.

	Viability (% of ST)	SEM	post hoc (Dunett) p (<ST)	p (>ST)
NST	91,46673	3,709197	0,166543	0,999692
ST	100	1,373162		
UA 2	100,3891	2,603849	0,907062	0,868297
UA 10	110,0242	5,546335	0,99991	0,091721
UA 50 µM	105,1696	2,525687	0,994954	0,450635
Mix **4a**/**4b** 2 µM	106,281	2,061571	0,997861	0,342196
Mix **4a**/**4b** 5 µM	106,1673	4,026725	0,997659	0,352808
Mix **4a**/**4b** 10 µM	102,4332	2,343859	0,968545	0,720091
Mix **4a**/**4b** 50 µM	39,26495	3,562343	0,000022	0,999963

post hoc (Tukey)

	NST	ST	UA 50 µM	Mix **4a**/**4b** 2 µM	Mix **4a**/**4b** 5 µM	Mix **4a**/**4b** 10 µM	
NST							
ST	0,664729						
UA 2	0,60937	1					
UA 10	0,005047	0,45165	0,506566				
UA 50 µM	0,097931	0,971479	0,982403	0,980621			
Mix **4a**/**4b** 2 µM	0,053776	0,914156	0,939156	0,996551	1		
Mix **4a**/**4b** 5 µM	0,057315	0,922047	0,945413	0,995751	1	1	
Mix **4a**/**4b** 10 µM	0,328794	0,999856	0,999962	0,788094	0,999655	0,995819	0,996608
Mix **4a**/**4b** 50 µM	0,000136	0,000136	0,000136	0,000136	0,000136	0,000136	0,000136

Table S1. Post hoc analysis of effects of UA and Mix **4a**/**4b** on THP-1 derived macrophage viability measured using MTT assay 3 hours after stimulation. Cells viability presented as a ratio of the experimental group to LPS stimulated cells. ST group. NST – non-stimulated control, ST – LPS stimulated control

	Viability (% of ST)	SEM	post hoc (Dunett) p (<ST)	p (>ST)
NST	106,5945	8,995372	0,945726	0,721615
ST	100,0000	4,749834		
UA 50 µM	107,1844	2,987045	0,950537	0,706223
Mix **4a**/**4b** 2 µM	113,2348	7,804671	0,971350	0,614843
Mix **4a**/**4b** 5 µM	150,2666	18,84261	0,999973	0,002693
Mix **4a**/**4b** 10 µM	147,2280	7,333491	0,999973	0,004823
Mix **4a**/**4b** 50 µM	4,217185	0,190066	0,000089	0,999973

post hoc (Tukey)

	NST	ST	UA 50 µM	Mix **4a**/**4b** 2 µM	Mix **4a**/**4b** 5 µM	Mix **4a**/**4b** 10 µM
NST						
ST	0,999744					
UA 50 µM	1,000000	0,999580				
Mix **4a**/**4b** 2 µM	0,999965	0,998053	0,999980			
Mix **4a**/**4b** 5 µM	0,179506	0,016282	0,191636	0,735161		
Mix **4a**/**4b** 10 µM	0,248497	0,028335	0,263756	0,803974	0,999991	
Mix **4a**/**4b** 50 µM	0,002947	0,006230	0,002757	0,001403	0,000136	0,000147

Table S2. Post hoc analysis of effects of UA and Mix **4a**/**4b** on THP-1 derived macrophage viability measured using MTT assay 24 hours after stimulation. Cells viability presented as a ratio of the experimental group to LPS stimulated cells. ST group. NST – non-stimulated control, ST – LPS stimulated control

	Viability (% of ST)	SEM	post hoc (Dunett) p (<ST)	post hoc (Dunett) p (>ST)
NST	110,9259	1,858783	0,999981	0,000065
ST	100,0000	0,975523		
UA 10 µM	102,4074	0,883277	0,996275	0,333029
UA 50 µM	102,7778	0,555556	0,998036	0,255639
Mix **4a/4b** 2 µM	102,1296	1,557649	0,994028	0,398113
Mix **4a/4b** 5 µM	102,9630	0,822981	0,998576	0,221591
Mix **4a/4b** 10 µM	104,5370	1,043466	0,999897	0,052107
Mix **4a/4b** 50 µM	91,20370	1,766554	0,000474	0,999981

post hoc (Tukey)

	NST	ST	UA 10 µM	UA 50 µM	Mix **4a/4b** 2 µM	Mix **4a/4b** 5 µM	Mix **4a/4b** 10 µM
NST							
ST	0,000459						
UA 10 µM	0,004170	0,868225					
UA 50 µM	0,006179	0,769927	0,999999				
Mix **4a/4b** 2 µM	0,003123	0,923573	1,000000	0,999942			
Mix **4a/4b** 5 µM	0,007535	0,712945	0,999979	1,000000	0,999689		
Mix **4a/4b** 10 µM	0,040871	0,248682	0,923573	0,970601	0,868225	0,983979	
Mix **4a/4b** 50 µM	0,000175	0,003123	0,000386	0,000318	0,000459	0,000293	0,000192

Table S3. Post hoc analysis of effects of UA and Mix **4a**/**4b** on THP-1 derived macrophage viability measured using NRU assay 3 hours after stimulation. Cells viability presented as a ratio of the experimental group to LPS stimulated cells. ST group. NST – non-stimulated control, ST – LPS stimulated control

	Viability (% of ST)	SEM	post hoc (Dunett) p (<ST)	p (>ST)
NST	109,0261	1,992042	0,996146	0,337522
ST	100,0000	0,411413		
UA 10 µM	102,7712	0,570950	0,949340	0,744263
UA 50 µM	99,20823	1,577582	0,843583	0,901621
Mix **4a/4b** 2 µM	100,0000	1,900238	0,874994	0,874994
Mix **4a/4b** 5 µM	100,3167	1,380491	0,886203	0,863011
Mix **4a/4b** 10 µM	98,89153	1,038391	0,829652	0,910992
Mix **4a/4b** 50 µM	72,05067	13,02039	0,002231	0,999980

post hoc (Tukey)

	NST	ST	UA 10 µM	UA 50 µM	Mix **4a/4b** 2 µM	Mix **4a/4b** 5 µM	Mix **4a/4b** 10 µM
NST							
ST	0,872769						
UA 10 µM	0,978886	0,999867					
UA 50 µM	0,821027	1,000000	0,999308				
Mix **4a/4b** 2 µM	0,872769	1,000000	0,999867	1,000000			
Mix **4a/4b** 5 µM	0,890910	1,000000	0,999941	1,000000	1,000000		
Mix **4a/4b** 10 µM	0,798015	1,000000	0,998808	1,000000	1,000000	0,999999	
Mix **4a/4b** 50 µM	0,001169	0,013955	0,006329	0,017489	0,013955	0,012743	0,019138

Table S4. Post hoc analysis of effects of UA and Mix **4a**/**4b** on THP-1 derived macrophage viability measured using NRU assay 24 hours after stimulation. Cells viability presented as a ratio of the experimental group to LPS stimulated cells. ST group. NST – non-stimulated control, ST – LPS stimulated control

post hoc (Dunett)

	% of PI (-) cells	SEM	p (<NST)	p (>NST)
NST	81,59444	1,249789		
UA 50 µM	76,06222	1,469574	0,004616	0,999968
Mix **4a/4b** 2 µM	81,98778	1,245794	0,914369	0,788404
Mix **4a/4b** 5 µM	82,03286	1,053212	0,916244	0,784895
Mix **4a/4b** 10 µM	79,30889	1,178412	0,295828	0,996675
Mix **4a/4b** 50 µM	5,827778	0,278487	0,000021	0,999968
Parthenolide 25 µm	60,84000	1,462561	0,000021	0,999968

post hoc (Tukey)

	NST	UA 50 µM	Mix **4a/4b** 2 µM	Mix **4a/4b** 5 µM	Mix **4a/4b** 10 µM	Mix **4a/4b** 50 µM
NST						
UA 50 µM	0,027443					
Mix **4a/4b** 2 µM	0,999986	0,014390				
Mix **4a/4b** 5 µM	0,999987	0,041670	1,000000			
Mix **4a/4b** 10 µM	0,819902	0,468627	0,686294	0,783358		
Mix **4a/4b** 50 µM	0,000136	0,000136	0,000136	0,000136	0,000136	
Parthenolide 25 µm	0,000136	0,000136	0,000136	0,000136	0,000136	0,000136

Table S5. Post hoc analysis of effects of UA and Mix **4a**/**4b** on THP-monocyte viability measured using PI flow cytometric assay after 24 hours incubation. ST group. NST – non-stimulated control, ST – LPS stimulated control

	TNF-α secretion (%)	SEM	post hoc (Dunett) p (<ST)	post hoc (Dunett) p (>ST)
NST	6,990748	1,806716	0,000022	0,999964
ST	100,0000	1,141368		
UA 2 µM	82,29229	6,969327	0,009414	0,999964
UA 10 µM	78,42103	2,034191	0,002734	0,999964
UA 50 µM	21,95156	0,749626	0,000022	0,999964
Mix **4a**/**4b** 2 µM	99,95509	3,715590	0,869749	0,873429
Mix **4a**/**4b** 5 µM	64,45318	5,397676	0,000022	0,999964
Mix **4a**/**4b** 10 µM	108,8595	4,459922	0,997793	0,284701

post hoc (Tukey)

	NST	ST	UA 2 µM	UA 10 µM	UA 50 µM	Mix **4a**/**4b** 2 µM	Mix **4a**/**4b** 5 µM
NST							
ST	0,000126						
UA 2 µM	0,000126	0,099028					
UA 10 µM	0,000126	0,019112	0,998471				
UA 50 µM	0,252905	0,000126	0,000126	0,000126			
Mix **4a**/**4b** 2 µM	0,000126	1,000000	0,100734	0,019515	0,000126		
Mix **4a**/**4b** 5 µM	0,000126	0,000131	0,094194	0,335546	0,000126	0,000131	
Mix **4a**/**4b** 10 µM	0,000126	0,840996	0,001580	0,000271	0,000126	0,837519	0,000126

Table S6. Post hoc analysis of effects of UA and Mix **4a**/**4b** on TNF-α secretion in THP-1 derived macrophages 3 hours after stimulation. TNF-α secretion presented as a ratio of the experimental group to LPS stimulated cells. ST group. NST – non-stimulated control, ST – LPS stimulated control

	Il-10 secretion (%)	SEM	post hoc (Dunett) p (<ST)	post hoc (Dunett) p (>ST)
NST	28,78721	3,154767	0,000022	0,999968
ST	100,0000	3,770853		
UA 50 µM	79,03216	15,46492	0,166068	0,999431
Mix **4a**/**4b** 2 µM	99,70232	1,429118	0,856240	0,864996
Mix **4a**/**4b** 5 µM	125,2743	7,250935	0,999872	0,079015
Mix **4a**/**4b** 10 µM	143,6136	8,562598	0,999968	0,001520

post hoc (Tukey)

	NST	ST	UA 50 µM	Mix **4a**/**4b** 2 µM	Mix **4a**/**4b** 5 µM
NST					
ST	0,000141				
UA 50 µM	0,002041	0,535340			
Mix **4a**/**4b** 2 µM	0,043415	1,000000	0,950454		
Mix **4a**/**4b** 5 µM	0,000137	0,326567	0,005406	0,885282	
Mix **4a**/**4b** 10 µM	0,000137	0,010147	0,000167	0,435253	0,630044

Table S7. Post hoc analysis of effects of UA and Mix **4a**/**4b** on Il-10 secretion in THP-1 derived macrophages 24 hours after stimulation. TNF-α secretion presented as a ratio of the experimental group to LPS stimulated cells. ST group. NST – non-stimulated control, ST – LPS stimulated control

	Il-6 secretion (% of ST)	SEM	post hoc (Dunett) p (<ST)	post hoc (Dunett) p (>ST)
NST	0,355629	0,203280	0,000022	0,999970
ST	100,0361	2,667569		
UA 50 µM	25,58801	4,647202	0,000022	0,999970
Mix **4a**/**4b** 2 µM	101,8447	6,602237	0,909063	0,725809
Mix **4a**/**4b** 5 µM	101,0198	3,198856	0,878477	0,778602
Mix **4a**/**4b** 10 µM	91,25331	4,478665	0,217340	0,997386

post hoc (Tukey)

	NST	ST	UA 50 µM	Mix **4a**/**4b** 2 µM	Mix **4a**/**4b** 5 µM
NST					
ST	0,000145				
UA 50 µM	0,001181	0,000145			
Mix **4a**/**4b** 2 µM	0,000145	0,999631	0,000145		
Mix **4a**/**4b** 5 µM	0,000145	0,999982	0,000145	0,999992	
Mix **4a**/**4b** 10 µM	0,000145	0,664977	0,000145	0,468793	0,557923

Table S8. Post hoc analysis of effects of UA and Mix **4a**/**4b** on Il-6 secretion in THP-1 derived macrophages 24 hours after stimulation. TNF-α secretion presented as a ratio of the experimental group to LPS stimulated cells. ST group. NST – non-stimulated control, ST – LPS stimulated control

scientific reports

Check for updates

OPEN

Author Correction: Conjugates of urolithin A with NSAIDs, their stability, cytotoxicity, and anti-inflammatory potential

Published online: 02 November 2022

Maciej Korczak, Piotr Roszkowski, Sebastian Granica & Jakub P. Piwowarski

Correction to: *Scientific Reports* https://doi.org/10.1038/s41598-022-15870-8, published online 08 July 2022

The original version of this Article contained an error in the grant number stated in the Acknowledgments section.

"Project financially supported by Polish National Science Centre research Grant Preludium Bis No. UMO-2019/35/B/NZ8/01388."

now reads:

"Project financially supported by Polish National Science Centre research Grant Preludium Bis No. UMO-2019/35/O/NZ7/00619."

The original Article has been corrected.

Publication No. 3

Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function

Maciej Korczak [a], Piotr Roszkowski [b], Weronika Skowrońska [c], Klaudia Małgorzata Żołdak [a], Dominik Popowski [a, d], Sebastian Granica [c], Jakub P Piwowarski [a]

[a] Microbiota Lab, Medical University of Warsaw, Warsaw, Poland.

[b] Faculty of Chemistry, University of Warsaw, Warsaw, Poland

[c] Department of Pharmaceutical Biology, Medical University of Warsaw, Warsaw, Poland

[d] Department of Food Safety and Chemical Analysis, Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology – State Research Institute, Warsaw, Poland

Biomedicine & Pharmacotherapy. 2023 Dec

169:115932

Biomedicine & Pharmacotherapy 169 (2023) 115932

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function

Maciej Korczak [a], Piotr Roszkowski [b], Weronika Skowrońska [c], Klaudia M. Żołdak [a], Dominik Popowski [a,d], Sebastian Granica [c], Jakub P. Piwowarski [a,*]

[a] Microbiota Lab, Medical University of Warsaw, Warsaw, Poland
[b] Faculty of Chemistry, University of Warsaw, Warsaw, Poland
[c] Department of Pharmaceutical Biology, Medical University of Warsaw, Warsaw, Poland
[d] Department of Food Safety and Chemical Analysis, Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology – State Research Institute, Warsaw, Poland

ARTICLE INFO

Keywords:
Urolithin A
NSAIDs
Glucuronidation
TEER
Tight junction proteins
Next-generation sequencing

ABSTRACT

Urolithin A (UA) is an ellagitannin-derived postbiotic metabolite which emerged as a promising health-boosting agent, promoting mitophagy, improving skeletal muscle function, and suppressing the inflammatory response. However, phase II intestinal metabolism severely limits its biopotency, leading to the formation of nonactive glucuronides. To address this constraint, a set of new UA derivatives (UADs), conjugated with nonsteroidal anti-inflammatory drugs (NSAIDs), was synthesized. The bioavailability and inhibitory activity of UADs against UA-glucuronidation were evaluated using differentiated Caco-2 cell monolayers. Parallelly, after the administration of tested substances, the transepithelial electrical resistance (TEER) of the cell monolayers was continuously monitored using the CellZscope device. Though investigated UADs did not penetrate Caco-2 monolayers, all of them significantly suppressed the glucuronidation rate of UA, while conjugates with diclofenac increased the concentration of free molecule on the basolateral side. Moreover, esters of UA with diclofenac (DicloUA) and aspirin (AspUA) positively influenced cell membrane integrity. Western blot analysis revealed that some UADs, including DicloUA, increased the expression of pore-sealing tight junction proteins and decreased the level of pore-forming claudin-2, which may contribute to their beneficial activity towards the barrier function. To provide comprehensive insight into the mechanism of action of DicloUA, Caco-2 cells were subjected to transcriptomic analysis. Next-generation sequencing (NGS) uncovered substantial changes in the expression of genes involved, for instance, in multivesicular body organization and zinc ion homeostasis. The results presented in this study offer new perspectives on the beneficial effects of modifying UA's structure on its intestinal metabolism and bioactivity in vitro.

1. Introduction

Natural products serve as a valuable source of lead molecules and new drugs, despite observed advances in pure medicinal chemistry and biologics [1]. Metabolites of natural origin are still an underexplored source of various pharmacophores; however, they often require chemical modifications, including reduction of size and complexity or removal of chiral centers to provide safe and cost-effective nature-derived drug candidates [2]. Structural derivatization appears to be an especially appropriate strategy for the modification of natural polyphenols, as it was with relatively simple acetylation of salicylic acid to diminish the occurrence of adverse effects [3]. Additionally, the conjugation of compounds of diverse pharmacological profiles may overcome the limitations of maternal molecules and improve their pharmacodynamics, pharmacokinetics, and safety features [4].

Urolithin A (UA) is the ellagitannin-derived postbiotic metabolite that gained attention due to its interesting biological activities, mainly related to silencing inflammation-associated processes and senescence

Abbreviations: NGS, Next-generation sequencing; NSAIDs, Nonsteroidal antiinflammatory drugs; PBS, phosphate-buffered saline; UA, Urolithin A; UADs, Urolithin A derivatives; UAGs, Urolithin A glucuronides; UASs, Urolithin A sulfates; TJP, Tight-junction protein; UGT, UDP Glucuronosyltransferases; TEER, Transepithelial electrical resistance.

* Corresponding author.
E-mail address: jakub.piwowarski@wum.edu.pl (J.P. Piwowarski).

https://doi.org/10.1016/j.biopha.2023.115932
Received 1 August 2023; Received in revised form 11 November 2023; Accepted 20 November 2023
Available online 24 November 2023

[5]. In contrast to ellagitannins, for urolithins in vitro and in vivo studies confirmed good absorption in the gastrointestinal tract [6]. UA activity in vitro and its impact on intracellular signal transduction pathways have been widely investigated. UA influences NF-κB and ERK 1/2 signallings, promotes autophagy and mitophagy [7–10]. The biological effects of UA were studied in the context of inflammatory diseases, cancer, muscle dysfunctions, and many other conditions [11]. Particularly the anti-inflammatory properties of UA were confirmed using several in vitro and in vivo models [5]. The positive impact of UA on age-related muscle function suggests its potential application in the treatment of muscle weakness and loss [10]. Furthermore, it was shown that after intraperitoneal administration, UA reduced the memory impairment in rat model of Alzheimer's disease or prevented blood-brain barrier disruption following traumatic brain injury in mice [12,13]. However, inconsistency between in vitro and in vivo studies cast doubt on the potential advantageous effects of orally administrated UA. Glucuronidation, as part of the phase II metabolism of xenobiotics, is a common pathway for deactivating pharmacophores to facilitate their elimination from the body [14]. It was demonstrated that after absorption, UA undergoes extensive phase II metabolism already in the gut wall, which severely limits the favorable impact of the maternal compound. Produced metabolites, mainly UA glucuronides (UAGs), are marked with significantly lower activity; for instance, in contrast to unconjugated form, glucuronides of UA did not inhibit the production of proinflammatory cytokines from stimulated macrophages, and additionally, UA-phase II metabolites posess significantly lesser antiproliferative activity [7,15]. Moreover, it was demonstrated that UA upregulates the expression of UDP glucuronosyltransferases (UGTs) in vitro, which can further decrease the bioavailability of the free form of UA [16].

Taking into account the potent inhibitory activity of nonsteroidal anti-inflammatory drugs (NSAIDs) against UGTs, a set of new UA derivatives (UADs) conjugated with NSAIDs has recently been synthesized to address the aforementioned limits resulting from the intestinal metabolism of UA [17–19]. In the conducted studies, preliminary stability assessments of newly synthesized compounds were also performed, demonstrating, among other findings, the highest durability of esters with mefenamic acid and diclofenac against acid hydrolysis. Similarly to the postulated approach of modifying xenobiotic metabolism to prevent drug resistance in the treatment of cancers, the strategy behind the synthesis of UADs was to obtain compounds that could heighten the bioaccessibility of the bioactive form of UA by limiting intracellular detoxification mechanisms [20].

Caco-2 cells, derived from human colorectal adenocarcinoma, are commonly used for in vitro evaluation of intestinal drug absorption and metabolism, and well-plate insert cultures of these cells are considered gold-standard for permeability assays [21]. After differentiation, Caco-2 cells form polarized monolayers and express phase I and II metabolic enzymes along with tight junction proteins (TJPs) [22–24]. Thus, Caco-2 cell monolayers not only act as a semi-permeable membrane but also simulate biotransformation occurring in vivo in the intestinal wall. Additionally, several compounds can modify Caco-2 barrier properties; therefore, the transepithelial electrical resistance (TEER) parameter is monitored to evaluate their effect on the integrity of the cell monolayer [25].

TJPs play a crucial role in maintaining the barrier function of intestinal epithelial cells, controlling the paracellular flux, and preventing the absorption of harmful substances. Among TJPs, certain families of proteins are listed, including ccluding, claudins, tricellulin, cingulin, zonula occludens (ZO), and junctional adhesion molecules (JAMs) [26]. While most TJPs are transmembrane barrier proteins required to seal the paracellular space, ZO and cingulin stabilize the plasma membrane and are involved in the interaction between peripheral membrane proteins and cytoskeletal proteins [27]. Despite being the first transmembrane TJP discovered, occludin's role in intestine function has not been fully clarified yet. Its overexpression resulted in increased TEER value; however, knockdown in Caco-2 cells did not affect cell monolayer permeability [28]. It is suggested that ccluding may not directly influence intestinal integrity but affects claudins' stability [29]. Claudins are classified into two subcategories: the "pore sealing" group (Claudin-1, −3, −4, −5, −7), enhancing the tightness of intercellular connections, and the "pore-forming" group (Claudin-2, −15), regulating small ion permeability and decreasing epithelial resistance [30]. Disruptions in claudins' expression and functioning have been linked with several conditions, including cancer, renal diseases, and even psychiatric disorders [31–33].

The presented study aimed to investigate the intestinal permeability of newly synthesized UADs and their impact on UA glucuronidation rate in vitro. Considering the positive effect of some UADs on the Caco-2 monolayer integrity, the expression of TJPs was additionally examined using Western blot technique. Finally, transcriptome analysis using next-generation sequencing (NGS) was performed to provide a comprehensive view on the influence of UA and selected UADs on intestinal epithelial cells.

2. Methods

2.1. Synthesis of UA and UADs

The chemical structures of synthesized compounds are represented in Fig. 1. UA was synthesized according to Bialonska et al. [34]. Firstly, UA 8-methyl ether was synthesized by heating resorcinol 2-bromo-5-methoxybenzoic acid and NaOH, followed by adding 5% $CuSO_4$. The dried intermediate product was then demethylated with $AlCl_3$ in chlorobenzene during heating under reflux. After completion of the demethylation process, confirmed with UHPLC-DAD-MS/MS analysis, the mixture was cooled on ice and extracted 3 times with diethyl ether. Finally, the organic solvent was removed on a vacuum rotary evaporator. A detailed description of UADs' synthesis and assessment of their purity and stability were previously reported by Korczak et al. [19]. Briefly, to obtain a mixture of UA monoesterified either with ibuprofen or acetylsalicylic acid, a solution of appropriate acyl chlorides in CH_2Cl_2 was added to the mixture of UA and triethylamine in dioxane and stirred for 2 h (for IbuUA) or 3 h (for AspUA) at 50 °C. Column chromatography on silica gel was used to isolate the final product. For the synthesis of MefUA and DicloUA DCC/DMAP were used as a coupling system. Suspension of UA, dioxane, CH_2Cl_2, appropriate NSAID, DCC, and DMAP were stirred for 24 h at room temperature. Like IbuUA and AspUA, the final products were purified using silica gel column chromatography. The schematic synthesis of UADs is presented in Fig. S1. The identity and purity of obtained compounds were confirmed using UHPLC-DAD-MS/MS and NMR methods.

2.2. Cell culture

Caco-2 cells (DSMZ, Braunschweig, Germany) were studied between 22 and 42 passages and cultured in 75 mL culture bottles with the seeding density of 40 000 cells/mL in the culture medium containing DMEM, 10% FBS, 100 IU/mL penicillin and 100 μg/mL streptomycin. All chemicals of the culture medium were purchased from Biowest (Nuaillé, France). Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. A medium change occurred at least 2 times per week. Cells were subcultured when the confluency reached 80–90%.

2.3. Caco-2 monolayer integrity assay

For permeability assays, Caco-2 cells were seeded on the porous, transparent inserts (0.4 μM pore diameter; ThinCert, Greiner Bio One, Kremsmünster, Austria) at 272 000 cells/mL density. Inserts were placed in the 6-well plates, and culture medium was added to the apical and basolateral sides. Cells were left for 21 days, with culture medium

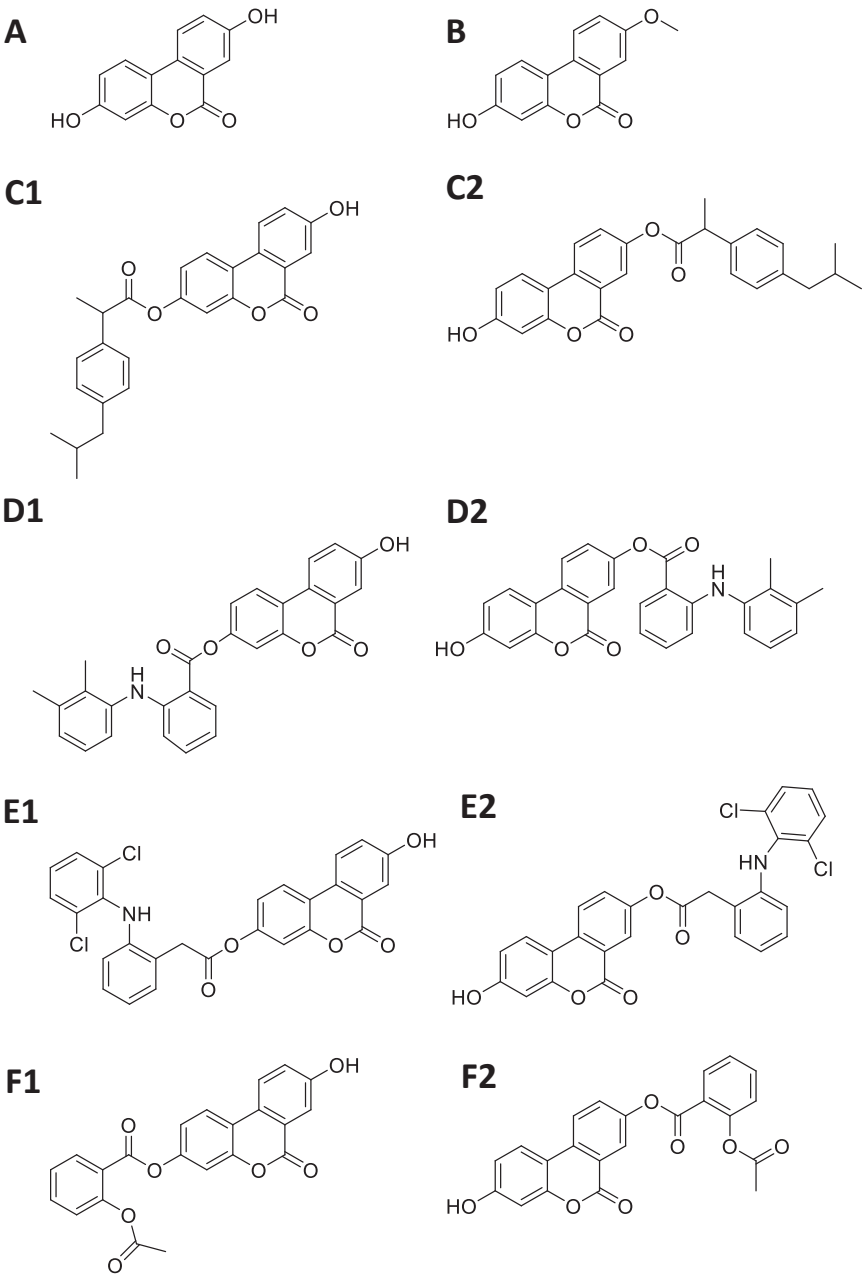


Fig. 1. Chemical structures of synthesized compounds. (A) UA, (B) UA 8-methyl ether, (C1 and C2) IbuUA, (D1 and D2) MefUA, (E1 and E2) DicloUA, (F1 and F2) AspUA.

change twice weekly, until the cell monolayer was formed and Caco-2 cells were fully differentiated. 14 days after seeding, inserts with Caco-2 cells were transferred into the cellZscope device (nanoAnalytics, Münster, Germany), the fresh culture medium was added to the apical and basolateral side, and the TEER value was automatically monitored hourly. The achievement of the TEER plateau value above 200 Ω/cm^2 indicated the completed monolayer formation and differentiation of Caco-2 cells. Before experiments, tested compounds were dissolved in DMSO (Avantor Performance Material, Gliwice, Poland). The final concentration of DMSO in culture medium did not exceed 0.5%. As a control condition, culture medium with 0.5% DMSO was used. 21 days after seeding, the culture medium was removed, fresh medium containing desired UA/UADs concentration was added to the apical side of the Caco-2 cell monolayer, while the basolateral side was filled with the fresh medium without tested compounds. Subsequently, 24-hour TEER value measurement in the cellZscope device was started. During the treatment, TEER level was monitored to observe potential cytotoxic activity, visualized by a sudden drop in electrical resistance of the cell monolayer. After that time, the measurement was stopped, and samples from the apical and basolateral sides were taken and subjected to the UHPLC-DAD-MS/MS analysis.

2.4. Cytotoxicity assay

Viability was determined by the colorimetric method by measuring mitochondrial dehydrogenase activity using the dye MTT (3-(4,5-dimethylthiazol-2-yl)− 2,5-diphenyltetrazolium bromide) (Merck, Darmstadt, Germany) according to the previously described protocol [35]. Briefly, cells were seeded in 24-well plates (1×10^5 cells per well) in a medium containing 20% FBS. After 24 h of incubation, the medium was changed to one containing 10% FBS, and the cells were incubated for a further 48 h. The cells were then washed with phosphate-buffered

saline (PBS), and the test compounds, negative (culture medium + 0.5% DMSO) and positive ((0.1% TritonX (Thermo Fisher Scientific, Waltham, MA, USA) in PBS)) controls, were applied. After 24 h of incubation, the cells were washed with PBS, and MTT solution in medium (0.5 mg/mL) was applied. After 1 h of incubation, the solution above the cells was aspirated, and the residue was dissolved in DMSO. All solutions were applied in a volume of 1 mL per well. Subsequently, 100 μL of the solution from each well was transferred to a 96-well plate, and the absorbance was measured at 560 nm (test) and 620 nm (reference). Four independent experiments were performed in triplicate. Cells in passages 33, 36, and 37 were used for the study.

2.5. Apoptosis assay

The effect of compounds on cell membrane integrity was determined using the BD PharmingenTM FITC Annexin V Apoptosis Detection Kit (BD, Franklin Lakes, NJ, USA) I according to the manufacturer's recommendations. Briefly, cells prepared as for the MTT viability assay were incubated with test compounds and appropriate controls for 24 h. The cells were washed with PBS (1 mL/well), and 300 μL TrypLe Express (Thermo Fisher Scientific) was added and incubated for 6 min to dissociate the cells from the surface. Subsequently, 1 mL of medium was added to each well, and the suspension was transferred to centrifuge tubes and centrifuged (125 rcf, 5 min, 37 °C). The cell pellet was suspended in 100 μL Annexin Binding Buffer, 5 μL FITC Annexin V, and Propidium Iodide Staining Solution (BD) were added. After 15 min of incubation at room temperature, 400 μL of Annexin Binding Buffer was added to the samples and assayed on a BD FACSCelestaTM flow cytometer using BD FACSDivaTM v.9.2 software. Durig each sample analysis, data from 10000 events were recorded. The assay was performed in three independent experiments with a total of seven replicates. Cells in passages 33, 36, and 37 were used for the study.

2.6. UHPLC-DAD-MS/MS analysis

Before UHPLC-DAD-MS/MS analyses, samples from permeability experiments were centrifuged for 15 min at a relative centrifugal force of 14.000 g. Obtained supernatants were collected and subjected to chromatographic determination. UHPLC-DAD-MS/MS analysis of samples was performed on UHPLC-3000 RS system (Dionex, Leipzig, Germany), connected to DAD detector and coupled with AmaZon SL ion trap mass spectrometer with an ESI interface (Bruker Daltonik GmbH, Bremen, Germany). 10 μL of samples were injected and separated on analytical column Kinetex XB-C_{18} (Phenomenex, Torrance, CA, 150 mm × 2.1 mm×1.7 μm) at a temperature 25 °C. For elution, mobile phase A containing H_2O/HCOOH (100:01, *v/v*), and mobile phase B containing acetonitrile/HCOOH (100:01, *v/v*) were used. Before in vitro experiments, synthesized UA and UADs were analyzed using gradient program 0–20 min 35–95% B, 20–25 min 95% B, and total flow 0.3 mL/min (Fig. S2). Following Caco-2 experiments, sufficient separation of sample components was achieved with a gradient program 0–60 min 0–95% mobile phase B and total flow 0.3 mL/min. The mass spectrometer parameters were: nitrogen gas temperature 300 °C, drying gas flow rate: 9 L/min, 40 psi, and capillary voltage: 4.5 kV. The mass spectra were registered by scanning from *m/z* 70–2200. The identity of compounds was confirmed by UV-Vis spectra, molecular mass, and fragmentation profile.

To determine the relative content of UA following apical administration of UA or UADs, a calibration curve of UA signal intensity was generated plots of synthesized UA's peak area vs amount injected to the HPLC column. For this analysis, accurately weighted UA was dissolved in 10% DMSO in PBS to obtain 50 μg/mL standard solution. The amount of UA injected to the column was controlled by modification of the injection volume (0.1–6 μL). Each injection volume was performed in triplicates. The quantified content of UA on the basolateral side of the Caco-2 cell monolayer after the administration of the tested substances was expressed as a percentage of the concentration observed after apical treatment with 100 μM UA.

2.7. Next-generation RNA sequencing

For transcriptome analysis, Caco-2 cells were cultured in a similar way as for the integrity assays. 24 h after incubation of differentiated Caco-2 monolayers with tested compounds, the culture medium was aspirated, and cells were washed with PBS. Afterward, PBS was removed, and the RNALater solution (Thermo Fisher Scientific) was added to the inserts. Cells were scratched using pipette tips, transferred to centrifuge tubes, and left at 4 °C overnight. In the next step, centrifuge tubes were placed in the freezer at − 20 °C until they were shipped for RNA isolation and Illumina paired-end (read length 2 ×150 bp) next-generation sequencing (NGS) performed by Eurofins Genomics (Ebersberg Germany). The Gene Ontology (GO) knowledgebase, UniProt database, andShinyGo 0.76.3 tools were used for bioinformatic analysis [36–39]. Genes with adjusted p-value ≤ 0.1 were selected as differentially expressed genes (DEGs) between groups and were used as a search input for enrichment analysis. GO Biological Processes with FDR cut-off ≤ 0.05 were considered as significantly changed.

2.8. Western blot

For Western blot analysis, Caco-2 cells were cultured and treated as described for the integrity assays. After 24-hour incubation of differentiated Caco-2 monolayers with tested compounds, cells were washed twice with cold PBS and lysed using cOmplete™ Lysis-M (Roche, Basel, Switzerland) containing proteases and phosphatases inhibitor cocktail (Roche). Lysates were centrifuged for 15 min at a relative centrifugal force of 14.000 g at 4 °C. Supernatants were collected and standardized on total protein concentration using a Quick Start™ Bradford Protein Assay kit Buffer (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instruction. Standardized samples were boiled with 4xLaemmli Sample Buffer (Bio-Rad) and kept at − 20 °C. Then, samples were subjected to SDS polyacrylamide gel electrophoresis (SDS-PAGE) using 12.5% polyacrylamide gel for ccluding (OCLN), claudin-1 (CLDN-1), claudin-2 (CLDN-2) and 8% polyacrylamide gel for zonula occludens-1 (ZO-1). For claudins separation, initially, 80 V was used. After 30 min, the voltage was increased to 100 V. OCLN was separated using 200 V during electrophoresis. For ZO-1, initially, 50 V was used until proteins entered the running gel. Then, electrophoresis was continued using 150 V. After separation, proteins were transferred to a nitrocellulose membrane (0.45 μm, Bio-Rad), which was afterwards blocked with Tris-Buffered saline (TBS; Promega, Madison, WI, USA) buffer containing 3% of skim milk (Merck). Electrotransfer conditions were as follows: 250 mA and 2 h for OCLN, 200 mA and 50 min for claudins and 250 mA for 3 h for ZO-1. In the next step, membranes were incubated either 2 h at room temperature (for ß-actin) or overnight at 4 °C with appropriate primary rabbit monoclonal antibodies: anti-ß-actin, anti-claudin-1, anti-claudin-2, anti-occludin, anti-ZO-1 obtained from Cell Signaling Technology (Danvers, MA, USA). Afterwards, membranes were washed 3 times with 3% skim milk TBS solution, followed by the addition of HRP-linked secondary antibody diluted 1:2000 (*v/v*) (Cell Signaling Technology, Danvers, MA, USA). Membranes were then incubated for 1 h at room temperature and washed 3 times with 3% skim milk TBS solution and 3 times with Tris-Buffered saline solution containing 0.1% Tween 20 detergent (Merck). For the protein bands detection, the SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Thermo Fisher Scientific) was used. Visualization of protein bands was performed on ChemiDoc MP imaging system (Bio-Rad). B-actin was selected as a loading control, and all proteins' concentrations were normalized for β-actin expression. The impact of tested compounds on protein levels was presented as a ratio of the protein band to its control band (derived from cells exposed to culture medium + 0.5% DMSO). A fold increase over the control band represented changes in protein levels.

2.9. Statistical analysis

If not otherwise stated, results from three independent in vitro experiments were demonstrated as mean values ± SD. Differences between groups were assessed by using one-way ANOVA followed by Dunnett's post hoc test. Differences were considered statistically significant at $p < 0.05$. Statistica 13 software was used to perform all statistical analyses. For Western blot analyses, pixel intensity was quantified by using ImageJ software. For semi-quantitative analysis of basolateral UA concentration, the statistical significance of the linear model regression equation at a 99% confidence level was assessed using the F-test. The computations were conducted utilizing the LINEST function in MS Excel.

3. Results

3.1. Intestinal absorption of UADs and influence on UA glucuronidation in vitro

The analysis of UADs' transport from apical to basolateral side and the inhibition of UA glucuronidation was conducted using non-monolayer-disrupting concentrations of tested compounds; hence 100 μM for UA, IbuUA, DicloUA, AspUA and 50 μM for MefUA. Details regarding the effects of UA/UADs on cell monolayer integrity are described in the next subsection. 24-hour incubation of Caco-2 cell monolayers with UA resulted in the presence of UA-glucuronides (UAGs) as the main metabolites and free UA on the basolateral side (Fig. 2). Regarding UAGs, the molecular ion [M-H]- *m/z* 403 was detected, which further gave *m/z* 227 peak on the MS^2 spectrum. Confirmation of the identity of UA was based on the presence of molecular ion $[M-H]^-$ at *m/z* 227 and the same retention time as a synthesized reference compound. The in-depth analysis of UHPLC-DAD-MS/MS data revealed the formation of a small amount of UA-sulfates (UASs) on the basolateral side of Caco-2 cell monolayers. The identity of UASs was confirmed by the presence of molecular ions [M-H]- at *m/z* 307 which gave *m/z* 227 peak on MS^2 spectrum (Figs. S3, S4).

None of the tested UADs penetrated through the Caco-2 monolayer, and only traces of maternal compounds were found in the medium from the apical side. Post-exposure to UADs, the same set of UA-derived metabolites on the basolateral side as for treatment with UA was observed; however, it should be noted that for 50 μM MefUA, considerably lesser amounts of metabolites were found. The low-intensity peak at around the 20th minute could be the result of an unknown metabolite (molecular ion [M+H]+ at *m/z* 139), which was not observed in the control conditions (culture medium + 0.5% DMSO) (Fig. S5). Vitaly, a substantial impact of UADs on UA's phase II metabolism was demonstrated. Following the application of UA, the ratio of the UA's peak area to its glucuronides on the basolateral side totaled approximately 0.3. Interestingly, treatment with all tested UADs significantly increased this proportion, with 100 μM DicloUA being the most potent inhibitor of glucuronidation, reaching nearly 0.8 UA/UAGs rate (Fig. 3 A).

During the semi-quantitative analysis of basolateral unconjugated UA content following apical administration of the tested substances, similar trends as observed with the degree of glucuronidation were noted. However, it is worth emphasizing that only 100 μM DicloUA significantly increased the relative bioavailability of the active form of UA compared to the basolateral concentration of free UA after administering its unmodified form to the apical side of the Caco-2 monolayers, while only traces of UA were observed following 50 μM MefUA treatment (Fig. 3B).

3.2. Impact of UADs on Caco-2 monolayer integrity

24 h of the incubation of differentiated Caco-2 cell monolayers with 100 μM UA resulted in a significantly increased TEER level (Fig. 4 A). Continuous changes in TEER value after apical administration of UA compared to the culture medium alone are presented in Fig. 4B. Interestingly, tested UADs exhibited diverse impacts on the Caco-2 monolayer. When a concentration of 100 μM was used, IbuUA showed a trend toward improved monolayer integrity; however, only DicloUA and AspUA significantly enhanced TEER value. Representative graphs with TEER value changes during treatment with IbuUA, DicloUA or AspUA are presented in Fig. 4 C. Contrary to the above-mentioned UADs, 100 μM MefUA negatively influenced Caco-2 cell monolayer integrity. After the initial increase, a sudden drop in TEER value occurred when 100 μM MefUA was tested, indicating impairment of cell monolayer integrity and cytotoxic activity during long-term incubation. This effect was not observed during treatment with 50 μM MefUA; hence, this concentration was selected for further research (Fig. 4D).

Observed changes in TEER values, especially the negative impact of 100 μM MefUA on the integrity of Caco-2 monolayers, correlated with

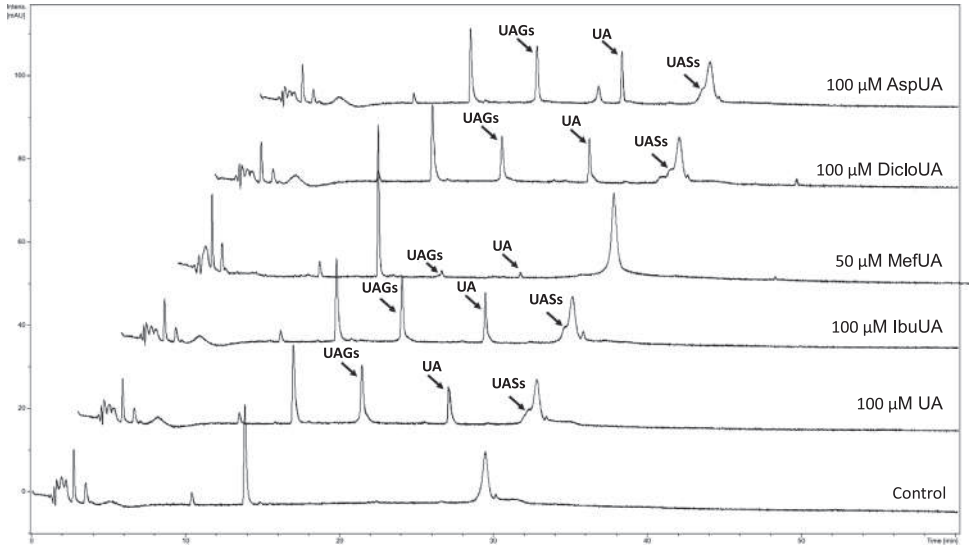


Fig. 2. Representative chromatograms of the basolateral side of Caco-2 cell monolayers 24 h after apical administration of UA or UADs. The chromatogram obtained after treatment with 50 μM MefUA was magnified to visualize faint UA and UAGs signals. Control - culture medium + 0.5% DMSO. Data acquired at λ = 305 nm. UA – urolithin A, UAGs – UA glucuronides, UASs – UA sulfates.

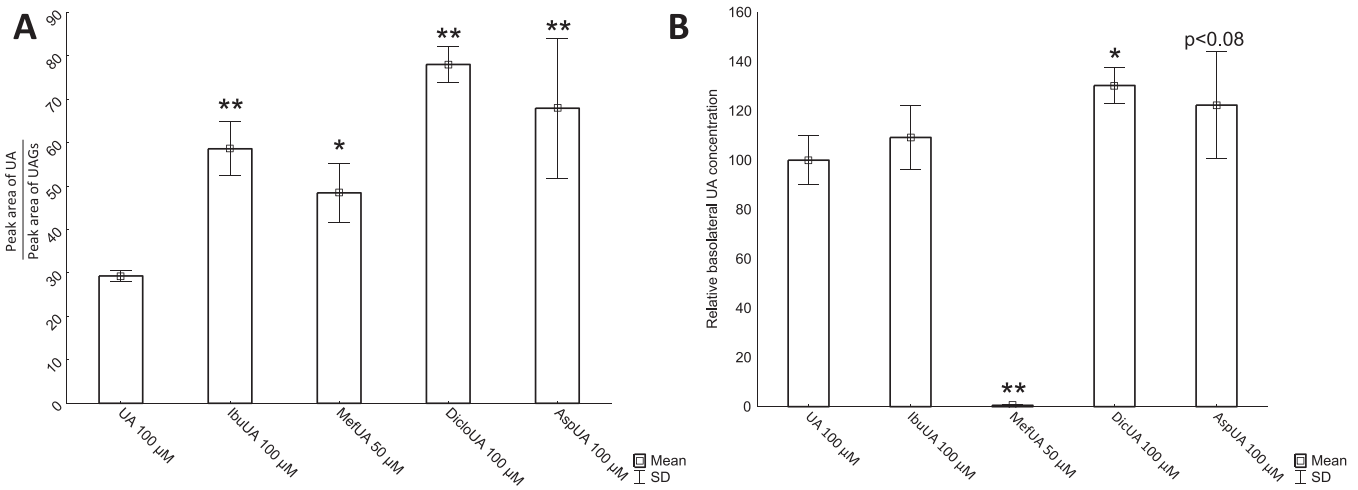


Fig. 3. (A) Inhibition of UA glucuronidation after UADs administration. Tested compounds were added to the apical side of Caco-2 cell monolayers, and after 24-hour incubation, the content of the basolateral side was analyzed using UHPLC-DAD-MS/MS. Values expressed as peak-area ratios of UA to UAGs acquired at $\lambda = 305$ nm. (B) The relative basolateral concentration of UA expressed as a percentage of basolateral content of unconjugated form after apical administration of 100 µM UA. * and * * represent significant differences compared to the administration of 100 µM UA ($p < 0.05$ and $p < 0.01$, respectively). UA – urolithin A, UAGs – UA glucuronides.

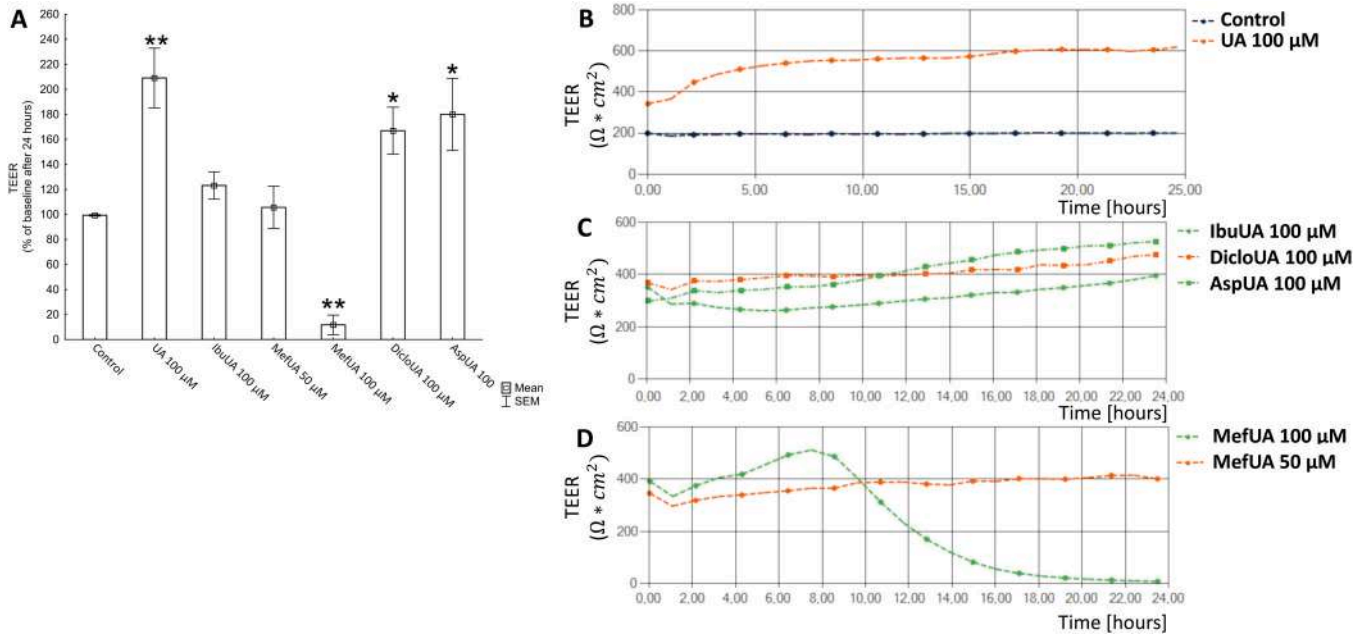


Fig. 4. (A) TEER values 24 h after apical administration of UA/UADs, compared to baseline and presented as percentages. Representative continuous TEER measurements during 24 incubation periods with (B) 100 µM UA and Control (culture medium + 0.5% DMSO), (C) 100 µM IbuUA, DicloUA or AspUA, (D) 50 and 100 µM MefUA. * and * * represent significant differences compared to TEER changes after 24 h of incubation in a culture medium alone ($p < 0.05$ and $p < 0.01$, respectively). Data expressed as a mean ± SEM.

results obtained during experiments evaluating the cytotoxic activity of tested compounds (MTT assay) and their pro-apoptotic properties measured using flow cytometry techniques (Fig. 5, S6). The observed decrease in the integrity of Caco-2 cell monolayers after the administration of 100 µM MefUA was reflected in both the cytotoxicity measurements and the pro-apoptotic effect on the cells. Other tested compounds did not exhibit similar detrimental effects on Caco-2 cells To exclude the potential influence of the tested compounds on the medium's pH and any resulting changes in TEER values, pH measurements were conducted using the FiveEasy Plus pH meter (Mettler-Toledo, Greifensee, Switzerland). It was observed that both the compounds and DMSO had similar effects on the pH values, with readings of 8.02, 7.97, 8.02, 8.02, 7.98, 7.87, and 7.9 for the control (culture medium + 0.5% DMSO), 100 µM UA, 100 µM IbuUA, 100 µM MefUA, 100 µM DicloUA, 100 µM AspUA, and 50 µM MefUA, respectively.

3.3. Impact of UADs on TJPs expression

Taking the positive influence of UA and its derivatives, especially AspUA and DicloUA, on Caco-2 monolayer integrity, the expression of TJPs after 24-hour treatment with UA or UADs was measured using the Western blot technique. Interestingly, despite possessing the same subunit of UA, UADs showed mixed effects on the regulation of TJPs expression. (Fig. 6 A, S7). Among tested compounds, MefUA, DicloUA, and AspUA significantly increased the ZO-1 protein level, with MefUA being the most potent compound in this aspect. Expression of ZO-1 after

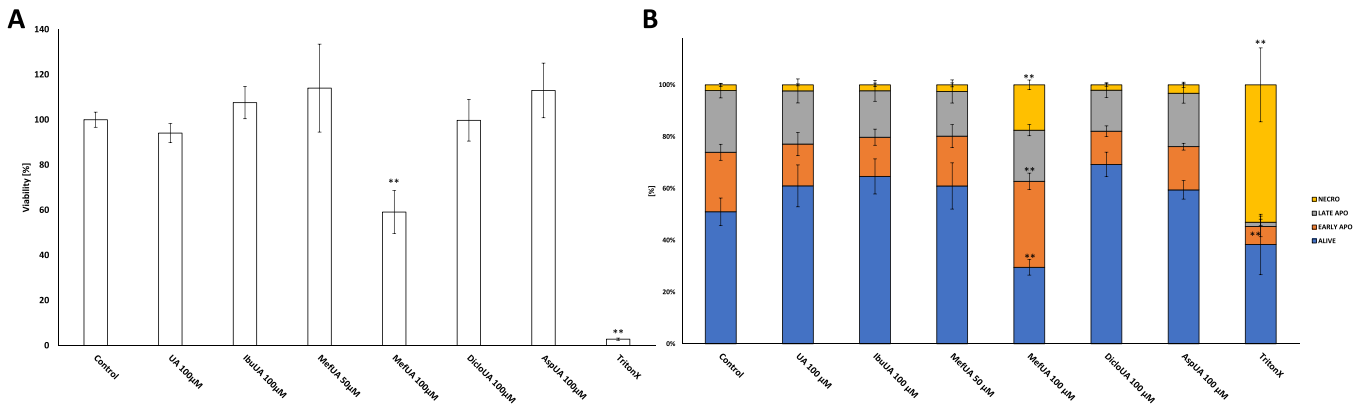


Fig. 5. (A) Viability of Caco-2 cells after 24 h of incubation with the tested compounds determined by the MTT assay. The negative control was culture medium + 0.5% DMSO (Control) and the positive control was 0.1% TritonX-100 (TritonX-100). Asterisks indicate a statistically significant reduction in cell viability compared to culture medium + (** $p < 0.01$). (B) The effect of the tested compounds on the integrity of the Caco-2 cell membrane determined by flow cytometry using FITC Annexin V and PI staining. The negative control was culture medium + 0.5% DMSO (Control) and the positive control was 0.1% TritonX-100 (TritonX). Asterisks indicate a statistically significant decrease in the number of live cells (ALIVE) and an increase in the number of necrotic cells (NECRO) and cells in late apoptosis (LATE APO) after 24 h of incubation compared to Medium (** $p < 0.01$). There are no significant differences in the number of cells in early apoptosis (EARLY APO) compared to Medium. Data expressed as a mean ± SD.

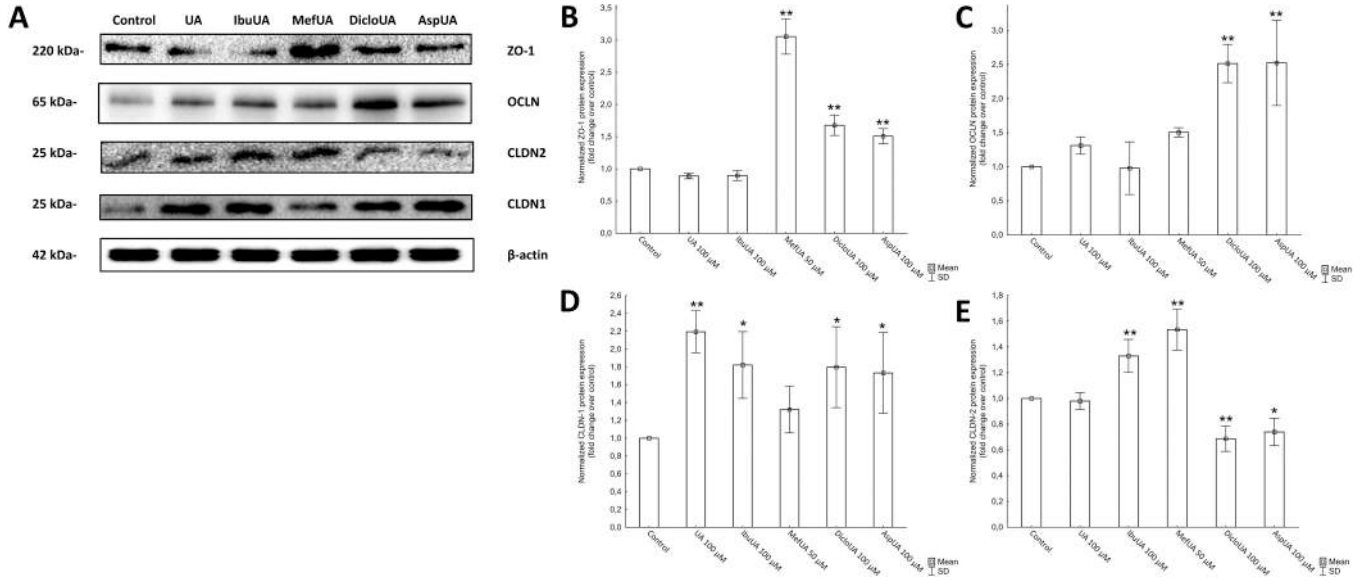


Fig. 6. (A) Effects of 100 µM UA, 100 µM IbuUA, 50 µM MefUA, 100 µM DicloUA, and 100 µM AspUA on TJPs expression in Caco-2 cell monolayers 24 h after apical administration of tested compounds. (B-E) Quantified changes in ZO-1, OCLN, CLDN-1, and CLDN-2 levels. * and * * represent significant differences compared to the culture medium alone ($p < 0.05$ and $p < 0.01$, respectively). All proteins' concentrations were normalized for β-actin expression.

exposure of Caco-2 cell monolayers to UA or IbuUA remained unaltered (Fig. 6B). Incubation with either 100 µM DicloUA or 100 µM AspUA led to up-regulation of OCLN, whereas UA and MefUA showed a positive trend towards increased expression, yet without statistically significant difference (Fig. 6 C). In monolayers exposed to UA, CLDN-1 protein level was increased by over 100% from the control (culture medium + 0.5% DMSO). The expression of CLDN-1 after apical administration of IbuUA, DicloUA, or AspUA was enhanced, however, to a lesser extent than observed after treatment with UA (Fig. 6D). Finally, the protein level of pore-forming CLDN-2 was evaluated. It was observed that UA did not influence the CLDN-2 expression, while the incubation with UADs presented opposing results. IbuUA and MefUA significantly upregulated CLDN-2. On the contrary, DicloUA and AspUA decreased CLDN-2 levels (Fig. 6E).

3.4. Transcriptomic changes after UADs administration

Subsequent investigation UADs' activity aimed to provide global insight into the UADs' mechanism of action and reveal whether modifications of the metabolic properties of Caco-2 cell monolayers were caused by the alternations in specific genes expression. In order to evaluate the influence of UA and UADs on transcriptomic changes in Caco-2 cells, next-generation RNA sequencing was performed. There were 107 genes differentially expressed between the UA and the control groups (culture medium + 0.5% DMSO), with 39 genes overexpressed in treated cells (Fig. 7A, Table S1). For NGS analysis of cells exposed to UADs, DicloUA was selected based on its potent inhibitory activity against UA glucuronidation, positive impact on Caco-2 monolayer integrity, and lowest degradation rate in the acidic environment, therefore potentially highest stability in gastric conditions and likelihood to influence intestinal epithelial cells after oral administration [19]. Compared to the cell monolayers incubated for 24 h in culture

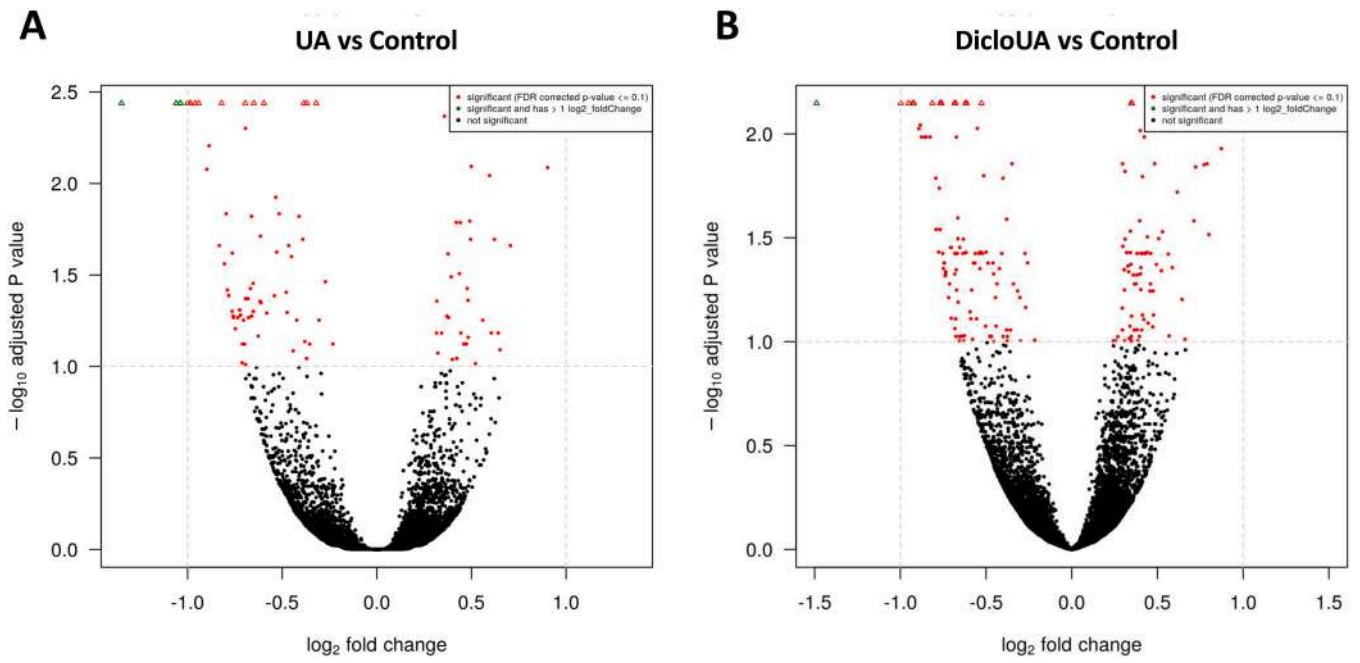


Fig. 7. Volcano plots representing gene distribution of genes in Caco-2 cell monolayers exposed either to (A) 100 μM UA or (B) 100 μM DicloUA versus culture medium with 0.5% DMSO (Control). Each dot in the diagram symbolizes one gene. Genes plotted on the graphs' right and left sides represent genes over- and underexpressed, respectively. Red dots represent significantly changed genes (adjusted p. value <0.1).

medium + 0.5% DMSO only, 184 genes were selected as DEGs, from which 81 were overexpressed in Caco-2 cells exposed to 100 μM DicloUA (Fig. 7B, Table S2). Curiously enough, the additional investigation revealed no DEGs between UA and DicloUA group, indicating that obtained differences in UA metabolism resulted rather from direct inhibition of glucuronidation than the influence on metabolic enzymes' expression. Interestingly, neither UA nor DicloUA influenced the expression of genes related to phase II metabolism.

Further studies of mRNA alternation caused by UA and DicloUA were performed using enrichment analysis utilizing GO database. Considering DEGs overexpressed in cells after 24 h of incubation with 100 μM UA, only one signaling pathway, glycoprotein metabolic process, was found to be significantly altered. (Fig. 8A). Treatment with DicloUA effected more extensive changes on the transcriptomic level than UA. 11 intracellular signaling pathways were significantly affected in total (Fig. 8B). Among these pathways, particularly genes involved in multivesicular body organization, regulation of exosomal secretion, and zinc ion homeostasis were influenced.

4. Discussion

Despite being an extremely promising health-promoting molecule, the medical use of UA is significantly narrowed by its glucuronidation. Consequently, herein proposed derivatization of UA structure aimed to bypass this limitation and contribute to higher bioavailability of bioactive form. The current study presents the first attempt to overcome the problem of UA phase II metabolism as well as intrinsically explores the influence of UA derivatives esterified with NSAIDs on Caco-2 monolayer integrity. The impact on TJPs and mRNA expression was measured to provide a profound understanding of their mechanism of action. Results obtained in this study show that synthesized UADs, similarly as UA, can potentially act in situ toward maintaining intestinal barrier integrity. Conducted experiments revealed that a portion of newly synthesized UADs reduced its degree of glucuronidation during in vitro intestinal metabolism, while DicloUA significantly increased the basolateral concentration of UA. The absence of changes at the mRNA level in the expression of genes encoding UGTs suggests a post-translational influence of UADs on the activity of these enzymes rather than alterations in glucuronidation levels due to changes in mRNA

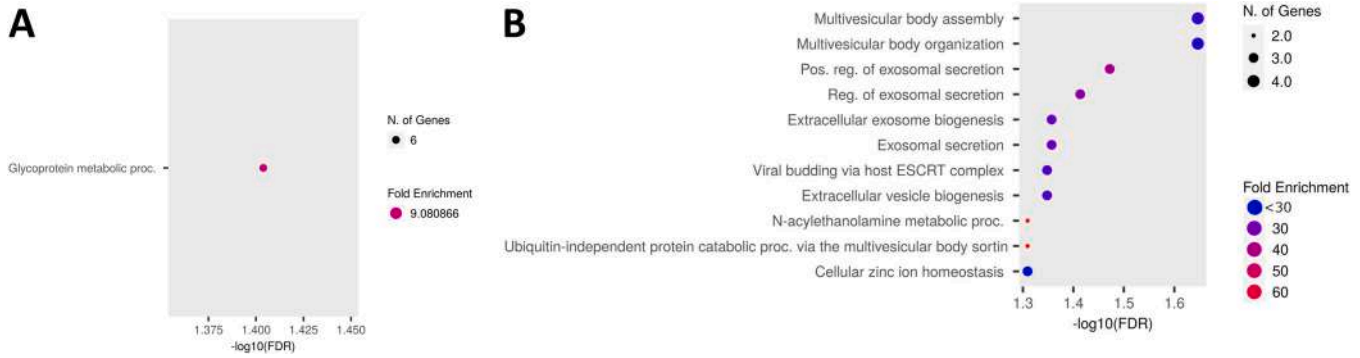


Fig. 8. Functional GO enrichment analysis of Caco-2 cell monolayers exposed for 24 h either to (A) 100 μM UA or (B) 100 μM DicloUA versus culture medium alone. Fold enrichment is defined as the percentage of DEGs involved in the biological process divided by the percentage of all genes involved in a certain action among genes identified in a model. FDR – False Discovery Rate.

expression. Taken together, presented results highlight UA conjugates with NSAIDs as potentially beneficial compounds, increasing the intestinal bioavailability of bioactive molecule in vitro.

One of the study's main results are the findings regarding the effect of UADs on UA glucuronidation. Phase II metabolism plays a crucial role in preserving the homeostasis of a healthy organism by generating metabolites that can be readily eliminated from the body. Ellagitannin-rich plants represent a widespread source of bioactive compounds; hence, their consumption without an efficient detoxification mechanism could lead to serious disruption in the inflammatory response [40]. In this regard, intestinal conjugation of UA with glucuronic acid offers a protective mechanism against immune system impairment and maintains the organism's readiness for exposure to harmful xenobiotics. It should be noted that the concentrations of UA used in the presented study are unattainable through the consumption of ellagitannin-rich plants, but rather may be more achievable following direct supplementation of UA/UADs. The oral administration of bioactive postbiotic metabolite is especially relevant for a part of the population (around 10% of society) belonging to metabotype 0, whose gut bacteria do not produce urolithin A after exposure to ellagitannins [41]. Performed experiments revealed that synthesized UADs, although they could not penetrate through Caco-2 cell monolayer, increased the ratio of UA to its glucuronides on the basolateral side. The relatively low concentration of UA and its metabolites on the basolateral siNde following administration of 50 μM MefUA could be potentially explained by the high accumulation of the compound in the intracellular compartments. Interestingly, previous studies evaluating the bioavailability in vitro of another mefenamic acid's derivative, ester-linked with guaiacol, revealed considerable accumulation within Caco-2 cells [42].

It is well-documented that some NSAIDs are strong inhibitors of UGT activity; hence the presence of these compounds, as free compounds or subunits of bigger molecules, may increase basolateral levels of the non-conjugated form of UA [17,18]. The lack of esterified forms of UADs and the presence of unconjugated UA and metabolites on the basolateral side after 24 h of incubation imply the hydrolysis of tested compounds. The synthesis of ester-containing prodrugs is a useful and common approach in active substance designing [43]. After absorption, endogenous, nonspecific esterases cleave ester bonds, releasing the active molecules. In the case of presented UADs, implementing the esterase-mediated strategy could ensure in situ interaction of NSAIDs (inhibition of UGT enzymes) and UA (active compound) in epithelial cells. Moreover, taking distinct molecular targets of UA and NSAIDs, administration of their conjugates could limit the required doses of the latter to achieve desirable anti-inflammatory activity and consequently reduce the prevalence of adverse drug reactions. However, more intrinsic studies including isobolographic analysis are required to evaluate UA's synergistic effects with NSAIDs fully.

One of the most frequently used methods of in vitro intestinal integrity measurement is monitoring TEER value after administration of tested compounds. In the presented study, TEER value was additionally used to evaluate the cytotoxic activity of tested compounds. The decrease in TEER value, similarly to MTT or NRU assays, reflects acute cytotoxicity of tested compounds on Caco-2 cell monolayers [44]. For substances negatively influencing cell membranes, rapid reduction of TEER value is a simple and more sensitive method than releasing lactate dehydrogenase to evaluate cytotoxic activity [45]. Moreover, the usage of Caco-2 cells seeded on porous inserts more accurately mimics the conditions occurring in vivo in the intestine. In the presented study, results obtained from continuous TEER measurements reflected the cytotoxic and pro-apoptotic properties of tested compounds measured using MTT assay and flow cytometric analysis of PE-Annexin V staining. All experiments evaluating the Caco-2 monolayer integrity in the presented study were run on the cellZscope device, enabling continuous TEER value measurement in real-time. It was particularly useful during the examination of 100 μM MefUA influence on Caco-2 monolayers when a sudden decrease after 8 h of surveys followed the initial gain of TEER value. The application of 24-hour continuous TEER measurements was used to exclude the cytotoxic concentration of tested compounds from further research.

The results presented here demonstrate for the first time the positive effect of UA on the barrier function of the fully differentiated Caco-2 monolayer. This outcome positively correlates with increased CLDN-1 expression following UA treatment. It was previously reported that the administration of UA prevented the negative impact of alternariol and deoxynivalenol on the integrity of Caco-2 monolayers after 24-hour incubation [46]. During experiments conducted in our study, a substantial increase in TEER value was observed not only for UA but also for its conjugates- DicloUA and AspUA. Positive trends in TEER changes after adding IbuUA were also observed, yet without statistically significant differences compared to baselines.

The beneficial activity of UA on intestinal 'TJPs' levels was previously established using various in vitro and in vivo models [47–50]. On the other hand, it was demonstrated that some NSAIDs significantly reduce the expression of these proteins and increase intestinal permeability [51–53]. Notably, the conjugation of NSAID molecules with urolithin A prevented the previously observed negative effects of these substances on the expression of TJPs [54]. Considering the preventive role of nitric oxide in protecting the intestinal barrier from NSAIDs-induced damage and UA's ability to enhance its production by epithelial cells, this mechanism may underlie counteracting the negative impact of NSAIDs on TJPs levels in the case of UADs [51,55]. Experiments conducted with newly synthesized UADs showed that, in most cases, tested compounds improved 'TJPs levels, while UA alone strongly increased CLDN-1 content. The inhibition of UA glucuronidation presented for all tested UADs may be the reason for their stronger activity towards the expression of certain TJP. Phase II metabolites of UA are characterized by limited bioactivity compared to the unconjugated form [7,56,57]. However, a detailed comparison between UA and its metabolites and UAGs' impact on 'TJPs' expression in differentiated Caco-2 cells has not been performed yet. TJPs are essential for maintaining intestinal integrity; hence, the upregulation of these proteins, except CLDN-2, may lead to TEER value elevation.

CLDN-2 is a pore-forming TJP, and its overexpression leads to increased junctional permeability [58]. It was found that starvation-induced autophagy stimulated the degradation of CLDN-2 and enhanced barrier function in Caco-2 model [59]. On the contrary, rapamycin, an autophagy activator, evoked disruption in TJPs, significantly decreasing certain TJPs' levels and increasing CLDN-2 expression [60]. The autophagic properties of UA were described and summarized previously; thus, the potential role of autophagy in the beneficial impact of this postbiotic metabolite on intestinal barrier integrity required additional research [61]. Overexpression of CLDN-2 after 24-hour treatment with IbuUA and MefUA may be partially responsible for their lower TEER value compared to UA, DicloUA, and AspUA.

In HT29 cells, UA's positive influence on OCLN and ZO-1 expression is mediated by activating the aryl hydrocarbon receptor-nuclear factor erythroid 2–related factor 2 pathway [50]. However, in the presented study, treating Caco-2 cell monolayers with UA significantly increased the level of only one TJP, CLDN-1. A similar, positive effect on CLDN-1 expression was detected for IbuUA, DicloUA, and AspUA. Of the note, depletion of CLDN-1 from Caco-2 cells was shown to result in the increased permeability of the monolayer and decreased TEER value, indicating its essential role for the integrity and barrier properties [62]. Another TJP, ZO-1, was found unaffected after administration of UA; however, conjugates of UA with mefenamic acid, diclofenac, and acetylsalicylic acid positively influenced the expression of ZO-1.

Similarly, except for DicloUA and AspUA, none of the tested compounds significantly increased OCLN levels. Analogously to CLDN-2, starvation-induced autophagy is associated with OCLN expression, yet in this case, autophagy induction reduced the degradation of OCLN in Caco-2 cells [63]. Moreover, researchers demonstrated that phosphorylation of ERK1/2 kinases is essential for the stability of OCLN and

enhancement of tight junction barrier. Independently, scientists presented the crucial role of activation of the ERK1/2 in barrier protection and protection against the loss of ZO-1 in in vitro model of colitis [64]. These findings might be particularly significant in the light of the potent activity of UA (but not UAGs) towards phosphorylation of ERK 1/2 in the THP-1 macrophages [7]. Both TJPs', OCLN and ZO-1, roles are not limited to the epithelial barrier function, and their involvement in epithelial proliferation and apoptosis should not be overlooked and may be the subject of further research focusing on UADs' role in cell cycle regulation [65].

The possibilities of high-throughput NGS provide detailed insight into cellular changes on the mRNA expression level. 100 μM DicloUA caused a significant upregulation of intracellular pathways involved in exosomal secretion. The impact of diclofenac on exosomal secretion was evaluated using the rat hepatocytes model [66]. The study revealed that diclofenac increased the number of secreted exosomes; therefore, enrichment of signaling pathways on the transcriptome level may result either from unconjugated diclofenac or diclofenac subunits in DicloUA. DicloUA noteworthy upregulated genes participating in zinc ions homeostasis compared to control (culture medium + 0.5% DMSO). It was previously reported that zinc ions play a vital role in maintaining Caco-2 monolayer integrity and can increase the expression of TJP [67,68]. Since DicloUA elevated the level of ZO-1, OCLN, and CLDN-1, the function of zinc ions in this phenomenon could be a subject of further investigation. Noteworthy, 24 h of treatment with UA did not induce considerable changes on the mRNA level, except upregulation of genes involved in the glycoprotein metabolic process. Hitherto conducted research used microarray analysis and RT-PCR to estimate changes in genes expression in Caco-2 cells exposed to UA; however, this study is the first to use NGS, which is a superior technique in terms of discovery power, to evaluate transcriptomic alternations after UA administration [69]. The relatively short incubation time with UA (24 vs 72 h in the previous study) potentially explains the limited outcomes of the transcriptome analysis and, finally, changes evoked by specific treatment may not be observable at the transcript level but result from post-translational modification or enhanced autophagy. Similarly, discrepancies in the observed changes in the expression of certain UGTs in Caco-2 cells after UA administration compared to the previously published work may stem from variations in the cultivation method of Caco-2 cells (monolayers vs plate-cultured cells), the employed techniques for detecting gene expression changes (NGS vs Microarray Analysis and RT-PCR), and primarily, the different incubation times (24 h vs 72 h) [16]. On the other hand, the 24-hour incubation period of Caco-2 cells with the tested compounds, relatively long for transcriptome-level investigations, may potentially lead to the oversight of certain alterations. Nevertheless, this timepoint is commonly employed for assessing changes associated with the barrier function of intestinal epithelial cells using RNA-seq; hence, it was selected for studying the impact of UADs on Caco-2 monolayers to enable potential cross-validation of results between different studies [70–72]. The sequencing data provided in the supplementary materials can serve as a guide for further studies focused on investigating the health-promoting properties of UA.

5. Conclusions

Results presented herein expose the profound impact of newly synthesized esters of NSAIDs and UA on Caco-2 monolayers. The inhibition of UA glucuronidation along with the enhancement of intestinal barrier integrity in vitro reveal novel treatment strategies incorporating the synthesis of bioactive postbiotic metabolites conjugated with inhibitors of their metabolism. The detailed investigation indicated that certain UADs positively influence the expression of genes involved in zinc ion homeostasis and regulate the expression of TJPs, which may contribute to improved TJPs' barrier function. Taken together, reported findings indicate that UA-NSAIDs conjugates emerge as promising therapeutic agents, increasing the bioavailability of the bioactive form of UA and lacking the harmful effects of NSAIDs on intestinal wall barrier proteins.

CRediT authorship contribution statement

Maciej Korczak: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Piotr Roszkowski**: Investigation. **Weronika Skowrońska**: Investigation. **Klaudia M. Żołdak**: Investigation. **Dominik Popowski**: Methodology. **Sebastian Granica**: Conceptualization, Methodology. **Jakub P. Piwowarski**: Conceptualization, Writing – original draft, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Project financially supported by Polish National Science Centre research grant Preludium Bis No. UMO-2019/35/O/NZ7/00619.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.115932.

References

[1] D.J. Newman, G.M. Cragg, Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019, J. Nat. Prod. 83 (2020) 770–803, https://doi.org/10.1021/acs.jnatprod.9b01285.
[2] Z. Guo, The modification of natural products for medical use, Acta Pharm. Sin. B 7 (2017) 119–136, https://doi.org/10.1016/j.apsb.2016.06.003.
[3] D. Lin, S. Jiang, A. Zhang, T. Wu, Y. Qian, Q. Shao, Structural derivatization strategies of natural phenols by semi-synthesis and total-synthesis, Nat. Prod. Bioprospect. 12 (2022), https://doi.org/10.1007/s13659-022-00331-6.
[4] M. Hasan, R.K. Leak, R.E. Stratford, D.P. Zlotos, P.A. Witt-Enderby, Drug conjugates-an emerging approach to treat breast cancer, Pharm. Res Perspect. 6 (2018), e00417, https://doi.org/10.1002/prp2.417.
[5] R. García-Villalba, J.A. Giménez-Bastida, A. Cortés-Martín, M.Á. Ávila-Gálvez, F. A. Tomás-Barberán, M.V. Selma, J.C. Espín, A. González-Sarrías, Urolithins: a comprehensive update on their metabolism, bioactivity, and associated gut microbiota, Mol. Nutr. Food Res (2022), 2101019, https://doi.org/10.1002/mnfr.202101019.
[6] M. Zhang, S. Cui, B. Mao, Q. Zhang, J. Zhao, H. Zhang, X. Tang, W. Chen, Ellagic acid and intestinal microflora metabolite urolithin A: a review on its sources, metabolic distribution, health benefits, and biotransformation, Crit. Rev. Food Sci. Nutr. 63 (2023) 6900–6922, https://doi.org/10.1080/10408398.2022.2036693.
[7] A. Bobowska, S. Granica, A. Filipek, M.F. Melzig, T. Moeslinger, J. Zentek, A. Kruk, J.P. Piwowarski, Comparative studies of urolithins and their phase II metabolites on macrophage and neutrophil functions, Eur. J. Nutr. 60 (2021) 1957–1972, https://doi.org/10.1007/s00394-020-02386-y.
[8] W. Komatsu, H. Kishi, K. Yagasaki, S. Ohhira, Urolithin A attenuates pro-inflammatory mediator production by suppressing PI3-K/Akt/NF-κB and JNK/AP-1 signaling pathways in lipopolysaccharide-stimulated RAW264 macrophages: Possible involvement of NADPH oxidase-derived reactive oxygen species, Eur. J. Pharm. 833 (2018) 411–424, https://doi.org/10.1016/j.ejphar.2018.06.023.
[9] H. Sahashi, A. Kato, M. Yoshida, K. Hayashi, I. Naitoh, Y. Hori, M. Natsume, N. Jinno, K. Kachi, G. Asano, T. Toyohara, Y. Kito, S. Ammanamanchi, H. Kataoka, Urolithin A targets the AKT/WNK1 axis to induce autophagy and exert anti-tumor effects in cholangiocarcinoma, Front Oncol. 12 (2022) 1–12, https://doi.org/10.3389/fonc.2022.963314.
[10] D. Ryu, L. Mouchiroud, P.A. Andreux, E. Katsyuba, N. Moullan, A.A. Nicolet-Dit-Félix, E.G. Williams, P. Jha, G. Lo Sasso, D. Huzard, P. Aebischer, C. Sandi, C. Rinsch, J. Auwerx, Urolithin A induces mitophagy and prolongs lifespan in C. elegans and increases muscle function in rodents, Nat. Med 22 (2016) 879–888, https://doi.org/10.1038/nm.4132.
[11] D. D'Amico, P.A. Andreux, P. Valdés, A. Singh, C. Rinsch, J. Auwerx, Impact of the natural compound urolithin A on health, disease, and aging, Trends Mol. Med 27 (2021) 687–699, https://doi.org/10.1016/j.molmed.2021.04.009.
[12] H.-J. Tu, C.-J. Su, C.-S. Peng, T.E. Lin, W.-C. Huang Fu, K.-C. Hsu, T.-L. Hwang, S.-L. Pan, Urolithin A exhibits a neuroprotective effect against Alzheimer's disease by inhibiting DYRK1A activity, J. Food Drug Anal. 31 (2023) 358–370, https://doi.org/10.38212/2224-6614.3462.

[13] Q.-Y. Gong, L. Cai, Y. Jing, W. Wang, D.-X. Yang, S.-W. Chen, H.-L. Tian, Urolithin A alleviates blood-brain barrier disruption and attenuates neuronal apoptosis following traumatic brain injury in mice, Neural Regen. Res 17 (2022) 2007, https://doi.org/10.4103/1673-5374.335163.
[14] Z. Mazerska, A. Mróz, M. Pawłowska, E. Augustin, The role of glucuronidation in drug resistance, Pharm. Ther. 159 (2016) 35–55, https://doi.org/10.1016/j.pharmthera.2016.01.009.
[15] M.Á. Ávila-Gálvez, J.C. Espín, A. González-Sarrías, Physiological relevance of the antiproliferative and estrogenic effects of dietary polyphenol aglycones versus their phase-II metabolites on breast cancer cells: a call of caution, J. Agric. Food Chem. 66 (2018) 8547–8555, https://doi.org/10.1021/acs.jafc.8b03100.
[16] A. González-Sarrías, M. Azorín-Ortuño, M.-J. Yáñez-Gascón, F.A. Tomás-Barberán, M.-T. García-Conesa, J.-C. Espín, Dissimilar in vitro and in vivo effects of ellagic acid and its microbiota-derived metabolites, urolithins, on the cytochrome P450 1A1, J. Agric. Food Chem. 57 (2009) 5623–5632, https://doi.org/10.1021/jf900725e.
[17] Y.B. Jarrar, D.H. Kim, S.-J. Lee, J.-G. Shin, Inhibition of 20-hydroxyeicosatetraenoic acid (20-HETE) glucuronidation by non-steroidal anti-inflammatory drugs in human liver microsomes and recombinant UDP-glucuronosyltransferase enzymes, Prostaglandins Leukot. Ess. Fat. Acids 153 (2020), 102055, https://doi.org/10.1016/j.plefa.2020.102055.
[18] J. Joo, Y.-W. Kim, Z. Wu, J.-H. Shin, B. Lee, J.C. Shon, E.Y. Lee, N.M. Phuc, K.-H. Liu, Screening of non-steroidal anti-inflammatory drugs for inhibitory effects on the activities of six UDP-glucuronosyltransferases (UGT1A1, 1A3, 1A4, 1A6, 1A9 and 2B7) using LC-MS/MS, Biopharm. Drug Dispos. 36 (2015) 258–264, https://doi.org/10.1002/bdd.1933.
[19] M. Korczak, P. Roszkowski, S. Granica, J.P. Piwowarski, Conjugates of urolithin A with NSAIDs, their stability, cytotoxicity, and anti - inflammatory potential, Sci. Rep. (2022) 1–15, https://doi.org/10.1038/s41598-022-15870-8.
[20] E.A. Zaal, C.R. Berkers, The influence of metabolism on drug response in cancer, Front Oncol. 8 (2018), https://doi.org/10.3389/fonc.2018.00500.
[21] S. Youhanna, V.M. Lauschke, The past, present and future of intestinal in vitro cell systems for drug absorption studies, J. Pharm. Sci. 110 (2021) 50–65, https://doi.org/10.1016/j.xphs.2020.07.001.
[22] X. Ding, X. Hu, Y. Chen, J. Xie, M. Ying, Y. Wang, Q. Yu, Differentiated Caco-2 cell models in food-intestine interaction study: Current applications and future trends, Trends Food Sci. Technol. 107 (2021) 455–465, https://doi.org/10.1016/j.tifs.2020.11.015.
[23] E.F.A. Brandon, T.M. Bosch, M.J. Deenen, R. Levink, E. van der Wal, J.B.M. van Meerveld, M. Bijl, J.H. Beijnen, J.H.M. Schellens, I. Meijerman, Validation of in vitro cell models used in drug metabolism and transport studies; genotyping of cytochrome P450, phase II enzymes and drug transporter polymorphisms in the human hepatoma (HepG2), ovarian carcinoma (IGROV-1) and colon carcinoma (CaCo-2, LS180) cell lines, Toxicol. Appl. Pharm. 211 (2006) 1–10, https://doi.org/10.1016/j.taap.2005.05.004.
[24] N. Li, P. Lewis, D. Samuelson, K. Liboni, J. Neu, Glutamine regulates Caco-2 cell tight junction proteins, Am. J. Physiol. -Gastrointest. Liver Physiol. 287 (2004) G726–G733, https://doi.org/10.1152/ajpgi.00012.2004.
[25] B. Srinivasan, A.R. Kolli, M.B. Esch, H.E. Abaci, M.L. Shuler, J.J. Hickman, TEER measurement techniques for in vitro barrier model systems, SLAS Technol. 20 (2015) 107–126, https://doi.org/10.1177/2211068214561025.
[26] C. Chelakkot, J. Ghim, S.H. Ryu, Mechanisms regulating intestinal barrier integrity and its pathological implications, Exp. Mol. Med 50 (2018), https://doi.org/10.1038/s12276-018-0126-x.
[27] C.M. Van Itallie, J.M. Anderson, Architecture of tight junctions and principles of molecular composition, Semin Cell Dev. Biol. 36 (2014) 157–165, https://doi.org/10.1016/j.semcdb.2014.08.011.
[28] S.M. Krug, J.D. Schulzke, M. Fromm, Tight junction, selective permeability, and related diseases, Semin Cell Dev. Biol. 36 (2014) 166–176, https://doi.org/10.1016/j.semcdb.2014.09.002.
[29] A. Monaco, B. Ovryn, J. Axis, K. Amsler, The epithelial cell leak pathway, Int J. Mol. Sci. 22 (2021), https://doi.org/10.3390/ijms22147677.
[30] M.J. Kozieł, M. Ziaja, A.W. Piastowska-Ciesielska, Intestinal barrier, claudins and mycotoxins, Toxins (Basel) 13 (2021) 758, https://doi.org/10.3390/toxins13110758.
[31] D.W. Wang, W.H. Zhang, G. Danil, K. Yang, J.K. Hu, The role and mechanism of claudins in cancer, Front Oncol. 12 (2022), https://doi.org/10.3389/fonc.2022.1051497.
[32] C. Prot-Bertoye, P. Houillier, Claudins in renal physiology and pathology, Genes (Basel) 11 (2020), https://doi.org/10.3390/genes11030290.
[33] C. Greene, N. Hanley, M. Campbell, Claudin-5: gatekeeper of neurological function, Fluids Barriers CNS 16 (2019), https://doi.org/10.1186/s12987-019-0123-z.
[34] D. Bialonska, S.G. Kasimsetty, S.I. Khan, F. Daneel, Urolithins, intestinal microbial metabolites of pomegranate ~ellagitannins, exhibit potent antioxidant activity in a cell-based assay, J. Agric. Food Chem. 57 (2009) 10181–10186, https://doi.org/10.1021/jf9025794.
[35] D. Popowski, K.A. Pawłowska, M. Deipenbrock, A. Hensel, A. Kruk, M.F. Melzig, J. P. Piwowarski, S. Granica, Antiadhesive activity of hydroethanolic extract from bean pods of Phaseolus vulgaris (common bean) against uropathogenic E. coli and permeability of its constituents through Caco-2 cells monolayer, J. Ethnopharmacol. 274 (2021), 114053, https://doi.org/10.1016/j.jep.2021.114053.
[36] S.X. Ge, D. Jung, R. Yao, ShinyGO: a graphical gene-set enrichment tool for animals and plants, Bioinformatics 36 (2020) 2628–2629, https://doi.org/10.1093/bioinformatics/btz931.
[37] M. Ashburner, C.A. Ball, J.A. Blake, D. Botstein, H. Butler, J.M. Cherry, A.P. Davis, K. Dolinski, S.S. Dwight, J.T. Eppig, M.A. Harris, D.P. Hill, L. Issel-Tarver, A. Kasarskis, S. Lewis, J.C. Matese, J.E. Richardson, M. Ringwald, G.M. Rubin, G. Sherlock, Gene ontology: tool for the unification of biology, Nat. Genet 25 (2000) 25–29, https://doi.org/10.1038/75556.
[38] S.A. Aleksander, J. Balhoff, S. Carbon, J.M. Cherry, H.J. Drabkin, D. Ebert, M. Feuermann, P. Gaudet, N.L. Harris, D.P. Hill, R. Lee, H. Mi, S. Moxon, C. J. Mungall, A. Muruganugan, T. Mushayahama, P.W. Sternberg, P.D. Thomas, K. Van Auken, J. Ramsey, D.A. Siegele, R.L. Chisholm, P. Fey, M.C. Aspromonte, M. V. Nugnes, F. Quaglia, S. Tosatto, M. Giglio, S. Nadendla, G. Antonazzo, H. Attrill, G. dos Santos, S. Marygold, V. Strelets, C.J. Tabone, J. Thurmond, P. Zhou, S. H. Ahmed, P. Asanitthong, D. Luna Buitrago, M.N. Erdol, M.C. Gage, M. Ali Kadhum, K.Y.C. Li, M. Long, A. Michalak, A. Pesala, A. Pritazahra, S.C. C. Saverimuttu, R. Su, K.E. Thurlow, R.C. Lovering, C. Logie, S. Oliferenko, J. Blake, K. Christie, L. Corbani, M.E. Dolan, H.J. Drabkin, D.P. Hill, L. Ni, D. Sitnikov, C. Smith, A. Cuzick, J. Seager, L. Cooper, J. Elser, P. Jaiswal, P. Gupta, P. Jaiswal, S. Naithani, M. Lera-Ramirez, K. Rutherford, V. Wood, J.L. De Pons, M. R. Dwinell, G.T. Hayman, M.L. Kaldunski, A.E. Kwitek, S.J.F. Laulederkind, M. A. Tutaj, M. Vedi, S.-J. Wang, P. D'Eustachio, L. Aimo, K. Axelsen, A. Bridge, N. Hyka-Nouspikel, A. Morgat, S.A. Aleksander, J.M. Cherry, S.R. Engel, K. Karra, S.R. Miyasato, R.S. Nash, M.S. Skrzypek, S. Weng, E.D. Wong, E. Bakker, T. Z. Berardini, L. Reiser, A. Auchincloss, K. Axelsen, G. Argoud-Puy, M.-C. Blatter, E. Boutet, L. Breuza, A. Bridge, C. Casals-Casas, E. Coudert, A. Estreicher, M. Livia Famiglietti, M. Feuermann, A. Gos, N. Gruaz-Gumowski, C. Hulo, N. Hyka-Nouspikel, F. Jungo, P. Le Mercier, D. Lieberherr, P. Masson, A. Morgat, I. Pedruzzi, L. Pourcel, S. Poux, C. Rivoire, S. Sundaram, A. Bateman, E. Bowler-Barnett, H. Bye-A-Jee, P. Denny, A. Ignatchenko, R. Ishtiaq, A. Lock, Y. Lussi, M. Magrane, M.J. Martin, S. Orchard, P. Raposo, E. Speretta, N. Tyagi, K. Warner, R. Zaru, A.D. Diehl, R. Lee, J. Chan, S. Diamantakis, D. Raciti, M. Zarowiecki, M. Fisher, C. James-Zorn, V. Ponferrada, A. Zorn, S. Ramachandran, L. Ruzicka, M. Westerfield, S.A. Aleksander, J. Balhoff, S. Carbon, J.M. Cherry, H.J. Drabkin, D. Ebert, M. Feuermann, P. Gaudet, N.L. Harris, D.P. Hill, R. Lee, H. Mi, S. Moxon, C.J. Mungall, A. Muruganugan, T. Mushayahama, P.W. Sternberg, P.D. Thomas, K. Van Auken, J. Ramsey, D.A. Siegele, R.L. Chisholm, P. Fey, M.C. Aspromonte, M. V. Nugnes, F. Quaglia, S. Tosatto, M. Giglio, S. Nadendla, G. Antonazzo, H. Attrill, G. dos Santos, S. Marygold, V. Strelets, C.J. Tabone, J. Thurmond, P. Zhou, S. H. Ahmed, P. Asanitthong, D. Luna Buitrago, M.N. Erdol, M.C. Gage, M. Ali Kadhum, K.Y.C. Li, M. Long, A. Michalak, A. Pesala, A. Pritazahra, S.C. C. Saverimuttu, R. Su, K.E. Thurlow, R.C. Lovering, C. Logie, S. Oliferenko, J. Blake, K. Christie, L. Corbani, M.E. Dolan, H.J. Drabkin, D.P. Hill, L. Ni, D. Sitnikov, C. Smith, A. Cuzick, J. Seager, L. Cooper, J. Elser, P. Jaiswal, P. Gupta, P. Jaiswal, S. Naithani, M. Lera-Ramirez, K. Rutherford, V. Wood, J.L. De Pons, M. R. Dwinell, G.T. Hayman, M.L. Kaldunski, A.E. Kwitek, S.J.F. Laulederkind, M. A. Tutaj, M. Vedi, S.-J. Wang, P. D'Eustachio, L. Aimo, K. Axelsen, A. Bridge, N. Hyka-Nouspikel, A. Morgat, S.A. Aleksander, J.M. Cherry, S.R. Engel, K. Karra, S.R. Miyasato, R.S. Nash, M.S. Skrzypek, S. Weng, E.D. Wong, E. Bakker, T. Z. Berardini, L. Reiser, A. Auchincloss, K. Axelsen, G. Argoud-Puy, M.-C. Blatter, E. Boutet, L. Breuza, A. Bridge, C. Casals-Casas, E. Coudert, A. Estreicher, M. Livia Famiglietti, M. Feuermann, A. Gos, N. Gruaz-Gumowski, C. Hulo, N. Hyka-Nouspikel, F. Jungo, P. Le Mercier, D. Lieberherr, P. Masson, A. Morgat, I. Pedruzzi, L. Pourcel, S. Poux, C. Rivoire, S. Sundaram, A. Bateman, E. Bowler-Barnett, H. Bye-A-Jee, P. Denny, A. Ignatchenko, R. Ishtiaq, A. Lock, Y. Lussi, M. Magrane, M.J. Martin, S. Orchard, P. Raposo, E. Speretta, N. Tyagi, K. Warner, R. Zaru, A.D. Diehl, R. Lee, J. Chan, S. Diamantakis, D. Raciti, M. Zarowiecki, M. Fisher, C. James-Zorn, V. Ponferrada, A. Zorn, S. Ramachandran, L. Ruzicka, M. Westerfield, The gene ontology knowledgebase in 2023, Genetics 224 (2023), https://doi.org/10.1093/genetics/iyad031.
[39] A. Bateman, M.-J. Martin, S. Orchard, M. Magrane, S. Ahmad, E. Alpi, E.H. Bowler-Barnett, R. Britto, H. Bye-A-Jee, A. Cukura, P. Denny, T. Dogan, T. Ebenezer, J. Fan, P. Garmiri, L.J. da Costa Gonzales, E. Hatton-Ellis, A. Hussein, A. Ignatchenko, G. Insana, R. Ishtiaq, V. Joshi, D. Jyothi, S. Kandasaamy, A. Lock, A. Luciani, M. Lugaric, J. Luo, Y. Lussi, A. MacDougall, F. Madeira, M. Mahmoudy, A. Mishra, K. Moulang, A. Nightingale, S. Pundir, G. Qi, S. Raj, P. Raposo, D.L. Rice, R. Saidi, R. Santos, E. Speretta, J. Stephenson, P. Totoo, E. Turner, N. Tyagi, P. Vasudev, K. Warner, X. Watkins, R. Zaru, H. Zellner, A.J. Bridge, L. Aimo, G. Argoud-Puy, A.H. Auchincloss, K.B. Axelsen, P. Bansal, D. Baratin, T.M. Batista Neto, M.-C. Blatter, J.T. Bolleman, E. Boutet, L. Breuza, B.C. Gil, C. Casals-Casas, K. C. Echioukh, E. Coudert, B. Cuche, E. de Castro, A. Estreicher, M.L. Famiglietti, M. Feuermann, E. Gasteiger, P. Gaudet, S. Gehant, V. Gerritsen, A. Gos, N. Gruaz, C. Hulo, N. Hyka-Nouspikel, F. Jungo, A. Kerhornou, P. Le Mercier, D. Lieberherr, P. Masson, A. Morgat, V. Muthukrishnan, S. Paesano, I. Pedruzzi, S. Pilbout, L. Pourcel, S. Poux, M. Pozzato, M. Pruess, N. Redaschi, C. Rivoire, C.J.A. Sigrist, K. Sonesson, S. Sundaram, C.H. Wu, C.N. Arighi, L. Arminski, C. Chen, Y. Chen, H. Huang, K. Laiho, P. McGarvey, D.A. Natale, K. Ross, C.R. Vinayaka, Q. Wang, Y. Wang, J. Zhang, UniProt: the universal protein knowledgebase in 2023, Nucleic Acids Res. 51 (2023) D523–D531, https://doi.org/10.1093/nar/gkac1052.
[40] J.P. Piwowarski, S. Granica, M. Zwierzyńska, J. Stefańska, P. Schopohl, M. F. Melzig, A.K. Kiss, Role of human gut microbiota metabolism in the anti-inflammatory effect of traditionally used ellagitannin-rich plant materials, J. Ethnopharmacol. 155 (2014) 801–809, https://doi.org/10.1016/j.jep.2014.06.032.
[41] A. Cortés-Martín, R. García-Villalba, A. González-Sarrías, M. Romo-Vaquero, V. Loria-Kohen, A. Ramírez-De-Molina, F.A. Tomás-Barberán, M.V. Selma, J. C. Espín, The gut microbiota urolithin metabotypes revisited: the human metabolism of ellagic acid is mainly determined by aging, Food Funct. 9 (2018) 4100–4106, https://doi.org/10.1039/c8fo00956b.

[42] V. Tantishaiyakul, K. Wiwattanawongsa, S. Pinsuwan, S. Kasiwong, N. Phadoongsombut, S. Kaewnopparat, N. Kaewnopparat, Y. Rojanasakul, Characterization of mefenamic acid-guaiacol ester: stability and transport across Caco-2 cell monolayers, Pharm. Res. 19 (2002) 1013–1018, https://doi.org/10.1023/A:1016470523923.
[43] L.D. Lavis, Ester bonds in prodrugs, ACS Chem. Biol. 3 (2008) 203–206, https://doi.org/10.1021/cb800065s.
[44] R. Konsoula, F.A. Barile, Correlation of in vitro cytotoxicity with paracellular permeability in Caco-2 cells, Toxicol. Vitr. 19 (2005) 675–684, https://doi.org/10.1016/j.tiv.2005.03.006.
[45] A. Narai, S. Arai, M. Shimizu, Rapid Decrease in Transepithelial Electrical Resistance of Human Intestinal Caco-2 Cell Monolayers by Cytotoxic Membrane Perturbents, 1997.
[46] J. Groestlinger, C. Seidl, E. Varga, G. Del Favero, D. Marko, Combinatory exposure to urolithin a, alternariol, and deoxynivalenol affects colon cancer metabolism and epithelial barrier Integrity in vitro, Front Nutr. 9 (2022), https://doi.org/10.3389/fnut.2022.882222.
[47] N.A. Hering, J. Luettig, B. Jebautzke, J.D. Schulzke, R. Rosenthal, The punicalagin metabolites ellagic acid and urolithin a exert different strengthening and anti-inflammatory effects on tight junction-mediated intestinal barrier function in vitro, Front Pharmacol. 12 (2021), https://doi.org/10.3389/fphar.2021.610164.
[48] S. Ghosh, B. Moorthy, B. Haribabu, Cytochrome P450 1A1 is essential for the microbial metabolite, Urolithin A-Mediat. Prot. Colitis (2022) 1–19, https://doi.org/10.3389/fimmu.2022.1004603.
[49] Y. Xiao, K. Li, J. Bian, H. Liu, X. Zhai, E. El-Omar, L. Han, L. Gong, M. Wang, Urolithin A attenuates diabetes-associated cognitive impairment by ameliorating intestinal barrier dysfunction via N-glycan biosynthesis pathway, Mol. Nutr. Food Res. (2022), 2100863, https://doi.org/10.1002/mnfr.202100863.
[50] R. Singh, S. Chandrashekharappa, S.R. Bodduluri, B.V. Baby, B. Hegde, N.G. Kotla, A.A. Hiwale, T. Saiyed, P. Patel, M. Vijay-Kumar, M.G.I. Langille, G.M. Douglas, X. Cheng, E.C. Rouchka, S.J. Waigel, G.W. Dryden, H. Alatassi, H.G. Zhang, B. Haribabu, P.K. Vemula, V.R. Jala, Enhancement of the gut barrier integrity by a microbial metabolite through the Nrf2 pathway, Nat. Commun. 10 (2019), https://doi.org/10.1038/s41467-018-07859-7.
[51] X. Wang, J. Shi, Z. Xu, D. Wang, Y. Song, G. Han, B. Wang, H. Cao, Y. Liu, J. Hou, Targeted delivery of nitric oxide triggered by α-glucosidase to ameliorate NSAIDs-induced enteropathy, Redox Biol. 59 (2023), https://doi.org/10.1016/j.redox.2022.102590.
[52] S. Chen, J. Jiang, G. Chao, X. Hong, H. Cao, S. Zhang, Pure total flavonoids from citrus protect against nonsteroidal anti-inflammatory drug-induced small intestine injury by promoting autophagy in vivo and in vitro, Front Pharmacol. 12 (2021), https://doi.org/10.3389/fphar.2021.622744.
[53] I. Bjarnason, K. Takeuchi, Intestinal permeability in the pathogenesis of NSAID-induced enteropathy, J. Gastroenterol. 44 (2009) 23–29, https://doi.org/10.1007/s00535-008-2266-6.
[54] G. Chao, X. Hong, S. Zhang, Effects of mast cells induced by NSAIDs impair intestinal epithelial barrier function in vivo and in vitro, Inflammation 44 (2021) 1396–1404, https://doi.org/10.1007/s10753-021-01424-z.
[55] Q. Han, C. Yan, L. Wang, G. Li, Y. Xu, X. Xia, Urolithin A attenuates ox-LDL-induced endothelial dysfunction partly by modulating microRNA-27 and ERK/PPAR-γ pathway, Mol. Nutr. Food Res. 60 (2016) 1933–1943, https://doi.org/10.1002/mnfr.201500827.
[56] A. González-Sarrías, J.A. Giménez-Bastida, M.Á. Núñez-Sánchez, M. Larrosa, M. T. García-Conesa, F.A. Tomás-Barberán, J.C. Espín, Phase-II metabolism limits the antiproliferative activity of urolithins in human colon cancer cells, Eur. J. Nutr. 53 (2014) 853–864, https://doi.org/10.1007/s00394-013-0589-4.
[57] J.A. Giménez-Bastida, M.Á. Ávila-Gálvez, J.C. Espín, A. González-Sarrías, The gut microbiota metabolite urolithin A, but not other relevant urolithins, induces p53-dependent cellular senescence in human colon cancer cells, Food Chem. Toxicol. 139 (2020), https://doi.org/10.1016/j.fct.2020.111260.
[58] S. Venugopal, S. Anwer, K. Szászi, Claudin-2: Roles beyond permeability functions, Int. J. Mol. Sci. 20 (2019), https://doi.org/10.3390/ijms20225655.
[59] P.K. Nighot, C.A.A. Hu, T.Y. Ma, Autophagy enhances intestinal epithelial tight junction barrier function by targeting claudin-2 protein degradation, J. Biol. Chem. 290 (2015) 7234–7246, https://doi.org/10.1074/jbc.M114.597492.
[60] Y. Feng, Y. Wang, P. Wang, Y. Huang, F. Wang, Short-chain fatty acids manifest stimulative and protective effects on intestinal barrier function through the inhibition of NLRP3 inflammasome and autophagy, Cell. Physiol. Biochem. 49 (2018) 190–205, https://doi.org/10.1159/000492853.
[61] A. Ahsan, M. Liu, Y. Zheng, W. Yan, L. Pan, Y. Li, S. Ma, X. Zhang, M. Cao, Z. Wu, W. Hu, Z. Chen, X. Zhang, Natural compounds modulate the autophagy with potential implication of stroke, Acta Pharm. Sin. B 11 (2021) 1708–1720, https://doi.org/10.1016/j.apsb.2020.10.018.
[62] I.M. Gohari, J. Li, M. Navarro, F. Uzal, B. McClane, Effects of claudin-1 on the action of Clostridium perfringens enterotoxin in caco-2 cells, Toxins (Basel) 11 (2019), https://doi.org/10.3390/toxins11100582.
[63] K. Saha, A.S. Ganapathy, A. Wang, N.M. Morris, E. Suchanec, W. Ding, G. Yochum, W. Koltun, M. Nighot, T. Ma, P. Nighot, Autophagy reduces the degradation and promotes membrane localization of occludin to enhance the intestinal epithelial tight junction barrier against paracellular macromolecule flux, J. Crohns Colitis (2022), https://doi.org/10.1093/ecco-jcc/jjac148.
[64] G.S. Seo, W.Y. Jiang, P.H. Park, D.H. Sohn, J.H. Cheon, S.H. Lee, Hirsutenone reduces deterioration of tight junction proteins through EGFR/Akt and ERK1/2 pathway both converging to HO-1 induction, Biochem. Pharmacol. 90 (2014) 115–125, https://doi.org/10.1016/j.bcp.2014.05.006.
[65] W.T. Kuo, M.A. Odenwald, J.R. Turner, L. Zuo, Tight junction proteins occludin and ZO-1 as regulators of epithelial proliferation and survival, Ann. N. Y. Acad. Sci. 1514 (2022) 21–33, https://doi.org/10.1111/nyas.14798.
[66] L. Palomo, J.E. Mleczko, M. Azkargorta, J. Conde-Vancells, E. González, F. Elortza, F. Royo, J.M. Falcon-Perez, Abundance of cytochromes in hepatic extracellular vesicles is altered by drugs related with drug-induced liver injury, Hepatol. Commun. 2 (2018), https://doi.org/10.1002/hep4.1210/full.
[67] Y. Shao, P.G. Wolf, S. Guo, Y. Guo, H.R. Gaskins, B. Zhang, Zinc enhances intestinal epithelial barrier function through the PI3K/AKT/mTOR signaling pathway in Caco-2 cells, J. Nutr. Biochem. 43 (2017) 18–26, https://doi.org/10.1016/j.jnutbio.2017.01.013.
[68] Y.X. Shao, Z. Lei, P.G. Wolf, Y. Gao, Y.M. Guo, B.K. Zhang, Zinc supplementation, via GPR39, upregulates PKCζ to protect intestinal barrier integrity in Caco-2 cells challenged by Salmonella enterica serovar typhimurium, J. Nutr. 147 (2017) 1282–1289, https://doi.org/10.3945/jn.116.243238.
[69] A. González-Sarrías, J.C. Espín, F.A. Tomás-Barberán, M.T. García-Conesa, Gene expression, cell cycle arrest and MAPK signalling regulation in Caco-2 cells exposed to ellagic acid and its metabolites, urolithins, Mol. Nutr. Food Res. 53 (2009) 686–698, https://doi.org/10.1002/mnfr.200800150.
[70] Y. He, X. Yin, J. Dong, Q. Yang, Y. Wu, Z. Gong, Transcriptome analysis of Caco-2 cells upon the exposure of mycotoxin deoxynivalenol and its acetylated derivatives, Toxins (Basel) 13 (2021), https://doi.org/10.3390/toxins13020167.
[71] J. Huo, W. Pei, G. Liu, W. Sun, J. Wu, M. Huang, W. Lu, J. Sun, B. Sun, Huangshui polysaccharide exerts intestinal barrier protective effects through the TLR4/MyD88/NF-κB and MAPK signaling pathways in Caco-2 cells, Foods 12 (2023) 450, https://doi.org/10.3390/foods12030450.
[72] J. Huo, M. Li, J. Wei, Y. Wang, W. Hao, W. Sun, J. Wu, M. Huang, RNA-seq based elucidation of mechanism underlying the protective effect of Huangshui polysaccharide on intestinal barrier injury in Caco-2 cells, Food Res. Int. 162 (2022), https://doi.org/10.1016/j.foodres.2022.112175.

Supplementary materials to:

Publication No. 3

Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function

Maciej Korczak [a], Piotr Roszkowski [b], Weronika Skowrońska [c], Klaudia Małgorzata Żołdak [a], Dominik Popowski [a, d], Sebastian Granica [c], Jakub P Piwowarski [a]

[a] Microbiota Lab, Medical University of Warsaw, Warsaw, Poland.

[b] Faculty of Chemistry, University of Warsaw, Warsaw, Poland

[c] Department of Pharmaceutical Biology, Medical University of Warsaw, Warsaw, Poland

[d] Department of Food Safety and Chemical Analysis, Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology – State Research Institute, Warsaw, Poland

Biomedicine & Pharmacotherapy. 2023 Dec

169:115932

Urolithin A conjugation with NSAIDs inhibits its glucuronidation and maintains improvement of Caco-2 monolayers' barrier function.

Maciej Korczak[a], Piotr Roszkowski[b], Weronika Skowrońska[c], Klaudia M. Żołdak[a], Dominik Popowski[a,d], Sebastian Granica[c], Jakub P. Piwowarski[a]

[a] Microbiota Lab, Medical University of Warsaw, Warsaw, Poland

[b] Faculty of Chemistry, University of Warsaw, Warsaw, Poland

[c] Department of Pharmaceutical Biology, Medical University of Warsaw, Warsaw, Poland

[d] Department of Food Safety and Chemical Analysis, Prof. Wacław Dąbrowski Institute of Agricultural and Food Biotechnology – State Research Institute, Warsaw, Poland

gene_id	gene_name	logfoldChange	adj_p_value	GO biological process
ENSG00000248592	TMEM110-MUSTN1	0,902380058	0,008185138	chondrocyte differentiation
				chondrocyte proliferation
				tissue regeneration
ENSG00000102312	PORCN	0,869234746	0,004737241	glycoprotein metabolic process
				lipid modification
				protein lipidation
				protein palmitoleylation
				protein palmitoylation
				regulation of postsynaptic membrane neurotransmitter receptor levels
				Wnt protein secretion
				Wnt signaling pathway
ENSG00000153982	GDPD1	0,863276543	0,002731031	glycerophospholipid catabolic process
				N-acylethanolamine metabolic process
ENSG00000196776	CD47	0,705966213	0,021853755	angiogenesis
				apoptotic process
				ATP export
				cell migration
				cellular response to interleukin-1
				cellular response to interleukin-12
				cellular response to type II interferon
				inflammatory response
				integrin-mediated signaling pathway
				negative regulation of Fc-gamma receptor signaling pathway involved in phagocytosis
				positive regulation of cell population proliferation
				positive regulation of cell-cell adhesion
				positive regulation of inflammatory response
				positive regulation of monocyte extravasation
				positive regulation of phagocytosis
				positive regulation of stress fiber assembly
				positive regulation of T cell activation
				regulation of interleukin-10 production

				regulation of interleukin-12 production
				regulation of interleukin-6 production
				regulation of nitric oxide biosynthetic process
				regulation of tumor necrosis factor production
				regulation of type II interferon production
ENSG00000179532	DNHD1	0,651283372	0,081089335	cilium movement
				flagellated sperm motility
				sperm flagellum assembly
ENSG00000127415	IDUA	0,641806119	0,065639753	dermatan sulfate catabolic process
				disaccharide metabolic process
				glycosaminoglycan catabolic process
				heparan sulfate proteoglycan catabolic process
				heparin catabolic process
ENSG00000267645	POLR2J2	0,621699121	0,020191511	DNA-templated transcription
				transcription by RNA polymerase II
ENSG00000196456	ZNF775	0,605153594	0,065639753	regulation of DNA-templated transcription
ENSG00000171798	KNDC1	0,596117641	0,009048719	cerebellar granule cell differentiation
				positive regulation of protein phosphorylation
				regulation of dendrite development
				regulation of dendrite morphogenesis
				small GTPase mediated signal transduction
ENSG00000270647	TAF15	0,572408366	0,000217607	mRNA stabilization
				positive regulation of DNA-templated transcription
				RNA splicing
ENSG00000143224	PPOX	0,560118569	0,055855647	heme biosynthetic process
				porphyrin-containing compound biosynthetic process
				protoporphyrinogen IX biosynthetic process
ENSG00000111752	PHC1	0,535862097	0,003643518	cellular response to leukemia inhibitory factor
				cellular response to retinoic acid
				chromatin remodeling

				negative regulation of DNA-templated transcription
ENSG00000183458	AC138932,1	0,521051943	0,096539125	mRNA transport
				protein transport
ENSG00000254681	PKD1P5	0,499284386	0,008064722	N/A
ENSG00000178467	P4HTM	0,495622737	0,020191511	peptidyl-proline hydroxylation to 4-hydroxy-L-proline
				regulation of erythrocyte differentiation
ENSG00000149541	B3GAT3	0,490875023	0,01604772	carbohydrate metabolic process
				chondroitin sulfate proteoglycan biosynthetic process
				dermatan sulfate proteoglycan biosynthetic process
				glycosaminoglycan biosynthetic process
				heparan sulfate proteoglycan biosynthetic process
				positive regulation of catalytic activity
				positive regulation of intracellular protein transport
				protein glycosylation
ENSG00000159363	ATP13A2	0,482339157	0,069287819	autophagosome organization
				autophagosome-lysosome fusion
				autophagy
				cellular response to manganese ion
				cellular response to oxidative stress
				cellular response to zinc ion
				extracellular exosome biogenesis
				intracellular calcium ion homeostasis
				intracellular iron ion homeostasis
				intracellular monoatomic cation homeostasis
				intracellular zinc ion homeostasis
				lipid homeostasis
				lysosomal transport
				monoatomic ion transmembrane transport
				negative regulation of lysosomal protein catabolic process
				negative regulation of neuron death
				peptidyl-aspartic acid autophosphorylation

				polyamine transmembrane transport
				positive regulation of exosomal secretion
				positive regulation of gene expression
				positive regulation of protein secretion
				protein autophosphorylation
				protein localization to lysosome
				regulation of autophagosome size
				regulation of autophagy of mitochondrion
				regulation of chaperone-mediated autophagy
				regulation of endopeptidase activity
				regulation of glucosylceramidase activity
				regulation of intracellular protein transport
				regulation of lysosomal protein catabolic process
				regulation of macroautophagy
				regulation of mitochondrion organization
				regulation of neuron apoptotic process
				regulation of protein localization to nucleus
				regulation of ubiquitin-specific protease activity
				spermine transmembrane transport
				transmembrane transport
ENSG00000164574	GALNT10	0,481511769	0,043479723	O-glycan processing
				protein O-linked glycosylation
ENSG00000100003	SEC14L2	0,477864017	0,037501597	positive regulation of DNA-templated transcription
				regulation of cholesterol biosynthetic process
ENSG00000126821	SGPP1	0,473109766	0,075391626	ER to Golgi ceramide transport
				extrinsic apoptotic signaling pathway
				intrinsic apoptotic signaling pathway
				phospholipid dephosphorylation
				regulation of epidermis development

				regulation of keratinocyte differentiation
				sphinganine-1-phosphate metabolic process
				sphingolipid biosynthetic process
				sphingosine metabolic process
ENSG00000172613	RAD9A	0,459890053	0,075363644	cellular response to ionizing radiation
				DNA damage checkpoint signaling
				DNA damage response
				DNA repair
				DNA replication checkpoint signaling
				intrinsic apoptotic signaling pathway in response to DNA damage
				mitotic intra-S DNA damage checkpoint signaling
				positive regulation of intrinsic apoptotic signaling pathway in response to DNA damage
ENSG00000081320	STK17B	0,457445206	0,000658467	apoptotic process
				intracellular signal transduction
				positive regulation of apoptotic process
				positive regulation of fibroblast apoptotic process
				protein autophosphorylation
				protein phosphorylation
ENSG00000244462	RBM12	0,451354533	0,004939868	regulation of RNA splicing
ENSG00000213780	GTF2H4	0,444005984	0,065663521	DNA repair
				nucleotide-excision repair
				transcription by RNA polymerase II
ENSG00000165915	SLC39A13	0,441498123	0,016338861	brown fat cell differentiation
				connective tissue development
				intracellular zinc ion homeostasis
				zinc ion transmembrane transport
				zinc ion transport
ENSG00000124243	BCAS4	0,43618839	0,031106949	N/A
ENSG00000179523	EIF3J-AS1	0,422708723	0,090429369	N/A
ENSG00000266173	STRADA	0,420038277	0,016338861	activation of protein kinase activity
				G1 to G0 transition
				protein export from nucleus
ENSG00000183426	NPIPA1	0,406272631	0,002731031	mRNA transport
				protein transport
ENSG00000197818	SLC9A8	0,399267814	0,091639527	acrosome assembly

				monoatomic ion transport
				potassium ion transmembrane transport
				proton transmembrane transport
				regulation of Golgi lumen acidification
				regulation of intracellular pH
				sodium ion transmembrane transport
				cellular response to interferon-beta
				negative regulation of retrograde protein transport, ER to cytosol
				protein K48-linked ubiquitination
				ubiquitin-dependent ERAD pathway
ENSG00000184787	UBE2G2	0,393471754	0,032358529	ubiquitin-dependent protein catabolic process
				bicarbonate transport
ENSG00000159348	CYB5R1	0,378897251	0,0540491	sterol biosynthetic process
				dolichol metabolic process
				GPI anchor biosynthetic process
				protein O-linked mannosylation
ENSG00000136908	DPM2	0,376726189	0,024248385	regulation of protein stability
ENSG00000163444	TMEM183A	0,369984972	0,053059612	regulation of protein stability
				DNA damage response
				DNA replication
				embryonic organ development
				in utero embryonic development
				mRNA processing
				multicellular organism growth
				protein ubiquitination
				regulation of DNA replication
				somite development
ENSG00000122257	RBBP6	0,357583084	0,00429274	ubiquitin-dependent protein catabolic process
				actin filament organization
				cytoplasmic sequestering of NF-kappaB
				negative regulation of cell cycle G1/S phase transition
				negative regulation of hematopoietic stem cell differentiation
				negative regulation of interleukin-8 production
ENSG00000205542	TMSB4X	0,342396249	0,065663521	negative regulation of NF-kappaB transcription factor activity

				negative regulation of NIK/NF-kappaB signaling
				negative regulation of RNA polymerase II regulatory region sequence-specific DNA binding
				positive regulation of ATP biosynthetic process
				positive regulation of blood vessel endothelial cell migration
				positive regulation of endothelial cell chemotaxis
				positive regulation of proton-transporting ATP synthase activity, rotational mechanism
				regulation of cell migration
				regulation of inflammatory response
				regulation of NIK/NF-kappaB signaling
				sequestering of actin monomers
				tumor necrosis factor-mediated signaling pathway
ENSG00000152133	GPATCH11	0,322787941	0,084501459	N/A
				cell cycle
				chromatin organization
				negative regulation of protein sumoylation
				positive regulation of neuron differentiation
				protein sumoylation
				regulation of gene expression
ENSG00000064961	HMG20B	0,317111072	0,043965503	skeletal muscle cell differentiation
				glycoprotein catabolic process
				glycoprotein metabolic process
				N-acetylglucosamine metabolic process
				protein deglycosylation
ENSG00000198408	MGEA5	0,315391609	0,065639753	protein O-linked glycosylation

Table S1. DEGs overexpressed in Caco-2 cells exposed to 24-hour incubation with 100 µM UA administered apically versus culture medium alone (Control)

gene_id	gene_name	log2_foldChange	adj_p_value	Biological Function
ENSG00000153982	GDPD1	0,89133896	0,001945932	glycerophospholipid catabolic process
				N-acylethanolamine metabolic process
ENSG00000125148	MT2A	0,872713005	0,011779256	cellular response to cadmium ion

				cellular response to copper ion
				cellular response to erythropoietin
				cellular response to interleukin-3
				cellular response to zinc ion
				detoxification of copper ion
				intracellular copper ion homeostasis
				intracellular zinc ion homeostasis
				negative regulation of growth
ENSG00000248592	TMEM110-MUSTN1	0,800498609	0,030559458	chondrocyte differentiation
				chondrocyte proliferation
				tissue regeneration
ENSG00000198208	RPS6KL1	0,788728026	0,01392577	protein phosphorylation
ENSG00000179532	DNHD1	0,771457714	0,014093271	cilium movement
				flagellated sperm motility
				sperm flagellum assembly
ENSG00000196776	CD47	0,723031499	0,014439676	angiogenesis
				apoptotic process
				ATP export
				cell migration
				cellular response to interleukin-1
				cellular response to interleukin-12
				cellular response to type II interferon
				inflammatory response
				integrin-mediated signaling pathway
				negative regulation of Fc-gamma receptor signaling pathway involved in phagocytosis
				positive regulation of cell population proliferation
				positive regulation of cell-cell adhesion
				positive regulation of inflammatory response
				positive regulation of monocyte extravasation
				positive regulation of phagocytosis

				positive regulation of stress fiber assembly
				positive regulation of T cell activation
				regulation of interleukin-10 production
				regulation of interleukin-12 production
				regulation of interleukin-6 production
				regulation of nitric oxide biosynthetic process
				regulation of tumor necrosis factor production
				regulation of type II interferon production
ENSG00000271601	LIX1L	0,712725467	0,026193837	autophagosome maturation
ENSG00000164574	GALNT10	0,705174661	0,00012453	O-glycan processing
				protein O-linked glycosylation
ENSG00000269897	COMMD3-BMI1	0,660888009	0,097455503	N/A
ENSG00000085563	ABCB1	0,64358102	0,062721535	carboxylic acid transmembrane transport
				ceramide translocation
				export across plasma membrane
				G2/M transition of mitotic cell cycle
				phospholipid translocation
				positive regulation of anion channel activity
				regulation of chloride transport
				regulation of response to osmotic stress
				response to xenobiotic stimulus
				stem cell proliferation
				terpenoid transport
				transepithelial transport
				transmembrane transport
				transport across blood-brain barrier
				xenobiotic detoxification by transmembrane export across the plasma membrane

				xenobiotic metabolic process
				xenobiotic transport across blood-brain barrier
ENSG00000081320	STK17B	0,619326485	2,12E-08	apoptotic process
				intracellular signal transduction
				positive regulation of apoptotic process
				positive regulation of fibroblast apoptotic process
				protein autophosphorylation
				protein phosphorylation
ENSG00000267645	POLR2J2	0,616030298	0,019059095	DNA-templated transcription
				transcription by RNA polymerase II
ENSG00000126821	SGPP1	0,595145241	0,007121677	ER to Golgi ceramide transport
				extrinsic apoptotic signaling pathway
				intrinsic apoptotic signaling pathway
				phospholipid dephosphorylation
				regulation of epidermis development
				regulation of keratinocyte differentiation
				sphinganine-1-phosphate metabolic process
				sphingolipid biosynthetic process
				sphingosine metabolic process
ENSG00000152763	WDR78	0,586612305	0,04404674	axonemal dynein complex assembly
				cilium movement
				hematopoietic progenitor cell differentiation
ENSG00000204267	TAP2	0,584402184	0,009651998	antigen processing and presentation of endogenous peptide antigen via MHC class I
				antigen processing and presentation of endogenous peptide antigen via MHC class I via ER pathway, TAP-dependent

				antigen processing and presentation of endogenous peptide antigen via MHC class Ib via ER pathway, TAP-dependent
				antigen processing and presentation of exogenous protein antigen via MHC class Ib, TAP-dependent
				cytosol to endoplasmic reticulum transport
				peptide antigen transport
				positive regulation of T cell mediated cytotoxicity
				protein transport
				response to molecule of bacterial origin
				T cell mediated cytotoxicity
				transmembrane transport
ENSG00000158292	GPR153	0,568245923	0,093885133	G protein-coupled receptor signaling pathway
ENSG00000183458	AC138932,1	0,563084115	0,037904532	mRNA transport
				protein transport
ENSG00000047578	KIAA0556	0,529509264	0,029613315	cerebrospinal fluid circulation
ENSG00000270647	TAF15	0,52523564	0,001292848	mRNA stabilization
				positive regulation of DNA-templated transcription
				RNA splicing
ENSG00000170634	ACYP2	0,523428453	0,045541796	phosphate-containing compound metabolic process
ENSG00000149541	B3GAT3	0,517815024	0,008506853	carbohydrate metabolic process
				chondroitin sulfate proteoglycan biosynthetic process
				dermatan sulfate proteoglycan biosynthetic process
				glycosaminoglycan biosynthetic process
				heparan sulfate proteoglycan biosynthetic process
				positive regulation of catalytic activity
				positive regulation of intracellular protein transport

				protein glycosylation
ENSG00000167778	SPRYD3	0,507317265	0,031938023	N/A
ENSG00000203993	ARRDC1-AS1	0,494215608	0,042508849	N/A
ENSG00000111752	PHC1	0,483616675	0,01392577	cellular response to leukemia inhibitory factor
				cellular response to retinoic acid
				chromatin remodeling
				negative regulation of DNA-templated transcription
ENSG00000109854	HTATIP2	0,476838618	0,074448066	angiogenesis
				apoptotic process
				cell differentiation
				import into nucleus
				negative regulation of apoptotic process
				positive regulation of programmed cell death
				positive regulation of transcription by RNA polymerase II
				protein autophosphorylation
				regulation of angiogenesis
				regulation of transcription by RNA polymerase II
ENSG00000159363	ATP13A2	0,474280935	0,057043886	autophagosome organization
				autophagosome-lysosome fusion
				autophagy
				cellular response to manganese ion
				cellular response to oxidative stress
				cellular response to zinc ion
				extracellular exosome biogenesis
				intracellular calcium ion homeostasis
				intracellular iron ion homeostasis
				intracellular monoatomic cation homeostasis
				intracellular zinc ion homeostasis
				lipid homeostasis

				lysosomal transport
				monoatomic ion transmembrane transport
				negative regulation of lysosomal protein catabolic process
				negative regulation of neuron death
				peptidyl-aspartic acid autophosphorylation
				polyamine transmembrane transport
				positive regulation of exosomal secretion
				positive regulation of gene expression
				positive regulation of protein secretion
				protein autophosphorylation
				protein localization to lysosome
				regulation of autophagosome size
				regulation of autophagy of mitochondrion
				regulation of chaperone-mediated autophagy
				regulation of endopeptidase activity
				regulation of glucosylceramidase activity
				regulation of intracellular protein transport
				regulation of lysosomal protein catabolic process
				regulation of macroautophagy
				regulation of mitochondrion organization
				regulation of neuron apoptotic process
				regulation of protein localization to nucleus
				regulation of ubiquitin-specific protease activity
				spermine transmembrane transport
				transmembrane transport

ENSG00000131437	KIF3A	0,472370581	0,085021881	anterograde axonal transport
				centriole-centriole cohesion
				cilium assembly
				microtubule anchoring at centrosome
				organelle organization
				plus-end-directed vesicle transport along microtubule
				protein localization to cell junction
				protein transport
ENSG00000213780	GTF2H4	0,460571209	0,037623212	DNA repair
				nucleotide-excision repair
				transcription by RNA polymerase II
ENSG00000172331	BPGM	0,459465352	0,052730749	carbohydrate derivative catabolic process
				carbohydrate metabolic process
				defense response to protozoan
				erythrocyte development
				establishment of blood-brain barrier
				glycolytic process
				neuroinflammatory response
				nucleoside phosphate metabolic process
				oxygen transport
				respiratory gaseous exchange by respiratory system
ENSG00000174373	RALGAPA1	0,455635569	0,057043886	activation of GTPase activity
				regulation of small GTPase mediated signal transduction
ENSG00000101844	ATG4A	0,445661157	0,081394895	autophagy
				lipid metabolic process
				protein delipidation
				protein transport
				proteolysis
ENSG00000125746	EML2	0,44126932	0,031314713	microtubule cytoskeleton organization
				negative regulation of microtubule polymerization
				regulation of microtubule nucleation
				sensory perception of sound
				visual perception

ENSG00000113360	DROSHA	0,439526161	0,037164705	defense response to Gram-negative bacterium
				defense response to Gram-positive bacterium
				miRNA metabolic process
				positive regulation of gene expression
				pre-miRNA processing
				primary miRNA processing
				regulation of inflammatory response
				regulation of miRNA metabolic process
				regulation of regulatory T cell differentiation
				rRNA processing
ENSG00000197818	SLC9A8	0,430422615	0,037623212	acrosome assembly
				monoatomic ion transport
				potassium ion transmembrane transport
				proton transmembrane transport
				regulation of Golgi lumen acidification
				regulation of intracellular pH
				sodium ion transmembrane transport
ENSG00000138190	EXOC6	0,423232382	0,010361777	exocytosis
				Golgi to plasma membrane transport
				intracellular protein transport
				membrane fission
				mitotic cytokinesis
				vesicle docking involved in exocytosis
				vesicle tethering involved in exocytosis
ENSG00000100003	SEC14L2	0,420513176	0,078111506	positive regulation of DNA-templated transcription
				regulation of cholesterol biosynthetic process
ENSG00000100612	DHRS7	0,415872854	0,037623212	retinol metabolic process
ENSG00000115145	STAM2	0,412788958	0,074448066	macroautophagy
				membrane fission
				multivesicular body assembly

				protein transport to vacuole involved in ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway
				signal transduction
ENSG00000134444	KIAA1468	0,412770815	0,016057265	intracellular cholesterol transport
ENSG00000186812	ZNF397	0,408330323	0,087809743	negative regulation of DNA-templated transcription
				regulation of transcription by RNA polymerase II
ENSG00000114316	USP4	0,40341823	0,04404674	negative regulation of protein ubiquitination
				protein deubiquitination
				protein localization to cell surface
				regulation of protein stability
				spliceosomal tri-snRNP complex assembly
				ubiquitin-dependent protein catabolic process
ENSG00000169718	DUS1L	0,403381734	0,056192088	tRNA dihydrouridine synthesis
ENSG00000205746	AC126755,1	0,403287351	0,037623212	N/A
ENSG00000124243	BCAS4	0,400593684	0,047190183	N/A
ENSG00000152133	GPATCH11	0,399909825	0,009651998	N/A
ENSG00000184787	UBE2G2	0,396695545	0,026193837	cellular response to interferon-beta
				negative regulation of retrograde protein transport, ER to cytosol
				protein K48-linked ubiquitination
				ubiquitin-dependent ERAD pathway
				ubiquitin-dependent protein catabolic process
ENSG00000204439	C6orf47	0,394495549	0,094422596	N/A
ENSG00000075790	BCAP29	0,385625477	0,099134361	apoptotic process
				endoplasmic reticulum to Golgi vesicle-mediated transport

				intracellular protein transport
				osteoblast differentiation
				protein localization to endoplasmic reticulum exit site
ENSG00000165915	SLC39A13	0,381785149	0,047754762	brown fat cell differentiation
				connective tissue development
				intracellular zinc ion homeostasis
				zinc ion transmembrane transport
				zinc ion transport
ENSG00000254681	PKD1P5	0,381116465	0,087646588	N/A
ENSG00000266173	STRADA	0,380872069	0,037623212	activation of protein kinase activity
				G1 to G0 transition
				protein export from nucleus
ENSG00000182224	CYB5D1	0,375155088	0,052730749	regulation of cilium beat frequency
ENSG00000159348	CYB5R1	0,365718513	0,052730749	bicarbonate transport
				sterol biosynthetic process
ENSG00000104290	FZD3	0,365130434	0,087809743	canonical Wnt signaling pathway
				cell proliferation in midbrain
				commissural neuron axon guidance
				dopaminergic neuron axon guidance
				establishment of planar polarity
				hair follicle development
				inner ear morphogenesis
				midbrain morphogenesis
				motor neuron migration
				negative regulation of execution phase of apoptosis
				negative regulation of mitotic cell cycle, embryonic
				neural tube closure
				neuron differentiation
				non-canonical Wnt signaling pathway
				planar cell polarity pathway involved in axon guidance

				positive regulation of neuroblast proliferation
				post-anal tail morphogenesis
				response to electrical stimulus
				response to xenobiotic stimulus
				serotonergic neuron axon guidance
				sympathetic ganglion development
				Wnt signaling pathway, planar cell polarity pathway
ENSG00000163444	TMEM183A	0,359114725	0,047754762	regulation of protein stability
ENSG00000122257	RBBP6	0,351117298	0,007121677	DNA damage response
				DNA replication
				embryonic organ development
				in utero embryonic development
				mRNA processing
				multicellular organism growth
				protein ubiquitination
				regulation of DNA replication
				somite development
				ubiquitin-dependent protein catabolic process
ENSG00000127980	PEX1	0,348807189	0,075457577	microtubule-based peroxisome localization
				peroxisome organization
				protein import into peroxisome matrix
				protein import into peroxisome matrix, receptor recycling
				protein targeting to peroxisome
				protein unfolding
ENSG00000131507	NDFIP1	0,347866322	0,094422596	CD4-positive, alpha-beta T cell proliferation
				intracellular iron ion homeostasis
				metal ion transport
				negative regulation of CD4-positive, alpha-beta T cell proliferation

				negative regulation of gene expression
				negative regulation of inflammatory response
				negative regulation of interleukin-4 production
				negative regulation of isotype switching to IgE isotypes
				negative regulation of protein transport
				negative regulation of transporter activity
				negative regulation of type 2 immune response
				positive regulation of I-kappaB kinase/NF-kappaB signaling
				positive regulation of protein catabolic process
				positive regulation of protein ubiquitination
				regulation of isotype switching to IgG isotypes
				regulation of lymphocyte differentiation
				regulation of myeloid leukocyte differentiation
				ubiquitin-dependent protein catabolic process
				vacuolar transport
ENSG00000176396	EID2	0,347847351	0,099176309	cell differentiation
				muscle organ development
				negative regulation of DNA-templated transcription
				negative regulation of transforming growth factor beta receptor signaling pathway
				regulation of cell population proliferation
				regulation of transforming growth factor beta receptor signaling pathway
				SMAD protein complex assembly
				transforming growth factor beta receptor complex assembly

ENSG00000114744	COMMD2	0,345673935	0,007121677	N/A
ENSG00000136908	DPM2	0,345247121	0,042508849	dolichol metabolic process
				GPI anchor biosynthetic process
				protein O-linked mannosylation
				regulation of protein stability
ENSG00000198408	MGEA5	0,342076864	0,029384673	dATP metabolic process
				glycoprotein metabolic process
				N-acetylglucosamine metabolic process
				necrotic cell death
				negative regulation of cardiac muscle adaptation
				negative regulation of protein glycosylation
				positive regulation of calcium ion transport
				positive regulation of calcium ion transport into cytosol
				positive regulation of cell killing
				positive regulation of DNA metabolic process
				positive regulation of glucose import
				positive regulation of growth hormone secretion
				positive regulation of insulin secretion
				positive regulation of mitochondrial depolarization
				positive regulation of protein-containing complex disassembly
				positive regulation of proteolysis
				protein deglycosylation
				protein targeting to membrane
ENSG00000189079	ARID2	0,342068918	0,037245607	cardiac muscle cell proliferation
				chromatin remodeling
				coronary artery morphogenesis
				embryonic organ development

				heart morphogenesis
				homeostatic process
				negative regulation of cell migration
				negative regulation of cell population proliferation
				nucleosome disassembly
				positive regulation of cell differentiation
				positive regulation of double-strand break repair
				positive regulation of double-strand break repair via homologous recombination
				positive regulation of myoblast differentiation
				positive regulation of T cell differentiation
				regulation of G0 to G1 transition
				regulation of G1/S transition of mitotic cell cycle
				regulation of mitotic metaphase/anaphase transition
				regulation of nucleotide-excision repair
				regulation of transcription by RNA polymerase II
ENSG00000123124	WWP1	0,339454915	0,084236698	central nervous system development
				monoatomic ion transmembrane transport
				negative regulation of DNA-templated transcription
				proteasome-mediated ubiquitin-dependent protein catabolic process
				protein ubiquitination
				signal transduction
				viral entry into host cell
ENSG00000123066	MED13L	0,335713396	0,096583308	regulation of transcription by RNA polymerase II
ENSG00000155975	VPS37A	0,332364576	0,046100712	macroautophagy
				membrane fission
				multivesicular body assembly

				protein targeting to membrane
				protein targeting to vacuole
				protein transport to vacuole involved in ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway
				ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway
				viral budding via host ESCRT
ENSG00000149182	ARFGAP2	0,328108591	0,04324741	COPI coating of Golgi vesicle
				protein transport
ENSG00000162613	FUBP1	0,319848934	0,037245607	positive regulation of gene expression
				regulation of gene expression
ENSG00000005175	RPAP3	0,311473101	0,03211716	protein stabilization
ENSG00000006007	GDE1	0,310849864	0,015159893	ethanolamine metabolic process
				glycerophospholipid catabolic process
				lipid metabolic process
				N-acylethanolamine metabolic process
				phospholipid metabolic process
ENSG00000186063	AIDA	0,308920929	0,099176309	determination of ventral identity
				dorsal/ventral pattern formation
				negative regulation of JNK cascade
				negative regulation of JUN kinase activity
				negative regulation of protein-containing complex assembly
ENSG00000166164	BRD7	0,306999468	0,056450976	cell cycle
				chromatin remodeling
				negative regulation of DNA-templated transcription
				negative regulation of G1/S transition of mitotic cell cycle
				positive regulation of cell differentiation

				positive regulation of double-strand break repair
				positive regulation of myoblast differentiation
				positive regulation of T cell differentiation
				regulation of G0 to G1 transition
				regulation of G1/S transition of mitotic cell cycle
				regulation of mitotic cell cycle
				regulation of mitotic metaphase/anaphase transition
				regulation of nucleotide-excision repair
				regulation of transcription by RNA polymerase II
				transcription initiation-coupled chromatin remodeling
				Wnt signaling pathway
ENSG00000123200	ZC3H13	0,305106875	0,045130926	mRNA methylation
				mRNA processing
				regulation of stem cell population maintenance
				RNA splicing
ENSG00000107854	TNKS2	0,298534599	0,034795955	negative regulation of telomere maintenance via telomere lengthening
				positive regulation of canonical Wnt signaling pathway
				positive regulation of telomere capping
				positive regulation of telomere maintenance via telomerase
				protein auto-ADP-ribosylation
				protein localization to chromosome, telomeric region
				protein poly-ADP-ribosylation
				protein polyubiquitination
				Wnt signaling pathway
ENSG00000115109	EPB41L5	0,296430608	0,01392577	actomyosin structure organization
				apical constriction

				axial mesoderm morphogenesis
				cellular response to transforming growth factor beta stimulus
				ectoderm development
				embryonic foregut morphogenesis
				endoderm development
				epithelial to mesenchymal transition
				in utero embryonic development
				left/right axis specification
				mesoderm migration involved in gastrulation
				negative regulation of cell-cell adhesion
				negative regulation of protein binding
				neural plate morphogenesis
				paraxial mesoderm development
				positive regulation of epithelial cell migration
				positive regulation of focal adhesion assembly
				positive regulation of protein binding
				post-transcriptional regulation of gene expression
				regulation of establishment of protein localization
				somite rostral/caudal axis specification
				substrate-dependent cell migration, cell attachment to substrate
				unidimensional cell growth
ENSG00000132612	VPS4A	0,295897124	0,087809743	abscission
				actomyosin contractile ring contraction
				autophagosome maturation
				autophagy
				cell division
				cytoskeleton-dependent cytokinesis
				endosomal transport

				endosomal vesicle fusion
				ESCRT complex disassembly
				ESCRT III complex disassembly
				intracellular cholesterol transport
				late endosomal microautophagy
				late endosome to lysosome transport via multivesicular body sorting pathway
				macroautophagy
				membrane fission
				midbody abscission
				mitotic cytokinesis checkpoint signaling
				mitotic metaphase plate congression
				mitotic nuclear membrane reassembly
				multivesicular body assembly
				multivesicular body sorting pathway
				negative regulation of cytokinesis
				nuclear envelope organization
				nuclear membrane reassembly
				nucleus organization
				plasma membrane repair
				positive regulation of exosomal secretion
				positive regulation of viral budding via host ESCRT complex
				protein targeting to lysosome
				regulation of protein localization
				regulation of protein localization to plasma membrane
				ubiquitin-dependent protein catabolic process via the multivesicular body sorting pathway
				ubiquitin-independent protein catabolic process via the multivesicular body sorting pathway

				vacuole organization
				vesicle budding from membrane
				vesicle uncoating
				vesicle-mediated transport
				viral budding from plasma membrane
				viral budding via host ESCRT complex
				viral release from host cell
ENSG00000144224	UBXN4	0,294513673	0,069001817	response to unfolded protein
				ubiquitin-dependent ERAD pathway
ENSG00000127870	RNF6	0,269408562	0,094682343	axon extension
				negative regulation of axon extension
				positive regulation of DNA-templated transcription
				protein K27-linked ubiquitination
				protein K48-linked ubiquitination
				protein K6-linked ubiquitination
				protein ubiquitination
				regulation of androgen receptor signaling pathway
				regulation of DNA-templated transcription
				ubiquitin-dependent protein catabolic process
ENSG00000132640	BTBD3	0,256945683	0,099176309	cerebral cortex development
				dendrite morphogenesis
				neurogenesis
ENSG00000111670	GNPTAB	0,252317751	0,099176309	carbohydrate phosphorylation
				lysosome organization
				N-glycan processing to lysosome
				secretion of lysosomal enzymes
ENSG00000170248	PDCD6IP	0,24318793	0,099134361	actomyosin contractile ring assembly
				apoptotic process
				bicellular tight junction assembly
				maintenance of epithelial cell apical/basal polarity
				midbody abscission

				mitotic cytokinesis
				multivesicular body assembly
				positive regulation of exosomal secretion
				positive regulation of extracellular exosome assembly
				protein homooligomerization
				protein transport
				regulation of centrosome duplication
				regulation of extracellular exosome assembly
				regulation of membrane permeability
				ubiquitin-independent protein catabolic process via the multivesicular body sorting pathway
				viral budding
				viral budding via host ESCRT complex

Table S2. DEGs overexpressed in Caco-2 cells exposed to 24-hour incubation with 100 µM DicloUA administered apically versus culture medium alone (Control)

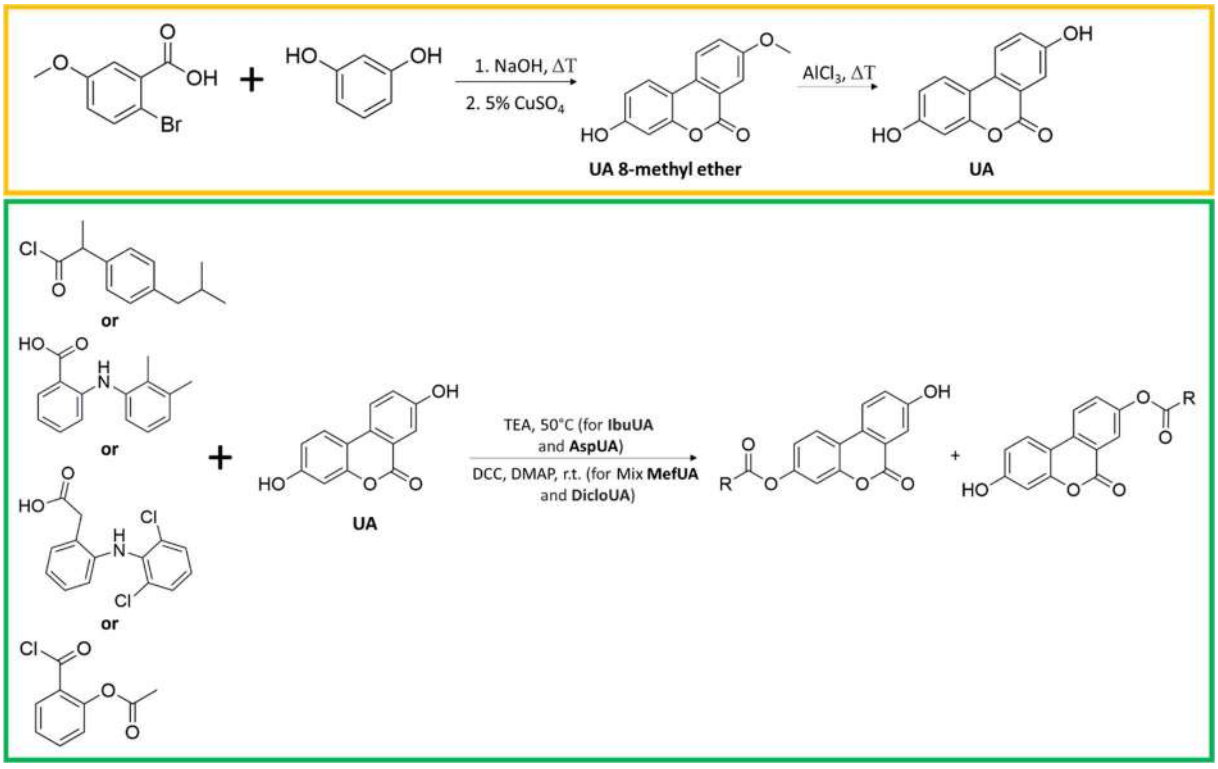


Figure S1. Schematic representation of UA and UADs' synthesis

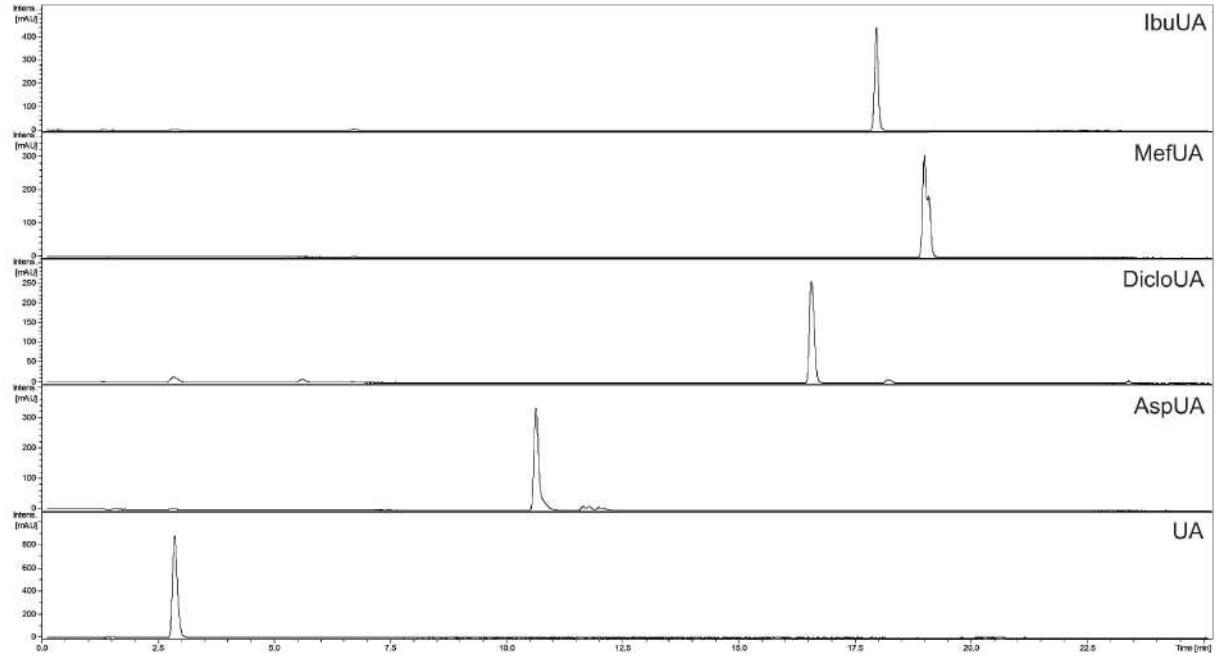


Figure S2. UHPLC profiles of UA and newly synthesized UADs (λ= 305 nm).

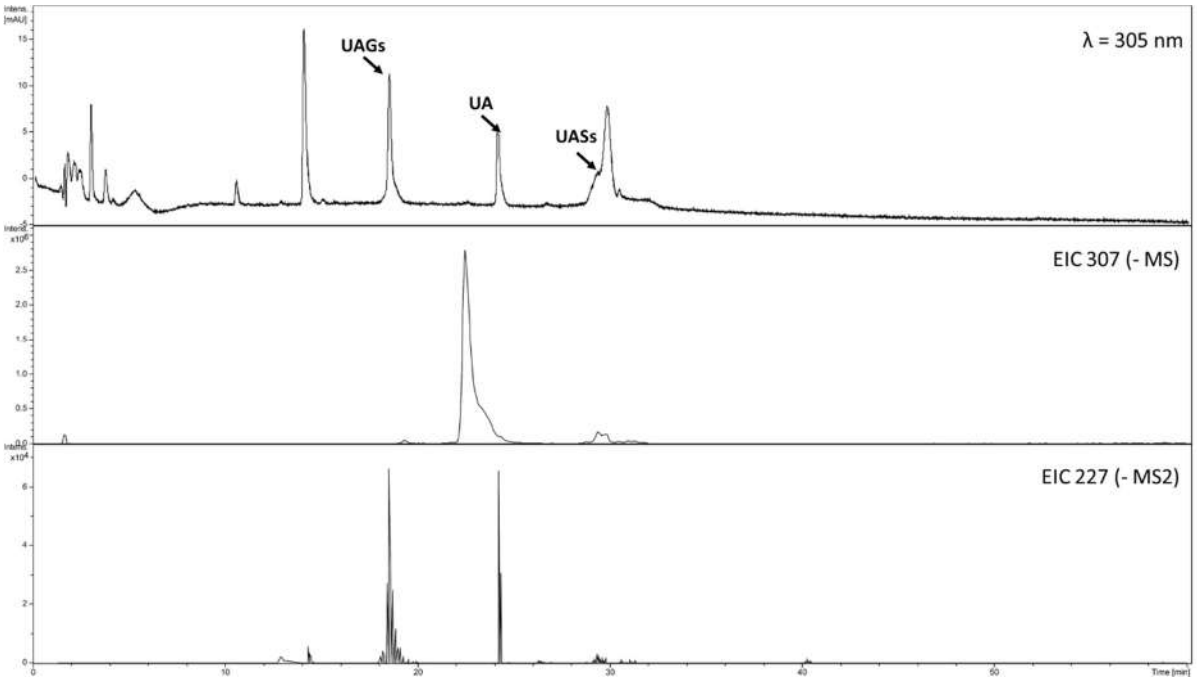


Figure S3. UHPLC-DAD-MS/MS analysis of basolateral side of Caco-2 cell monolayers exposed to 24-incubation with apically administered 100 µM UA. Presented a small amount of UA-sulphates (3) confirmed by extraction of molecular ion $[M-H]^-$ m/z = 307 and presence of fragments m/z = 227 $[UA-H]^-$ in a negative ion mode. UA – urolithin A, UAGs- UA glucuronides, UASs – UA sulfates. (λ= 305 nm).

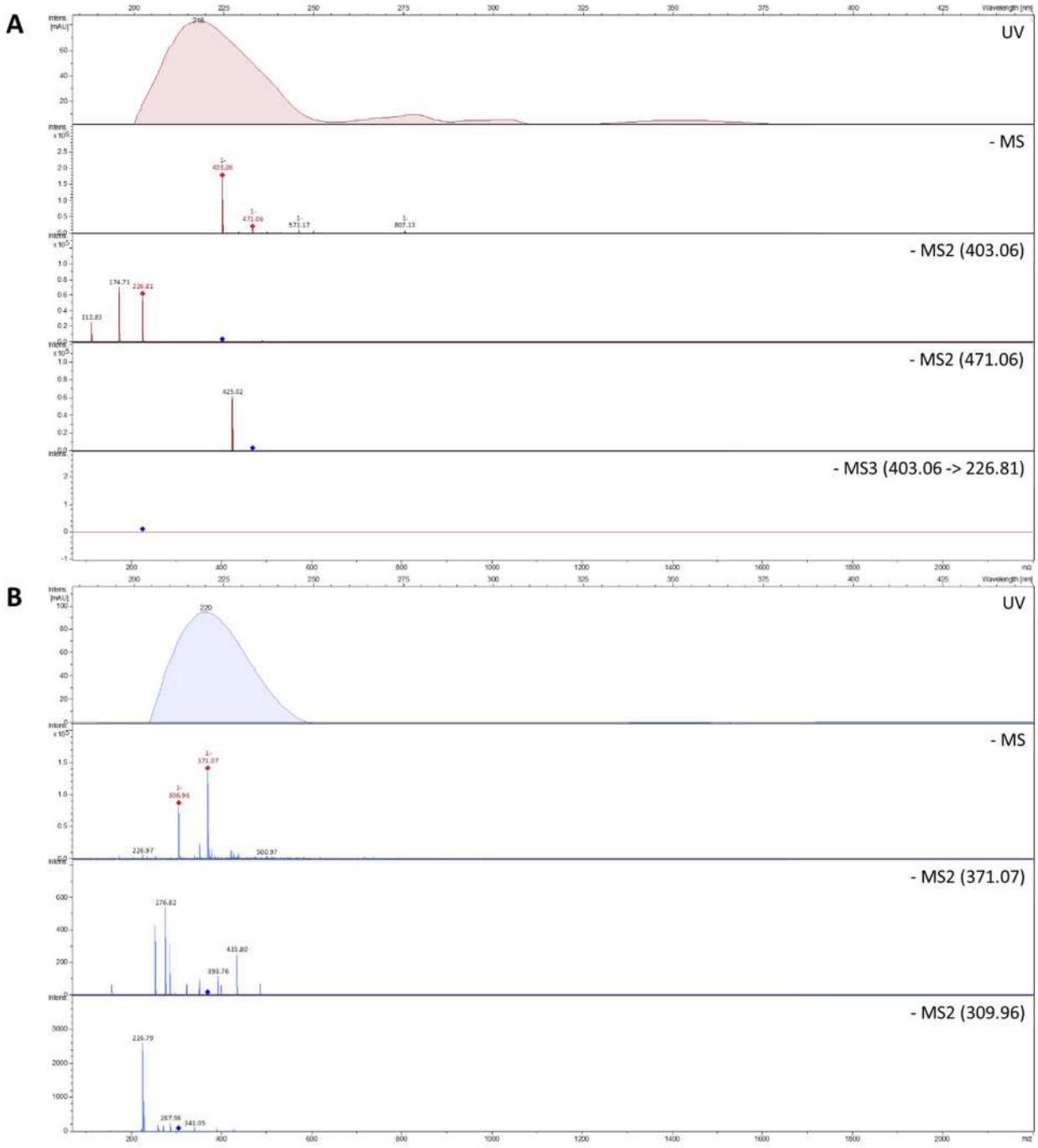


Figure S4. UV data and fragmentations of (A) UAGs and (B) UASs.

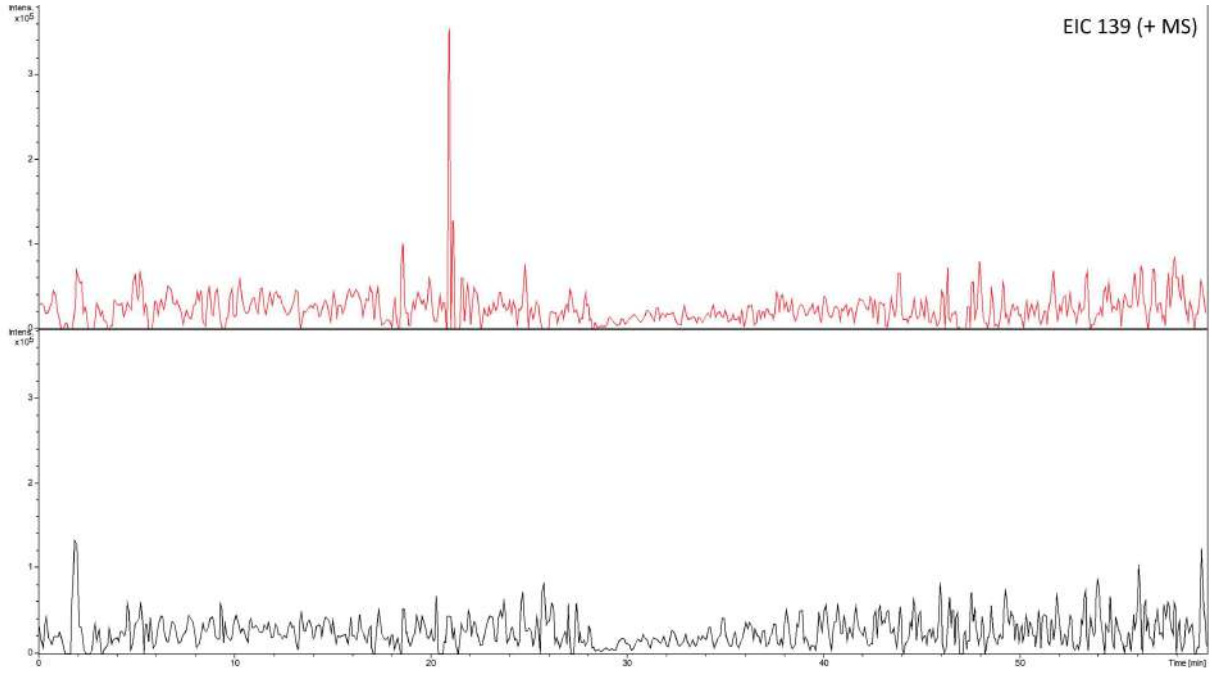


Figure S5. UHPLC-DAD-MS/MS analysis of basolateral side of Caco-2 cell monolayers exposed to 24-incubation with either apically administered 100 µM AspUA (red) or DMSO (black). Data acquired after extracting m/z = 139 $[M-H]^+$ in a positive ion mode.

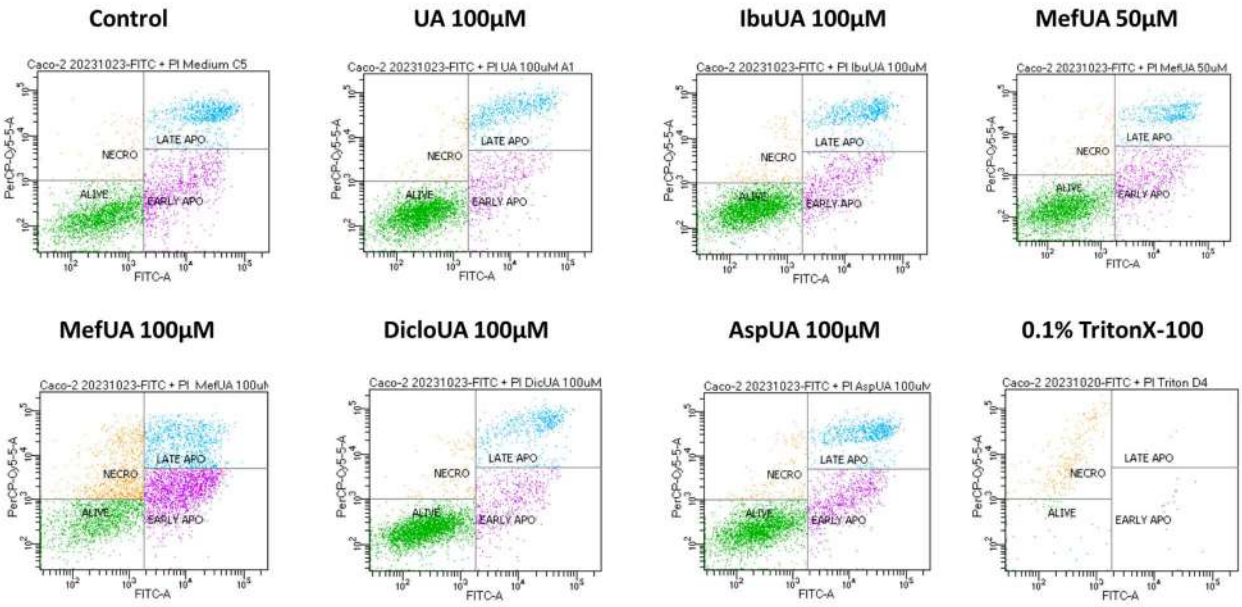
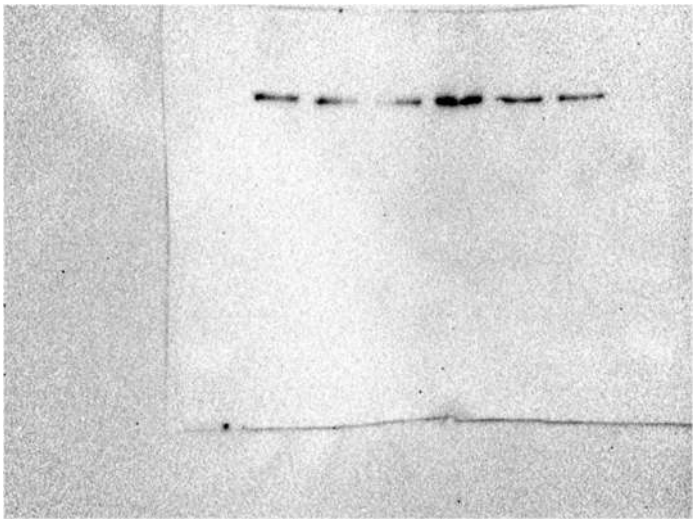
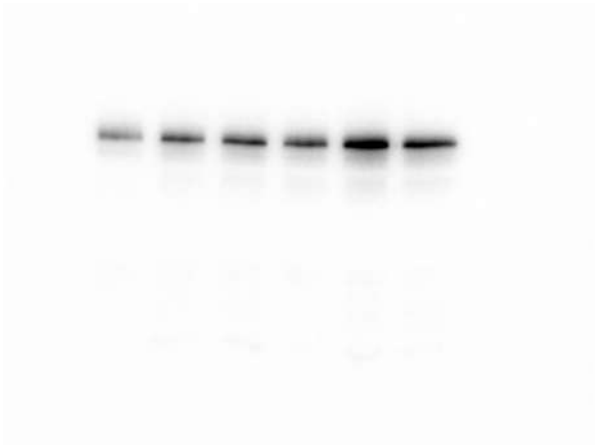


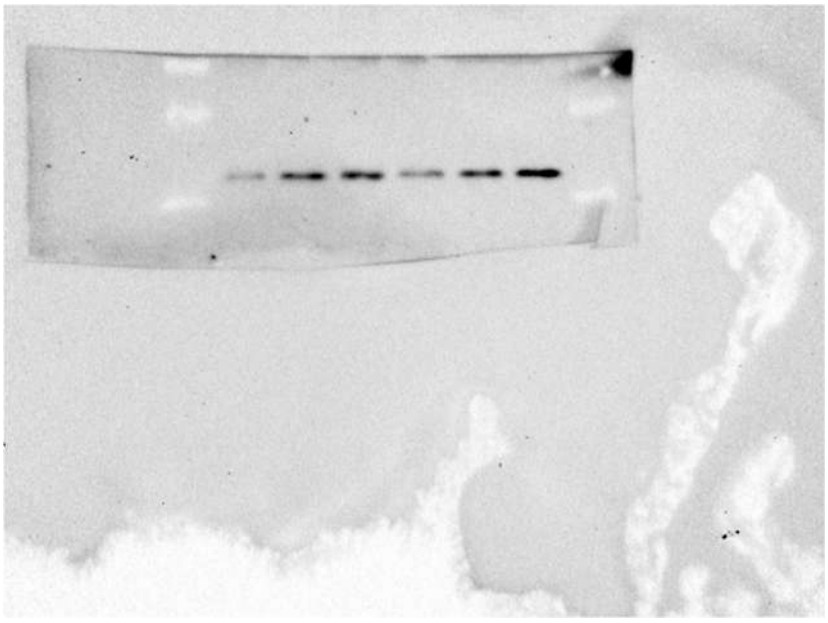
Figure S6. Representative dot plots from flow cytometric analysis evaluating proapoptopic properties of tested compounds using FITC Annexin V and PI staining.



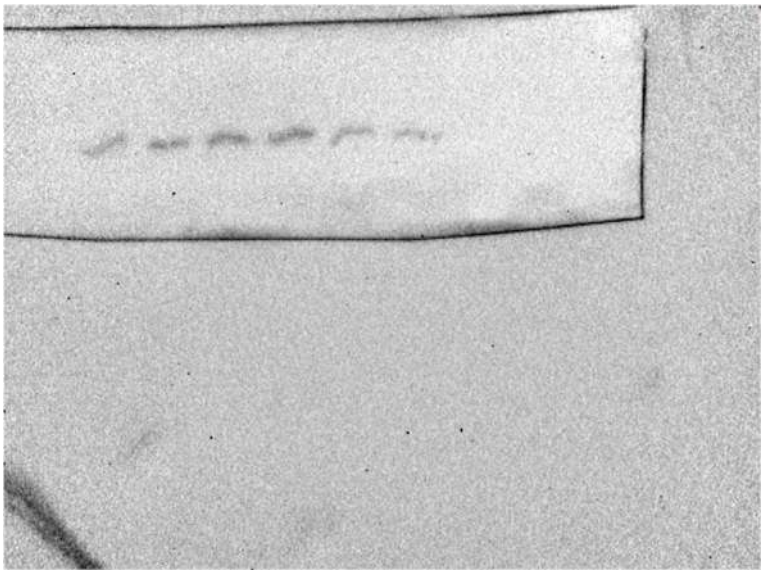
ZO-1



OCLN



CLDN-1



CLDN-2

B-actin

Figure S7. Original uncropped images of western blots presented in Figure 5A.