MEDICAL UNIVERSITY OF WARSAW

Dissertation for the degree of Doctor of Medicine and Health Sciences in the discipline of pharmaceutical sciences

Interactions between human skin microbiota and plant extracts traditionally used in the treatment of skin conditions

MPharm Natalia Melnyk

Supervisor: Prof. Sebastian Granica
Assistant supervisor: PhD Dominik Popowski

Department of Pharmaceutical Biology
Faculty of Pharmacy
Medical University of Warsaw

UNIVERSITAS MEDICA VARSOVIENSIS
1926
FACULTAS FARMACEUTICA

Defense of the doctoral dissertation to the Pharmaceutical Sciences Council of Medical University of Warsaw

Warsaw, 2026

WARSZAWSKI UNIWERSYTET MEDYCZNY

Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu

w dyscyplinie nauki farmaceutyczne

Interakcje mikrobioty skóry ludzkiej z ekstraktami roślinnymi tradycyjnie stosowanymi w leczeniu schorzeń skóry

mgr farm. Natalia Melnyk

Promotor: prof. dr hab. Sebastian Granica
Promotor pomocniczy: dr Dominik Popowski

Katedra i Zakład Biologii Farmaceutycznej
Wydział Farmaceutyczny
Warszawski Uniwersytet Medyczny

UNIVERSITAS MEDICA VARSOVIENSIS
1926
FACULTAS FARMACEUTICA

Obrona rozprawy doktorskiej przed Radą Dyscypliny Nauk Farmaceutycznych

Warszawskiego Uniwersytetu Medycznego

Warszawa, 2026

Acknowledgements

This work would not have been possible without the support, guidance, and encouragement of the many people who accompanied me through this demanding scientific journey.

First and foremost, I would like to express my sincere gratitude to my supervisor, **Prof. Sebastian Granica**, who believed in me at an early stage and supported me throughout every step of this challenging academic path. His mentorship extended beyond scientific supervision, as his openness, understanding, and respectful approach fostered a supportive academic environment that was essential for my development as both a researcher and a person.

I would also like to sincerely thank **Prof. Jakub Piwowarski** for his invaluable advice, support, and constructive feedback throughout my doctoral project.

My heartfelt thanks go to my co-supervisor, **Dominik Popowski**, whose willingness to help and ability to clearly explain complex issues were always greatly appreciated.

I am especially grateful to **Weronika Skowrońska**. I sincerely thank her for generously sharing her expertise, for her kindness and constant support, and for her invaluable scientific guidance throughout this research process.

I would like to thank **Maciej Korczak**, who became a guide in the world of Western blotting. The phrase “red to red, black to black” will always remain a memorable reminder of his practical instructions.

I am grateful to senior colleagues **Marcin Równicki**, **Małgorzata Kołtun-Jasion**, and **Aleksandra Tymoszewska** for their valuable advice and for sharing their experience, and **Aleksandra Kruk** and **Karolina Pawłowska** for the warm welcome when I joined the lab, for their kind words, and for their constant readiness to help.

I would also like to thank my colleagues **Diana Dolzhko**, **Yuliia Kostenko**, **Inna Vlasova**, **Zuzanna** and **Michał Markowscy**, **Maciej Obrębski,** and **Rafał Kiełkiewicz** for creating a friendly working environment.

I would like to express my sincere gratitude to the **technical staff of the department** for their invaluable assistance, reliability, and everyday support in laboratory work. Their professionalism greatly facilitated the completion of this research. I am also grateful to **all members of the department** for the opportunity to grow in such an inspiring academic environment.

I am deeply thankful to **Prof. Chantal Pichon** for the opportunity to complete a research internship in her laboratory, which provided me with invaluable experience and significantly broadened my scientific perspective. My sincere thanks go to my French colleagues and friends, **Juan Carlos Calderón de la Rosa**, **Manal Farhat**, **Ardita Rrahmanaj**, **Oumayma Ouchan**, **Mathéo Grand**, **Olivia Faillot,** and **Anne-Caroline Sangaret**, for their support both inside and outside the laboratory, which made this internship particularly meaningful.

I would like to thank **Aleksandra Zdanowicz** for her constant support throughout my doctoral path and for going this path together.

Finally, I would like to express my sincere gratitude to **my family** for their constant support, understanding, and unwavering faith in me.

Fundings

Doctoral project funded by the National Science Centre (NCN) under the Preludium Bis 2 funding scheme (grant No. 2020/39/O/NZ7/01109, “Interactions of plant extracts traditionally used in skin diseases with a human skin microbiota”), which provides long-term financial support for innovative research conducted by early-stage researchers, including funding for scholarship, research activities, and international mobility.

NATIONAL SCIENCE CENTRE POLAND

Two research internships completed during the doctoral program were funded by the Polish National Agency of Academic Exchange (NAWA) within the NAWA STER and NAWA Preludium Bis 2 programs, lasting one month and six months, respectively.

NAWA POLISH NATIONAL AGENCY FOR ACADEMIC EXCHANGE

1. List of publications included in the doctoral thesis

The application for the doctoral degree in Medical and Health Sciences, within the discipline of Pharmaceutical Sciences, is based on a series of three scientific papers published between 2022-2025 in peer-reviewed journals, with a total Impact Factor of 13.4 and a combined score of 300 according to the Ministry of Education and Science.

Publication 1 – Review Article

IF = 5.6; MEiN = 140

Melnyk N., Vlasova I., Skowrońska W., Bazylko A., Piwowarski J., Granica S. Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota. *International Journal of Molecular Sciences*. 2022, Vol. 23, No. 17, pp. 1-28. DOI: 10.3390/ijms23179644.

Publication 2 – Original Research Article

IF = 5.4; MEiN = 140

Melnyk N., Popowski D., Strawa J., Przygodzińska K., Tomczyk M., Piwowarski J., Granica S. Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.) *Journal of Ethnopharmacology*. 2024, Vol. 318, No. Pt B, pp. 1–10. DOI: 10.1016/j.jep.2023.116968.

Publication 3 – Original Research Article

IF = 2.4; MEiN = 20

Melnyk N., Skowrońska W., Popowski D., Piwowarski J., Granica S.

From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin. *Prospects in Pharmaceutical Sciences*. 2025, pp.1-15.DOI: 10.56782/pps.792.

2. Table of contents

3. Abstracts

3.1. Abstract in the English language

The human skin is a multifunctional organ that serves as a physical, chemical, and immunological barrier while simultaneously hosting a diverse and dynamic microbiota. Increasing evidence indicates that the skin microbiota is an integral component of cutaneous homeostasis, actively contributing to barrier integrity, immune regulation, and protection against pathogenic colonization. Alterations in microbiota composition or function have been associated with numerous inflammatory and infectious skin disorders, underscoring the clinical relevance of host-microbiota interactions in dermatology.

Medicinal plants have been traditionally used in the treatment of skin conditions due to their anti-inflammatory, antimicrobial, and wound-healing properties. However, the mechanisms underlying their therapeutic effects remain not fully understood. While extensive research has demonstrated the role of the gut microbiota in the biotransformation of natural compounds, the capacity of the skin microbiota to metabolize plant-derived substances applied typically has received comparatively little scientific attention.

This dissertation addresses this knowledge gap by examining the interactions between human skin microbiota and plant extracts traditionally used in dermatological conditions. Through a combination of literature analysis, pharmaceutical market evaluation, and experimental studies, the work investigates how selected plant materials interact with the skin microbiota and how these interactions influence chemical components of extracts, microbiota structure, and host responses. Emphasis is placed on microbiota-mediated metabolism of plant-derived compounds, an underexplored but potentially critical factor affecting the safety, stability, and biological behavior of topical herbal preparations.

The presented research demonstrates that skin microbiota-plant extract interactions are compound-dependent and that microbial biotransformation can selectively affect specific classes of phytochemicals without inducing global microbial dysbiosis. Furthermore, the findings support the concept that traditional medicinal plants may exert their effects not only through direct action on skin cells but also via

microbiota-related processes. Collectively, this work establishes an integrated framework for understanding skin microbiota-plant extract interactions and provides a scientific basis for the rational development of microbiota-conscious topical formulations in modern dermatology and pharmacognosy.

Keywords: skin microbiota; medicinal plants; plant extracts; microbiota-plant extract interactions; microbiota-mediated metabolism; microbial biotransformation; dysbiosis; skin health; topical phytotherapy.

3.2. Abstract in Polish

Skóra ludzka jest wielofunkcyjnym organem, który pełni funkcję bariery fizycznej, chemicznej i immunologicznej, a jednocześnie stanowi siedlisko różnorodnej i dynamicznej mikrobioty. Coraz więcej dowodów wskazuje, że mikrobiota skóry jest integralnym elementem homeostazy skóry, aktywnie przyczyniającym się do integralności bariery, regulacji odpowiedzi odpornościowej i ochrony przed kolonizacją organizmu przez patogeny. Zmiany w składzie lub funkcji mikrobioty są związane z licznymi zapalnymi i zakaźnymi schorzeniami skóry, co podkreśla kliniczne znaczenie interakcji między gospodarzem a mikrobiotą w schorzeniach dermatologicznych.

Rośliny lecznicze są tradycyjnie stosowane w leczeniu chorób skóry ze względu na ich właściwości przeciwzapalne, przeciwbakteryjne i wspomagające gojenie ran. Jednak mechanizmy leżące u podstaw ich działania terapeutycznego pozostają nie do końca poznane. Chociaż szeroko zakrojone badania wykazały rolę mikrobioty jelitowej w biotransformacji związków naturalnych, zdolność mikrobioty skóry do metabolizowania substancji pochodzenia naturalnego stosowanych zewnętrznie spotkała się ze stosunkowo niewielkim zainteresowaniem naukowców.

Niniejsza rozprawa wypełnia tę lukę w wiedzy, analizując interakcje między mikrobiotą skóry ludzkiej a ekstraktami roślinnymi tradycyjnie stosowanymi w terapii dermatologicznej. Poprzez połączenie analizy literatury, oceny rynku farmaceutycznego i badań eksperymentalnych, praca bada, w jaki sposób wybrane substancje roślinne oddziałują na mikrobiotę skóry i jak te interakcje wpływają na substancje zawarte w ekstraktach, strukturę mikrobioty i reakcje gospodarza. W ramach tej pracy szczególny nacisk kładzie się na metabolizm związków pochodzenia roślinnego, w którym pośredniczy mikrobiota, będący niedostatecznie zbadanym, ale potencjalnie krytycznym czynnikiem wpływającym na bezpieczeństwo, stabilność i zachowanie biologiczne preparatów roślinnych do stosowania miejscowego.

Przedstawione badania pokazują, że interakcje między mikrobiotą skóry a ekstraktami roślinnymi zależą od ich składu chemicznego i że biotransformacja pod wpływem mikroorganizmów może selektywnie wpływać na określone klasy związków pochodzenia naturalnego bez wywoływania dysbiozy. Ponadto wyniki badań

potwierdzają koncepcję, że tradycyjne rośliny lecznicze mogą wywierać swoje działanie nie tylko poprzez bezpośredni wpływ na komórki skóry, ale również poprzez procesy związane z mikrobiotą. Podsumowując, praca ta tworzy zintegrowane ramy dla zrozumienia interakcji między mikrobiotą skóry a substancjami roślinnymi i stanowi naukową podstawę dla racjonalnego opracowywania preparatów do stosowania miejscowego uwzględniających mikrobiotę w nowoczesnej dermatologii i farmakognozji.

Słowa kluczowe: mikrobiota skóry; rośliny lecznicze; ekstrakty roślinne; interakcje między mikrobiotą a ekstraktami roślinnymi; metabolizm za pośrednictwem mikrobioty; biotransformacja mikrobiologiczna; dysbioza; zdrowie skóry.

4. List of abbreviations

AD	atopic dermatitis
AMP	antimicrobial peptide
HPTLC	high-performance thin-layer chromatography
LC–DAD–ESI–MS/TOF	liquid chromatography (**LC**) coupled to electrospray ionization (**ESI**) mass spectrometry (**MS**) time-of-flight (**TOF**)
LTA	lipoteichoic acid
MAIT	mucosal-associated invariant T-cells
MAPK	mitogen-activated protein kinase
NF-κB	nuclear factor kappa-light-chain-enhancer of active B cells
NKT	natural killer T-cells
rDNA	recombinant deoxyribonucleic acid
SD	seborrheic dermatitis
TLR	toll-like receptor
UHPLC-DAD-MS^n	ultra-high performance liquid chromatography (**UHPLC**) coupled to diode array detection (**DAD**) and multi-stage mass spectrometry (**MS**)
SMM	skin microbiota metabolites
SM	skin microbiota
Ig	immunoglobulin
IL	interleukin
TNF	tumor necrosis factor
Th	type helper T cells

5. Scientific profile of the doctoral candidate

Master of Pharmacy Natalia Melnyk

I started a master's degree in pharmacy in 2014 at the National University of Pharmacy in Kharkiv, Ukraine, which I finished in 2019. During my studies, I was actively involved in the university's scientific life as a member of a student scientific society operating within the Departments of Biochemistry (2016/2017), Pharmacotherapy (2017/2018), and Pharmacognosy (2017/2019), where I gained broad and diverse experience, working across different areas of pharmaceutical and biomedical sciences. In the Biochemistry department, I investigated the feasibility of using coenzyme Q (ubiquinone) to correct statin-associated myopathy in a rabbit model of atherosclerosis. At the Pharmacotherapy, I explored modern therapeutic approaches, preparing projects on the “Pharmacotherapy of amebiasis” and “Modern directions of breast cancer medication”, which broadened my understanding of contemporary evidence-based pharmacotherapy and its clinical applications. Furthermore, my involvement in these activities provided me with a solid foundation for scientific thinking and academic development.

During my study, I also participated in numerous academic internships and educational excursions led by Prof. Oleh Koshovyi and Prof. Ihor Kireev, which enriched my practical understanding of pharmaceutical sciences and completed the theoretical training received at the university. My connection with the Medical University of Warsaw began during one of these visits, which introduced me to the Polish academic environment and sparked my interest in continuing my professional development in Poland.

Later, I had an opportunity to participate in the Erasmus+ mobility program under the supervision of Dr. Agnieszka Bazylko and Prof. Sebastian Granica, at the Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, during which I completed the practical part of my master's thesis titled “Determination of HPTLC parameters for identifying linden flowers”. The study aimed to determine standardization parameters for linden flowers using high-performance thin-layer chromatography, including the selection of a suitable mobile phase and the identification of species-specific chemical markers. The analysis

compared the chemical profiles of five *Tilia* species collected in Europe and was conducted using HPTLC to evaluate chromatographic parameters and separation efficiency. This experience strengthened my interest in pharmacognosy and played a decisive role in shaping the direction of my future research career.

After completing my studies, I received an invitation to undertake doctoral studies within a research project led by Prof. Sebastian Granica. Therefore, following graduation, I initiated the nostrification process of my diploma, and after receiving a positive decision, I relocated to Warsaw in 2021 to join MicrobiotaLab at the Medical University of Warsaw and begin my PhD research.

Between 2022 and 2026, I conducted laboratory classes for third-year Pharmacy students as part of the Pharmacognosy curriculum. I served as a tutor for five students during the completion of their master's theses and for three international students during their Erasmus+ mobility.

An essential component of my doctoral studies includes participation in two international research internships: a one-month internship in April 2023 at CBM (Centre de Biophysique Moléculaire), CNRS (Le Centre national de la recherche scientifique), and a six-month internship at ART ARNm (Accélérer le développement des technologies ARNm), INSERM (Institut national de la santé et de la recherche médicale) in February 2025, both under the supervision of Prof. Chantal Pichon in Orléans, France. During these internships, I conducted a part of the experimental work associated with the Preludium Bis project. Additionally, I expanded my methodological competencies, developed experimental skills, became familiar with advanced cellular techniques, and established international academic collaborations that significantly contributed to my scientific development.

A comprehensive description of my research achievements and contributions is outlined in the following sections.

5.1. Scientific achievements in numbers (07.01.2026)

ORCID: 0000-0003-0002-1197

Total number of publications: 8 (5 publications – first author, 1 publication – corresponding author)

- 4 publications – original
- 4 publications – review

Total Impact Factor: 27.431.

Total MEiN score: 560.

Total number of citations (according to the Scopus database): 45.

Hirsch Index (according to the Scopus database, excluding self-citations): 4.

5.2. Publications beyond the scope of the dissertation

- **Melnyk N.**, Nyczka A., Piwowarski J., Granica S. Traditional Use of Chamomile Flowers (*Matricariae flos*) in Inflammatory-Associated Skin Disorders. *Prospects in Pharmaceutical Sciences*. 2024, Vol. 22, No. 4, pp. 59-73. DOI: 10.56782/pps.215
- Dolzhko D., **Melnyk N.**, Kruk A., Granica S., Piwowarski J. Traditional use of polar extracts from lavender flowers – systematic review of literature data. *Prospects in Pharmaceutical Sciences.* 2024, Vol. 22, No. 3, pp. 92-101. DOI: 10.56782/pps.221.
- **Melnyk N.**, Pawłowska K.A., Ziaja M., Wojnowski W., Koshovyi O., Granica S., Bazylko A. Characterization of herbal teas containing lime flowers – *Tiliae flos* by HPTLC method with chemometric analysis. *Food Chemistry*. 2021, Vol. 346, pp. 1-9. DOI: 10.1016/j.foodchem.2020.128929.
- Romanenko Y., Koshovyi O., Ilyina T., Borodina N., **Melnyk N.** Standardization parameters of modified extracts from *Loenurus cardiaca* herb. *ScienceRise: Pharmaceutical Science.* 2019, No. 1, pp. 17-23. DOI: 10.15587/2519-4852.2019.157996.

Zagayko L., Briukhanova T., Shynkariov A., **Melnyk N.** Metabolic effects of carnitine, role in the development of pathologies and prospects for clinical application. *Ukrainian Biopharmaceutical Journal.* 2016, No. 6, pp. 17-22. DOI: 10.24959/ubphj.16.77.

5.3. Conference participation[1]

- **Natalia Melnyk**, Weronika Skowrońska, Dominik Popowski, Jakub Patryk Piwowarski3, Sebastian Granica. Crosstalk between natural products from Symphytum officinale root, skin cells, and human skin microbiota. *73rd International congress and annual meeting of the society for medical plant and natural product research (GA).* 2025, Naples, Italy.
- **Natalia Melnyk**, Weronika Skowrońska, Jakub Patryk Piwowarski, Sebastian Granica. Anti-inflammatory potential of chamomile flowers extract in topical applications. *International congress on natural products research.* 2024, Kraków, Poland.

- **Natalia Melnyk**, Dominik Popowski, Jakub Patryk Piwowarski, Sebastian Granica. The skin microbiota-mediated biodegradation of comfrey root extract. *12th probiotics, prebiotics & new food.* 2023, Rome, Italy.
- **Natalia Melnyk**, Dominik Popowski, Laura Peeters, Jakub Patryk Piwowarski, Sebastian Granica. Interaction of the extract from marigold flowers and comfrey root with human skin Microbiota (P-333). *70th International congress and annual meeting of the society for medical plant and natural product research (GA).* 2022, Thessaloniki, Greece.
- **Natalia Melnyk**, Maria Ziaja, Oleh Koshovyi, Sebastian Granica, Agnieszka Bazylko. High-performance thin-layer chromatography method for the analysis of lime flowers (Tiliae flos) preparations. *American Society of Pharmacognosy Meeting.* 2019, Madison, Wisconsin, USA.
- **Natalia Melnyk,** Oleh Koshovyi, Sebastian Granica, Agnieszka Bazylko. Comparison of solvent systems for qualitative analysis of Lime flowers of Tilia cordata using HPTLC. *Topical issues of new medicines development.* 2019, Kharkiv, Ukraine.

[1] The authors presenting the results are underlined.

- **Natalia Melnyk,** Oleh Koshovyi, Sebastian Granica, Agnieszka Bazylko. Development of qualitative analysis for lime flowers (Tiliae flos) using HPTLC. *Medical drugs for human, modern issues of pharmacotherapy, and prescription of medicine*. 2019, Kharkiv, Ukraine.
- **Natalia Melnyk,** Oleh Koshovyi, Sebastian Granica, Agnieszka Bazylko. Determination of solvents system for qualitative analysis of lime flower of Tilia cordata and Tilia platyphyllos using HPTLC methodology. 2019, *BaltPharm Forum*, Kaunas, Lithuania.

5.4. Scientific internships

- Participation in the Preludium Bis NAWA program, carried out at ART ARNm INSERM, Orleans, France, Feb – Aug 2025.
- Participation in the NAWA program "STER – Internationalization of Doctoral Schools," carried out at CBM (Centre de Biophysique Moléculaire), CNRS (Center National De La Recherche Scientifique), Orleans, France, Apr 13, 2023 – May 12, 2023.
- Participation in the Erasmus+ Program based on Higher Education Student and Staff Mobility, agreement 2018-2019, carried out at the Department of Pharmacognosy and Molecular Basis of Phytotherapy of the Medical University of Warsaw, December 3, 2018 – May 3, 2019.

5.5. Courses / Trainings

- Cell Signaling; Communication at the Molecular level, Udemy, online, Jan 20, 2026.
- The Comprehensive Course to Become a Professional in High Performance Liquid Chromatography (HPLC), Udemy, online, Dec 30, 2025.
- Writing in the Science, Stanford University, online, Dec 11, 2025.
- 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, offline, Feb 23, 2024.

- Basic Principles of Cell Signaling, KAIST (Korean Advanced Institute of Science and Technology, online, Apr 3, 2023.

5.6. Other significant information

- Preludium 24 project application, Jun 2025.

Title: *Amanita muscaria* as a controversial source of active lid molecules: From gut fermentation to immune and neuronal modulation.

- Program application L'Oréal-UNESCO for women in science, Mar 2025.
- Award of an increased doctoral scholarship granted to the top-performing PhD candidates in the Doctoral School in the academic years 2021/2022 and 2022/2023.

6. Introduction with justification of the research topic

6.1. Structure and functions of the skin

The skin represents the largest organ in the human body. It covers approximately 1.5-2 m^2 and accounts 3-5 kg of the mean individual weight [1]. It forms a dynamic interface between the internal milieu and external environment, providing many vital functions, including protective, sensory, metabolic, and immunological functions. Structurally, the skin is composed of three principal layers – the epidermis, dermis, and hypodermis (subcutis) – as well as its appendages (hair follicles, sweat glands, and sebaceous glands), each contributing unique functional properties [1,2].

The epidermis is the skin's outermost layer and is composed predominantly of keratinocytes (about 95%), which differentiate through the basal, spinous, granular, and cornified layers [3,4]. The remaining cells include melanocytes, Langerhans cells, and Merkel cells [5]. Melanocytes within the basal layer synthesize melanin and transfer it to neighboring keratinocytes, providing protection, while Langerhans cells and Merkel cells contribute to immune surveillance and mechanosensation, respectively [6,7]. Beneath the epidermis lies the dermo-epidermal junction, a thin but complex basement membrane zone that anchors the epidermis to the dermis. The dermis, varying in thickness from 0.5 to 5 mm, comprises a superficial papillary region rich in microvasculature and sensory receptors, and a deeper reticular layer dominated by collagen types I and III, elastic fibers, ground substance, and diverse resident cells, including fibroblasts, mast cells, and antigen-presenting cells [1,8]. This layer also contains lymphatic, vascular networks, and abundant sweat glands, predominantly eccrine glands responsible for thermoregulation. The deepest layer, the subcutis, consists of adipocyte lobules separated by fibrous septa and serves as the body's major fat reservoir, providing insulation, mechanical protection, and endocrine activity through hormones such as leptin involved in metabolic regulation [9].

Together, these components form an integrated system essential for maintaining homeostasis and ensuring survival. The skin performs numerous vital functions, including serving as a physical and chemical barrier against environmental

insults, mediating sensory perception, participating in metabolic processes, and regulating innate and adaptive immune responses.

6.2. The human skin as a complex ecosystem

The human skin is not composed solely of host-derived cells but also contains a diverse and abundant microbiota that constitutes an integral component of cutaneous biology. This skin-associated microbial ecosystem comprises bacteria, fungi, viruses, and microeukaryotes that are spatially distributed across distinct cutaneous niches [10]. The composition of the skin microbiota (SM) is shaped by local physicochemical factors, including moisture, sebum content, pH, temperature, and the presence of antimicrobial lipids and peptides, leading to characteristic microbial communities at sebaceous, moist, and dry skin sites [11].

Bacterial populations are largely composed of representatives of phyla *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*, with dominant genera differing according to anatomical site [12]. Sebum-rich areas are mainly colonized by *Propionibacterium* and *Staphylococcus*, moist regions are enriched in *Corynebacterium*, while dry sites exhibit higher bacterial diversity (**Fig. 1**) [11,13,14].

Beyond bacteria, the SM encompasses fungi, viruses, and arthropods [15]. Lipid-dependent fungi of the genus *Malassezia* prevail in sebaceous regions, whereas other areas of the skin host a broader range of fungal taxa. Arthropods such as *Demodex* mites are common residents of hair follicles and sebaceous glands. Although the skin virome remains less well characterized, metagenomic studies indicate substantial viral diversity, suggesting that viruses may also represent a regular component of the skin microbial ecosystem [10,13].

The composition of the SM differs markedly between individuals; however, within a given person, it remains relatively stable over time, reflecting a resilient and highly individualized microbial ecosystem. This temporal stability suggests the presence of regulatory mechanisms influenced by both host-related and environmental factors, which preserve microbial equilibrium while allowing adaptive responses to physiological changes and external challenges, thereby sustaining long-term skin homeostasis.

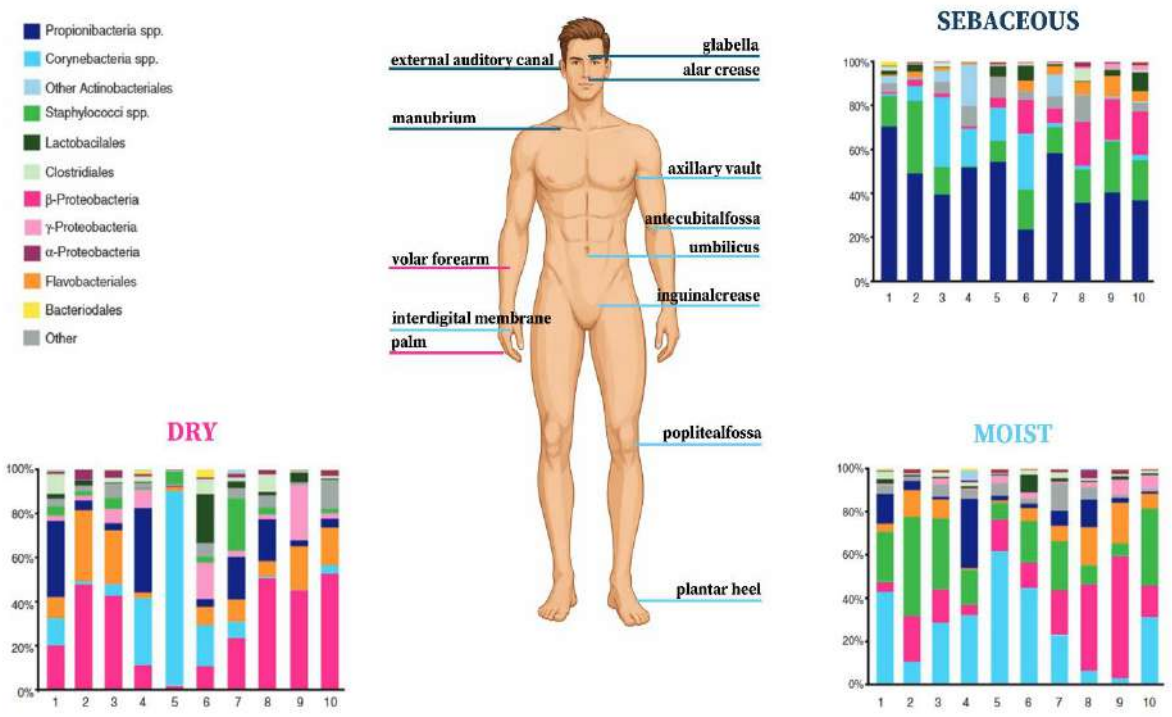


Fig. 1. Site-specific distribution of bacterial communities on human skin. Microbiome composition varies according to local skin microenvironments, with bacterial profiles shown for sebaceous (dark-blue), moist (blue), and dry (pink). Data from Crice et al.'s study [11].

6.3. Skin microbiota functions

Rather than acting as passive inhabitants, skin-resident microbes actively participate in skin functions, performing their functions through a dynamic network of interactions involving microbe-microbe, microbe-host, and environment-microbe relationships that collectively support cutaneous homeostasis [16].

Microbe-microbe interactions represent a fundamental function of the skin microbiome and play a crucial role in maintaining microbial balance on the skin surface. Commensal microorganisms compete with potential pathogens for nutrients, space, and adhesion sites, thereby limiting pathogen colonization [17]. In addition to direct competition, beneficial skin bacteria produce antimicrobial compounds, bacteriocins, and signaling molecules that inhibit the growth, virulence, and biofilm formation of pathogenic species. This ability is characteristic of Gram-positive bacteria such as *Lactococcus*, *Streptomyces,* and *Streptococcus* spp. [18].

Microbe-host interactions are essential for maintaining cutaneous homeostasis. Resident microorganisms engage in continuous bidirectional

communication with host skin cells, including keratinocytes and immune cells, through microbial-associated molecular patterns and secreted metabolites. These interactions activate innate defense mechanisms of the skin, among which antimicrobial peptides (AMPs) play a central role. AMPs, such as cathelicidin and β-defensins, are predominantly produced by keratinocytes [19–22]. On the other hand, SM actively influences host physiology by producing metabolites and signaling molecules that modulate epidermal differentiation, barrier integrity, and immune responses. Skin-resident microorganisms metabolize host-derived compounds, convert carbohydrates into lactic acids, breaking down sebum lipids into free fatty acids, and proteins into amino acids through their proteases [15,23,24]. These microbial activities regulate skin surface pH, lipid composition, and inflammatory signaling.

Moreover, the SM exerts profound immunomodulatory effects through direct molecular interactions with host immune and epithelial cells by pattern-recognition receptor-mediated signaling [17]. Commensal bacteria, particularly *Staphylococcus epidermis*, interact with keratinocytes via Toll-like receptor 2 (TLR2), leading to enhanced expression of antimicrobial peptides and increased resistance to pathogenic infections such as *Staphylococcus aureus* [25]. TLR2-dependent signaling from commensals not only promotes antimicrobial defense but also counteracts pathogen-induced suppression of immune pathways, including NF-κB signaling [26]. Beyond keratinocytes, microbe-derived signals influence other immune cell types, such as mast cells, enhancing their recruitment barrier integrity by regulating inflammatory response and strengthening tight junction function, thereby limiting excessive inflammation during tissue damage [25].

In addition to regulating local immune responses, the SM plays a fundamental role in immune education and establishment of immune tolerance, particularly during early life [12]. Microbial colonization of the skin contributes to the development of commensal-specific regulatory T-cells, which suppress excessive immune activation and prevent inappropriate inflammatory responses to resident microorganisms. Furthermore, microbiota-derived metabolites influence the differentiation and function of innate-like T-cell populations, such as natural killer T (NKT) cells and mucosal-associated invariant T (MAIT) cells, through presentation by

non-classical and pathogenic signals and establish long-lasting immune balance. Disruption of microbiota-driven immune education may impair tolerance mechanisms and predispose the skin to chronic inflammatory conditions [10,27].

Environment-microbe interactions consist of the fact that external factors such as temperature, humidity, ultraviolet radiation, hygiene practices, cosmetics, and topical therapeutics influence microbial composition by altering local physicochemical conditions, including pH, oxygen availability, and lipid content [27]. In turn, resident microorganisms adapt to these environmental pressures through metabolic activity and community restructuring, contributing to the resilience and stability of the skin microbiome.

In summary, the SM represents an integral component of the cutaneous barrier system, supporting skin protective function by acting simultaneously as a physical, chemical, and immunological barrier. Together, these interconnected functions highlight the SM as a dynamic and essential element of skin homeostasis and protection against environmental challenges.

6.4. Role of the human skin microbiota in skin health

The human SM plays a crucial role in maintaining skin health and is increasingly recognized as a key factor in dermatological conditions. Through continuous interactions with epidermal cells and the immune system, resident microorganisms contribute to barrier integrity, immune regulation, and protection against pathogenic colonization [28]. Alterations in microbiota composition or function (dysbiosis) have been associated with numerous dermatological conditions, including atopic dermatitis, acne, psoriasis, seborrheic dermatitis, and impaired wound healing [29].

6.5. Skin microbiota dysbiosis and its pathological consequences

Skin microbiota dysbiosis refers to qualitative and quantitative alterations in the composition and function of the skin microbial community resulting from the influence of intrinsic and extrinsic factors [30]. Such disturbances can compromise

colonization resistance, a key protective function of commensal microbiota that normally limits the establishment of pathogenic microorganisms. Although dysbiosis is consistently observed in various skin conditions, it remains unclear whether these microbial imbalances represent a primary causative factor or a secondary consequence of disease development [31]. Current research indicates that dysbiosis involves not only disrupted competition for nutrients and adhesion sites but also altered microbial signaling processes that influence host-microbe interactions.

In this context, modern intensified use of household detergents and antiseptics should be mentioned [10,32]. It represents an important external factor that may contribute to SM imbalance. Widespread and repeated topical application of these agents substantially reduces microbial exposure and directly affects the skin microbial community. The loss of effective colonization resistance during dysbiosis creates a permissive environment for pathogen overgrowth and contributes to disease onset and progression [33].

Atopic dermatitis (AD) is characterized by a profound dysbiosis of the **microbiota** of the skin that actively contributes to disease pathogenesis through intertwined effects on epidermal barrier integrity and immune regulation. During AD flares, reduced microbial diversity and overgrowth of *Staphylococcus aureus* dominate lesional skin, with *S. aureus* abundance strongly correlating with disease severity [34]. Mechanistically, *S. aureus* exploits the impaired barrier caused by filaggrin deficiency and elevated skin pH, which enhances bacterial adhesion and virulence factor expression. *S. aureus* secretes superantigens, proteases, phenol-soluble modulins, and exotoxins that directly disrupt tight junctions, degrade structural proteins such as filaggrin, and activate keratinocyte serine proteases, further weakening the barrier [35]. Concurrently, these microbial products stimulate innate immune receptors, including TLR receptor 2, and drive exaggerated Th2-skewed inflammation, characterized by increased IL-4, IL-13, and IL-22 signaling. Th2 cytokines suppress keratinocyte-derived antimicrobial peptides, including cathelicidin and β-defensins, creating a permissive environment for persistent microbial colonization [36]. Loss of protective commensals, particularly coagulase-negative *Staphylococci* that normally inhibit *S. aureus* via bacteriocins and immune-modulating signals, further amplifies dysbiosis [37]. In parallel, fungal components of the **microbiota**, especially *Malassezia*

species, contribute to AD pathogenesis by including IgE-mediated responses, mast cell activation, and cytokine release, particularly in the context of barrier disruption [38]. Together, these mechanisms establish a self-reinforcing cycle in which barrier defects, immune dysregulation, and microbial imbalance perpetuate chronic inflammation in atopic dermatitis.

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit in which alterations of the SM contribute to disease development through strain-level dysbiosis, microbial metabolic activity, and host immune activation rather than simple bacterial overgrowth [39,40]. Although *Cutibacterium acnes* is a dominant commensal of sebaceous follicles in both healthy and acne-prone skin, acne is associated with a loss of *C. acnes* strain diversity and enrichment of specific phylotypes with increased pro-inflammatory potential [41]. These acne-associated strains exhibit enhanced lipase, protease, and hyaluronidase activity, promoting sebum hydrolysis into free fatty acids that disrupt follicular keratinocyte differentiation and exacerbate comedone formation. *C. acnes* also activate innate immune pathways via TLR 2 on keratinocytes, sebocytes, and immune cells, including the release of IL-1β, IL-8, TNF-α, and AMPs, thereby driving neutrophil recruitment and follicular inflammation [39,42–44]. Biofilm formation by *C. acnes* within the follicular canal further enhances bacterial persistence, increases antibiotic tolerance, and promotes **microbiota** stability. In parallel, dysregulated interactions between *C. acnes* and other skin commensals, including *Staphylococcus epidermis* and *Corynebacterium* species, alter competitive and quorum-sensing networks that normally restrain inflammation [39]. These mechanisms indicate that acne pathogenesis arises from a complex interplay between sebaceous microenvironmental changes, strain-specific microbial virulence, biofilm-mediated persistence, and dysregulated host-microbe immune crosstalk, rather than from simple bacterial colonization alone.

Psoriasis is a chronic immune-mediated inflammatory skin disease in which cutaneous microbiome dysbiosis contributes to disease initiation and persistence by amplifying aberrant keratinocyte-immune cell crosstalk rather than acting as a single causative trigger [45,46]. Lesional psoriatic skin is characterized by reduced microbial diversity and reproducible shift in bacterial composition, including enrichment of *Firmicutes* and *Proteobacteria* and depletion of *Actinobacteria*, particularly

Cutibacterium and *Propionibacterium* species that are abundant in healthy skin [46]. This dysbiosis leads to lower colonization resistance and alters microbial-derived immune signals at the epidermal interface. Certain bacterial taxa, especially *Streptococcus* spp., have been implicated in psoriasis through molecular mimicry mechanisms, whereby streptococcal M proteins and superantigens activate autoreactive T cells that cross-react with keratinocytes' antigens, promoting Th1 and Th17 polarization [47,48]. In addition, microbial components and metabolites stimulate pattern-recognition receptors on keratinocytes and antigen-presenting cells, enhancing IL-23 and IL-17 axis activation, a central pathogenic pathway in psoriasis [47–49]. Keratinocyte hyperproliferation and impaired differentiation further modify the cutaneous microenvironment by altering lipid composition, pH, and AMP's expression, reinforcing dysbiosis and sustaining inflammation. Fungal members of the SM, particularly *Malassezia* species, also contribute to psoriatic inflammation by inducing keratinocyte cytokine release, IgG- and IgE-mediated immune responses, and heat-shock protein expression, thereby enhancing immune cell recruitment and epidermal thickening [45]. Collectively, these findings support a model in which psoriasis-associated **microbiota** alterations act as immune modulators that perpetuate chronic inflammation.

Rosacea is a chronic inflammatory dermatosis in which alterations of the SM contribute to disease expression primarily by amplifying innate immune dysregulation and neurovascular reactivity rather than through overt infection. Although the overall composition of the facial SM in rosacea partially overlaps with that of healthy skin, rosacea is associated with subtype-, age, and severity-dependent shifts in microbial community structure, including altered abundance of *Staphylococcus epidermis, Cutibacterium acnes*, *Corynebacterium* species, and *Proteobacteria* [50,51]. A hallmark of rosacea pathophysiology is exaggerated innate immune sensing, characterized by increased expression of TLR2 on keratinocytes, which enhances kallikrein 5 activity and adherent processing of cathelicidin into pro-inflammatory peptides. Microbial components derived from skin commensals and *Demodex*-associated bacteria, including *Bacillus oleronius*, are thought to act as persistent immune stimuli that activate TLR-mediated pathways, promoting cytokine release, mast cell activation, angiogenesis, and vasodilatation [52]. *Demodex*

folliculorum density is increased in many rosacea patients and may function as both a mechanical disruptor of the folliculorum unit and a vector facilitating microbial antigen delivery to deeper skin layers, thereby intensifying inflammatory response [52]. In parallel, environmental factors such as increased skin temperature and altered lipid composition influence microbial metabolism and virulence, particularly enhancing *S. epidermis* protein expression and inflammatory potential.

Seborrheic dermatitis (SD) is a chronic inflammatory dermatosis of sebaceous-gland-rich skin in which dysregulated interactions between the SM, sebum metabolism, epidermal barrier function, and host immune responses drive disease expression rather than simple microbial overgrowth [53–55]. Although *Malassezia* species, particularly *Malassezia restricta* and *Malassezia globosa*, are consistently enriched in lesional skin, their pathogenicity appears to depend on host susceptibility and microenvironmental conditions [54,56]. *Malassezia* species secrete lipases that hydrolyze sebum triglycerides into free fatty acids, including oleic and arachidonic acids, which disrupt keratinocyte differentiation, impair barrier integrity, and induce inflammatory signaling. These lipid metabolites activate immune pathways via pattern-recognition receptors, inflammasome signaling, and NF-κB-dependent cytokine production, leading to increased release of IL-1β, IL-6, IL-8, TNF-α, and IL-17-associated responses [53]. Also, bacterial dysbiosis accompanies fungal alterations, with lesional SD skin showing increased abundance of *Staphylococcus* species and reduced *Cutibacterium* and *Staphylococcus* ratio, a shift associated with elevated transepidermal water loss, higher skin pH, and compromised barrier function. Bacterial and fungal communities may synergistically contribute to sebum degradation and inflammatory amplification, reinforcing epidermal turnover and scaling [54,57].

Cutaneous **wound healing** is a highly coordinated, multistage process that is tightly regulated by dynamic interactions between host cells and SM, with microbial composition and activity critically influencing inflammatory resolution, tissue repair, and barrier restoration. In acute wounds, commensal skin bacteria, particularly coagulase-negative *Staphylococci* such as *Staphylococcus epidermis,* actively promote healing by modulating immune responses, enhancing keratinocyte migration, and stimulating AMP production through TLR-dependent signaling [58–60]. These

commensals also suppress pathogenic colonization via bacteriocin secretion, quorum-sensing interference, and maintenance of an acidic microenvironment, thereby limiting excessive inflammation. In contrast, chronic non-healing wounds are characterized by microbial dysbiosis, reduced bacterial diversity, and enrichment of pathogenic taxa, including *S.aureus* and *Pseudomonas aeruginosa*, which form polymicrobial biofilms that confer resistance to host immunity and antimicrobial therapy [58–60]. Pathogen-derived virulence factors, endotoxins, and proteases induce persistent activation of inflammatory pathways, excessive matrix metalloproteinase activity, and degradation of extracellular matrix components, collectively impairing re-epithelialization, angiogenesis, and fibroblast function. Furthermore, intracellular persistence of bacteria within keratinocytes and immune cells enables immune evasion and sustains chronic inflammation. Longitudinal studies indicate that wounds with great microbial diversity and dynamic microbial turnover exhibit improved healing outcomes, whereas stable, low-diversity microbial communities are associated with delayed repair [61].

6.6. Medicinal plants traditionally used in the treatment of skin conditions

Medicinal plants have played a central role in the treatment of skin conditions across cultures since antiquity and remain widely used in traditional and complementary medicine [62]. Ancient medical texts and ethnobotanical records describe the use of plant-based preparations – infusions, decoctions, poultices, ointments, and oils – for managing wounds, burns, infections, inflammatory dermatoses, and chronic skin disorders [63]. The popularity of these remedies was largely based on their observed therapeutic effects, including anti-inflammatory, antimicrobial, wound-healing, soothing, and protective actions on the skin. Many traditionally used plants were applied to reduce erythema, alleviate pruritus, accelerate tissue regeneration, and prevent infection, reflecting a holistic approach to skin care [64].

In modern dermatology and cosmetic science, medical plants continue to be widely used, with their applications increasingly supported by pharmaceutical and

clinical research. Plant extracts and isolated phytochemicals are incorporated into topical formulations, dermocosmetics, and adjunct therapies for inflammatory skin diseases, wound healing, acne, and skin barrier repair [65,66]. Contemporary use emphasizes standardized extracts, controlled dosing, and well-defined mechanisms of action, such as inflammatory signaling, antioxidant protection, regulation of keratinocytes and fibroblasts activity, and enhancement of tissue regeneration.

The long-standing and modern widespread use of medicinal plants in dermatological treatments underscores a valuable foundation for further scientific investigations of their biological activities.

6.7. Current experimental approaches to studying skin microbiota – plant extracts interactions

Current research on skin microbiota–plant extracts interactions is largely focused on evaluating the antimicrobial properties of natural products or their direct effects on skin cells, frequently overlooking microbiota-mediated processes. Experimental models employed in this field include *in vitro*, *ex vivo*, and *in vivo* systems, which differ substantially in their complexity, physiological relevance, and translational value, each presenting specific methodological limitation [28,67].

In vitro models offer high experimental control but fail to reproduce the complexity of the skin ecosystem, whereas *in vivo* approaches provide physiological relevance but are constrained by ethical considerations and interspecies differences. *Ex vivo* models represent an intermediate approach but remain underutilized [67].

Overall, there is a lack of standardized experimental frameworks capable of simultaneously addressing microbial metabolism, host cellular response, and chemical transformations of plant-derived compounds, highlighting a critical gap in current dermatological and pharmacognostic research [10].

6.8. Underexplored aspect: skin microbiota-mediated metabolism of plant-derived compounds

In recent years, increasing attention has been devoted to the role of the microbiota – predominantly the gut microbiota – in the biotransformation of ingested substances, and extensive evidence has demonstrated its impact on the metabolism and biological activity of natural compounds [68–72]. Although scientific databases are rich in studies addressing gut microbiota-mediated biotransformation, considerably less is known about the influence of the skin microbiota on the chemical fate of topically applied preparations (**Fig.2**).

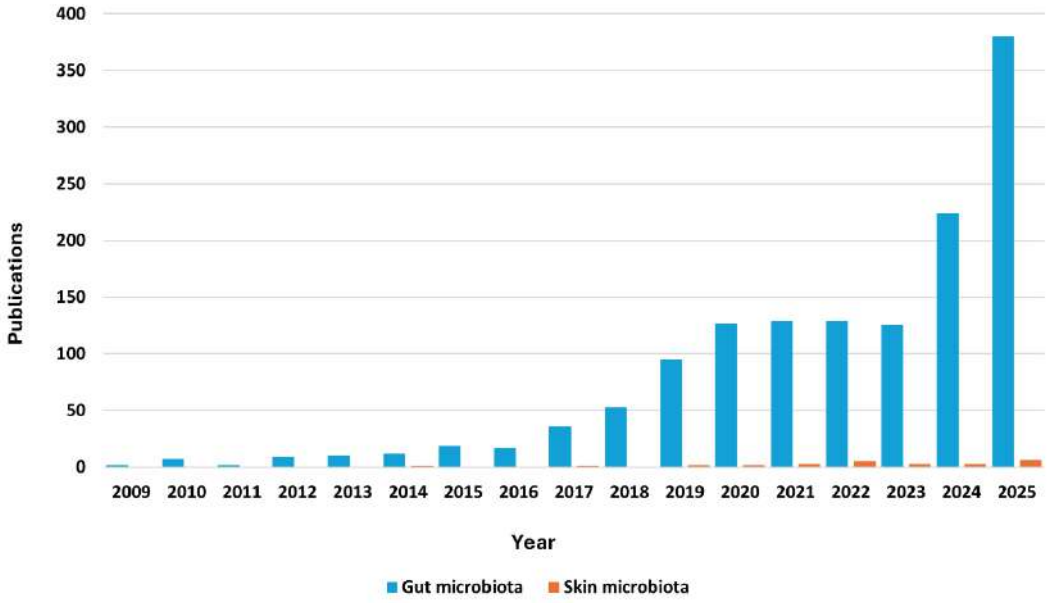


Fig. 2. Quantitative comparison of literature addressing microbiota-mediated metabolism and biotransformation of plant-derived compounds in the gut versus the skin, based on predefined Scopus search queries. The search query for gut microbiota was ("gut microbiota" AND ("metabolism" OR "biotransformation") AND "plant extract", while the search query for skin microbiota was ("skin microbiota" AND ("metabolism" OR "biotransformation") AND "plant extract").

Nevertheless, in recent years, increasing scientific attention has been directed toward the interactions between plant extracts and the skin microbiota. This growing interest is reflected in the rising number of publications addressing skin microbiota and plant-derived compounds, as illustrated in **Figure 3**.

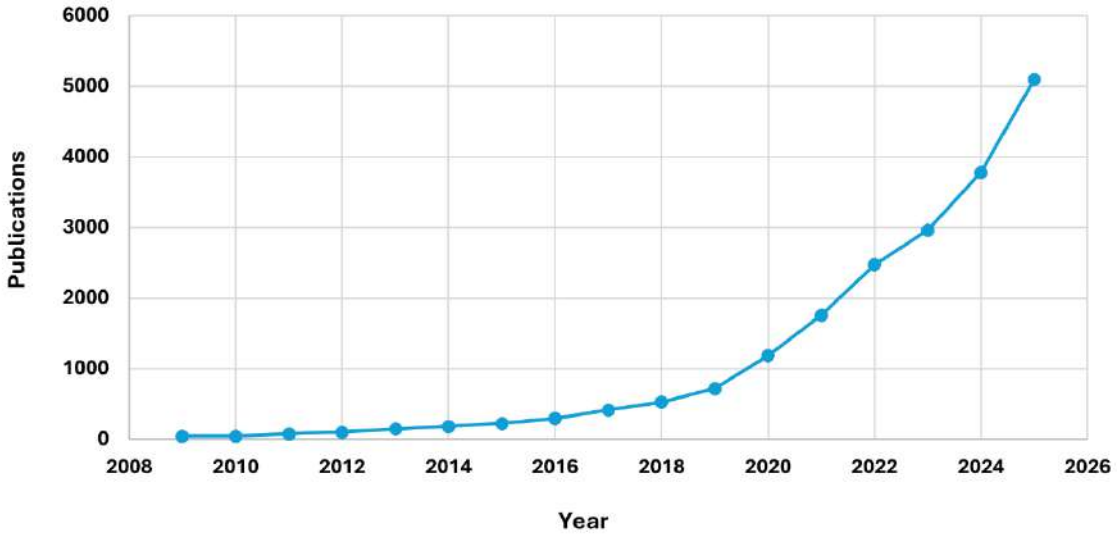


Fig. 3. Trends in scientific publications addressing extract-skin microbiota interactions over the last 15 years. A graph was created based on the Scopus database, using keywords such as plant extract, microbiota, and skin.

Despite this upward trend, the field remains at an early stage of development, and mechanistic studies exploring microbiota-mediated metabolism on the skin are still scarce. Moreover, most of these publications focus on the influence of plant-derived materials on skin composition, without considering how their chemical constituents may be transformed by skin microbiota [73]. Given that the mechanisms underlying the pharmacological effects of many plant extracts remain incompletely understood, it has been hypothesized that interactions between their chemical components and the human skin microbiota may contribute to their therapeutic effects in skin conditions.

In the context of skin diseases, this knowledge gap is particularly relevant, as the majority of medicinal plant preparations are applied topically, directly interacting with both skin cells and resident microorganisms. Therefore, understanding microbiota-mediated processes is essential for the rational evaluation of the efficacy, safety, and stability of plant-based dermatological products.

These considerations highlight the need for experimental models capable of capturing microbiota-driven chemical transformations occurring directly on the skin surface.

7. Aim of the thesis

The study aimed to investigate the interactions between plant extracts traditionally used in the treatment of skin conditions and skin microbiota, as well as their biological effects in skin cells. The specific objectives were as follows:

1. Analysis of the pharmaceutical market for plant-based materials traditionally applied in skin disorders treatments.
2. Selection of three plant materials with high traditional relevance and diverse chemical components.
3. Extraction of plant materials and characterization of their chemical profiles by UHPLC-DAD-MS^n.
4. Studies on the interaction of the chosen extracts with the human skin microbiota *ex vivo*.
    4.1. Evaluation of the extracts' influence on skin microbiota composition by 16S rDNA sequencing.
    4.2. Evaluation of microbiota-driven transformations of the chemical constituents of the extracts.
5. *In vitro* studies of the effects of extracts and skin microbiota metabolites (SMM) on skin cells (keratinocytes and fibroblasts).
    5.1. Assessment of cell proliferation and viability assays
    5.2. Evaluation of the modulatory effects of the extracts and SMM on the inflammatory response, measured as IL-6 and IL-8 secretion in:
        - Keratinocytes stimulated with TNF-α/IFN-γ
        - Fibroblasts stimulated with *S. aureus* LTA

8. Comments on publications

8.1. Publication No. 1

Melnyk N., Vlasova I., Skowrońska W., Bazylko A., Piwowarski J., Granica S.

Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota.

International Journal of Molecular Sciences. 2022, Vol. 23, No. 17, pp. 1-28.

DOI: 10.3390/ijms23179644.

Publication No. 1 is a review paper that presents the first comprehensive review of the current scientific knowledge on the interactions between plant materials traditionally used in dermatology in Poland and Ukraine and the human skin microbiota. This review integrates data retrieved through extensive searches of PubMed, Scopus, and Wiley Online Library, combined with an analysis of pharmaceutical products available in Poland and Ukraine. It provides an updated synthesis of chemical components, pharmacological properties, and traditional uses of twenty-six medicinal plants widely applied in skin inflammation, wounds, burns, and infectious conditions. Furthermore, it outlines known antibacterial, anti-inflammatory, antioxidant, wound-healing, and immunomodulatory activities, supported by *in vitro* and *in vivo* studies for such plants as:

1. *Allium cepa* L. - onion bulbs;
2. *Aloe vera* L. - aloes leaves;
3. *Arnica montana* L. - arnica flowers;
4. *Calendula officinalis* L. - marigold flowers;
5. *Chelidonium majus* L. - greater celandine herb;
6. *Hamamelis virginiana* L. - witch hazel leaves and bark;
7. *Hippophae rhamnoides* L. - sea-buckthorn fruits;
8. *Linum usitatissimum* L. – linseed;
9. *Matricaria chamomilla* L. - chamomile flowers;
10. *Potentilla erecta* L. - tormentil root;
11. *Quercus robur* L. - common oak bark;
12. *Salvia officinalis* L. - sage leaf;

13. *Sophora japonicum* L. - Japanese pagoda tree fruits ;

14. *Symphytum officinale* L. - comfrey root.

Importantly, this publication highlights a major research gap: although many studies describe how plant extracts influence keratinocytes, fibroblasts, cytokine production, wound healing, and inflammation, very limited evidence exists on how these plant materials interact with skin microbiota or how skin microorganisms metabolize compounds delivered topically. Only limited reports, such as the inhibitory effects of some plant materials on specific bacteria, suggest that some traditional remedies may act as prebiotics or modulators of the microbiota, yet systematic studies are largely missing. The review, therefore, emphasizes the need for targeted investigations into microbial biotransformation of plant compounds and the biological activity of potential postbiotic metabolites formed on the skin surface.

In addition to summarizing existing data, the article identifies specific methodological challenges in the field, such as the scarcity of validated models for studying plant-microbiota interactions and the lack of data on metabolic transformations by skin microbiota (compared to the gut microbiota). By comparing traditional plant use with modern biochemical and microbiological evidence, the review provides a framework for designing future studies that address these gaps and incorporate microbiota-focused endpoints.

Overall, this work lays the foundation for an emerging research direction that integrates phytotherapy with skin microbiota science, suggesting that understanding microbial metabolism of topical plant preparations may uncover new bioactive molecules, explain therapeutic effects observed in folk medicine, and support the development of more effective and microbiota-friendly dermatological products.

The analysis of the pharmaceutical market for topical products containing plant-derived ingredients traditionally used for dermatological indications served as the basis for selecting plant materials for subsequent studies. By examining product composition, declared therapeutic uses, and frequency of occurrence across both the Polish and Ukrainian markets, the review identified plant materials most utilized in modern topical formulations. **These selected plants included comfrey roots, marigold flowers, and chamomile flowers.**

8.2. Publication No. 2

Melnyk N., Popowski D., Strawa J., Przygodzińska K., Tomczyk M., Piwowarski J., Granica S.

Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)

Journal of Ethnopharmacology. 2024, Vol. 318, No. Pt B, pp. 1–10.

DOI: 10.1016/j.jep.2023.116968.

Publication No. 2 presents the first *ex vivo* investigation of how human skin microbiota metabolizes natural products contained in *Symphytum officinale* (comfrey) root extract (**Fig. 4**). Although comfrey is a widely used traditional medicinal plant with well-documented topical application for wound healing, inflammatory conditions, contusions, and musculoskeletal pain, little was previously known about how its bioactive compounds interact with microorganisms inhabiting human skin. This study addresses an important research gap by analyzing not only the chemical transformations mediated by skin microbiota but also the impact of the extract on the microbial community itself.

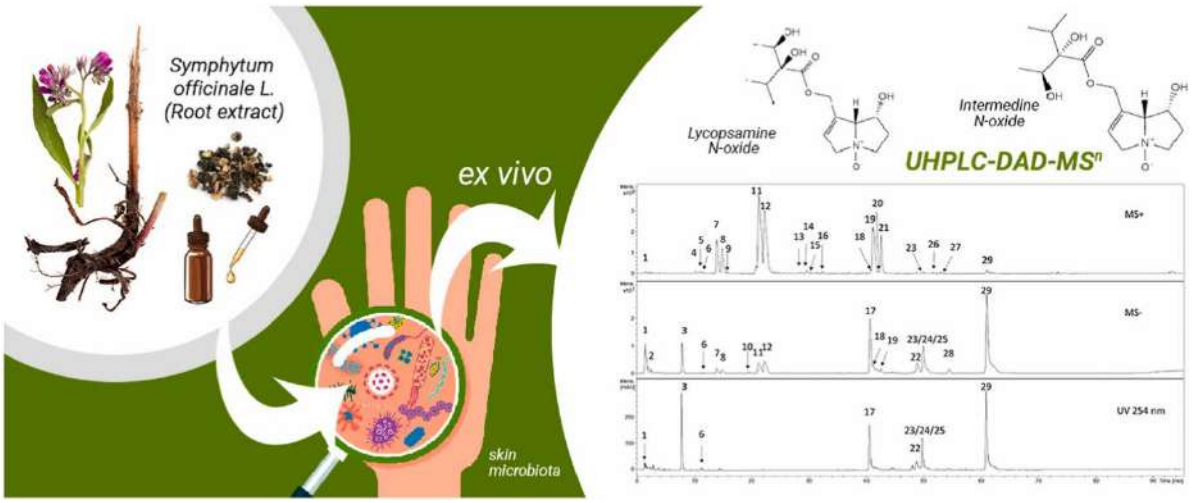


Fig. 4. Graphical abstract of publication No. 2.

Using UHPLC-DAD-MS^n and LC–DAD–ESI–MS/TOF profiling, the paper characterizes twenty-nine constituents of comfrey extract, including phenolic acids, lignans, and a rich spectrum of pyrrolizidine alkaloids and their N-oxide derivatives. The *ex vivo* incubation of these extracts with human skin microbiota from ten healthy donors revealed a microbiota-driven biodegradation pathway largely focused on pyrrolizidine alkaloid derivatives. Specifically, the microbiota catalyzed deacetylation

and deesterification reactions, converting 7-acetylintermedine N-oxide, 7-acetyllycopsamine N-oxide, symphytine N-oxide, and symladine N-oxide into their corresponding intermedine and lycopsamine N-oxides (**Fig. 5**). Notably, none of these transformations led to the formation of free pyrrolizidine alkaloids, which are known to be associated with systemic toxicity. This finding is crucial for the safety evaluation of topical comfrey preparations and supports the hypothesis that intermittent dermal use does not pose a substantial toxicological risk.

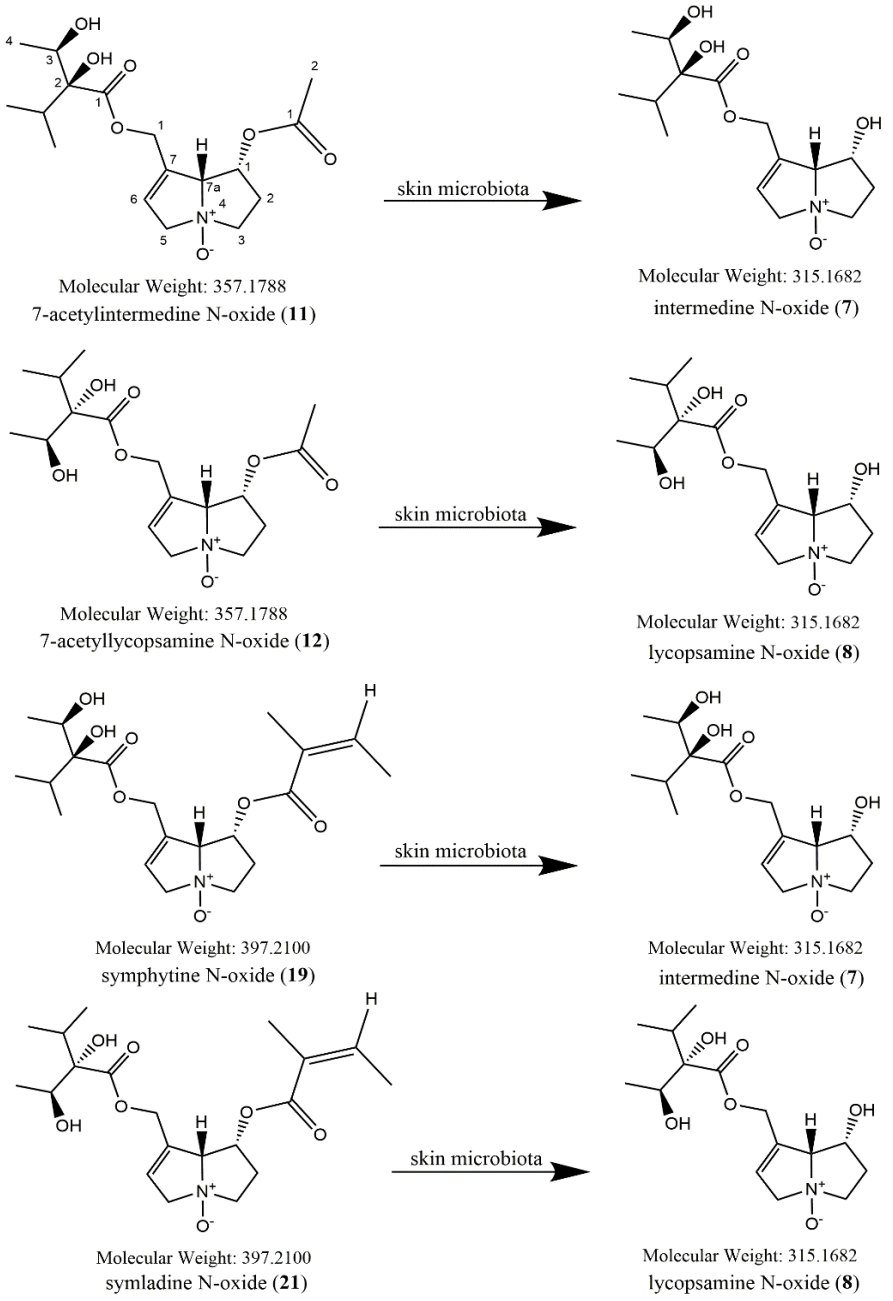


Fig. 5. Biotransformation of comfrey root extract constituents by the human skin microbiota. 7, 8, 11, 12, 19, and 21 – peak numbers on the chromatograms.

The second key component of this publication involves an analysis of skin microbiota composition using 16S rDNA amplicon sequencing. The study demonstrates that comfrey extract does not induce dysbiosis in *ex vivo* cultures: alpha-diversity indices remained stable, and no major disruptions of bacterial community structure were observed after exposure to the extract. However, subtle shifts in the abundance of several families, such as *Staphylococcaceae, Enterococcaceae, Bacillaceae,* and *Micrococcaeae,* were detected, and interpersonal variability among donors was shown to influence differences in metabolic outcomes meaningfully. This underscores the importance of considering donor-specific microbiota architecture when evaluating topically applied natural products.

Overall, this publication introduces an innovative methodological framework for studying topical phytotherapeutics in the context of the skin microbiota. As one of the earliest systematic investigations in this field, it expands the scientific basis for evaluating traditional herbal products within the context of the skin microbiota and clarifies how natural compounds behave after their application to the skin surface.

8.3. Publication No.3

Melnyk N., Skowrońska W., Popowski D., Piwowarski J., Granica S.

From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin.

Prospects in Pharmaceutical Sciences. 2025, pp.1-15.

DOI: 10.56782/pps.792.

Publication No. 3 delivers the integrated experimental evaluations of *Calendula officinalis* and *Matricaria recutita* extracts, combining phytochemical profiling, *ex vivo* skin microbiota analysis, and cellular anti-inflammatory assays (**Fig. 6**). A key strength of the study is the demonstration that both extracts remain chemically stable when exposed to human skin microbiota, with no formation of new metabolites. This finding provides important methodological value: it confirms that their biological effects under tested conditions originate directly from native phytochemicals rather than microbiota-derived transformation products.

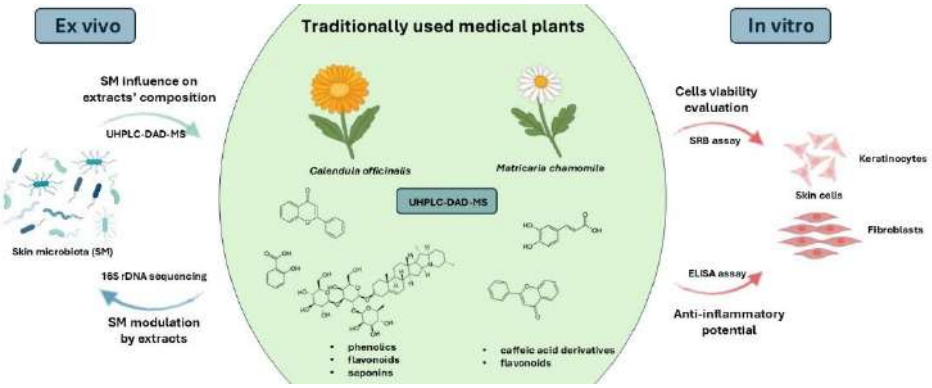


Fig. 6. Graphical abstract of publication No. 3 (not published).

The work also established clear evidence of selective microbiota modulation. Both extracts preserved overall community complexity while shifting the relative abundance of specific bacterial families. Particularly relevant is the observed decrease in taxa commonly linked with inflammatory skin states (*Staphylococcaceae, Enterococcaceae, Corynebacteriaceae*) and the parallel increase in *Bacillales*, groups potentially associated with protective or stabilizing functions. Such selective rebalancing supports the concept that traditional topical herbs may contribute to skin homeostasis not through antimicrobial suppression, but via gentle ecological modulation.

Equally important are the cellular findings, which confirm that both extracts are biocompatible with keratinocytes and fibroblasts at concentrations relevant to topical use. The extracts effectively attenuated LTA-induced cytokine release in fibroblasts, and chamomile extract also demonstrated a clear capacity to reduce IL-6 secretion in keratinocytes. Together, these results provide mechanistic evidence for their traditional anti-inflammatory use.

Overall, the publication establishes a mechanistic basis for the topical effectiveness of *Calendula officinalis* and *Matricaria recutita*, showing that they maintain chemical stability on the skin surface, modulate microbiota composition in a selective and non-disruptive manner, and exert direct anti-inflammatory effects on skin cells. This multidimensional dataset significantly strengthens the scientific validation of two widely used ethnopharmacological species.

9. Summary and Conclusions

The research encompassed in the three presented publications provides a comprehensive and multidisciplinary analysis of the interactions between traditionally used topical herbal preparations, their chemical constituents, and the human skin microbiota. Together, these works create an integrated scientific framework that connects ethnopharmacology knowledge with modern analytical chemistry, microbiology, and cell-based assays, thereby strengthening the evidence base for the topical use of selected medicinal plants.

This dissertation highlights several important contributions:

1. **Identification of widely used medicinal plants for dermatological applications** through systematic analysis of pharmaceutical markets in two countries, ensuring that subsequent laboratory studies reflect real-world therapeutic use.
2. **Establishment of the selective influence of skin microbiota on the chemical components of plant extracts *Symphytum officinale*, *Calendula officinalis,* and *Matricaria recutita,*** demonstrating that **microbial transformation is compound-dependent**: pyrrolizidine N–oxides underwent measurable biodegradation, whereas saponins, flavonoids, and phenolic acids remained chemically stable under the tested *ex vivo* conditions. This distinction provides important insight into the chemical stability of topical preparations, supports more accurate safety assessment, and improves the prediction of how different phytochemicals behave on the skin surface following application.
3. **Assessment of microbiota-compatible properties of traditional herb extracts**, showing that the studied preparations do not disrupt the overall microbial balance and preserve key diversity parameters of the skin microbiota. The *Symphytum officinale*, *Calendula officinalis,* and *Matricaria recutita* extracts induced only selective and non-dysbiotic shifts in the relative abundance of certain bacterial taxa, indicating that they act in a manner consistent with maintaining stability on the skin surface. **These findings**

highlight the potential of such preparations to support skin homeostasis without compromising the integrity of the resident microbial community.

4. **Mechanistic confirmation of anti-inflammatory activity**, linking traditional applications with experimentally validated cellular effects. The studies demonstrated that **the extracts effectively reduced pro-inflammatory cytokine release in keratinocytes and fibroblasts**, establishing a clear biological rationale for their long-standing ethnopharmacological use in inflammatory skin conditions.

In summary, the presented research creates a coherent scientific foundation for understanding the chemical behavior of microbiota interactions and the biological effects of traditional herbal preparations used on the skin. It introduces validated experimental tools, generates novel data on skin microbiota biotransformation, and supports the rational and evidence-based use of comfrey root, marigold flower, and chamomile flower in dermatology.

Collectively, these studies provide a scientific basis for the development of microbiota-focused topical formulations and reinforce the relevance of pharmacognosy within contemporary integrative dermatological research.

In addition, it should be emphasized that the overall scope of experimental work conducted within this doctoral project extends beyond the results presented in this dissertation. *Symphytum officinale* (comfrey) was selected as a model plant, as it demonstrated clear susceptibility to skin microbiota-driven biotransformation, as well as anti-inflammatory properties. These characteristics rendered comfrey root a suitable and rational candidate for further mechanistic investigations. Accordingly, additional studies were undertaken, including co-culture experiments involving keratinocytes and fibroblasts, as well as analyses of the modulation of key intracellular signaling pathways, particularly of MAPK and NF-κB. Furthermore, selected individual compounds were isolated from the comfrey root extract and are being subjected to subsequent investigations to elucidate their specific contribution to the observed anti-inflammatory effects. The results of these extended studies are

intended to form the basis for a separate future scientific publication, independent of the present dissertation.

Bibliography

[1] McGrath J.A., Uitto J. Structure and Function of the Skin. In *Rook's Textbook of Dermatology;* Barker J, Griffiths C, Bleiker T, et al., **2016.**

[2] Gilaberte Y., Prieto-Torres L., Pastushenko I., et al. In *Anatomy and Function of the Skin,* Hamblin M, Avci P, Prow T; Elsevier Inc.; **2016.**

[3] Lai-cheong J.E., McGrath J.A.. Structure and Function of Skin, Hair, and Nails. *Medicine* **2021**, *49*, 337–342. doi:10.1016/j.mpmed.2021.03.001

[4] Celleno L., Tamburi F. Structure and Function of the Skin. In *Nutritional Cosmetics*; Tabor A, Blair R; William Andrew, **2009**; 3–45.

[5] Maksimovic S., Nakatani M., Baba Y., et al. Epidermal Merkel cells are mechanosensory cells that tune mammalian touch receptors. *Nature* **2014**, *000*, 1–5. doi:10.1038/nature13250

[6] Chopin M., Nutt S.L. Establishing and Maintaining the Langerhans Cell Network. *Semin Cell Dev Biol* **2015**; *41*, 23–29. doi:10.1016/j.semcdb.2014.02.001

[7] Lang D., Mascarenhas J.B., Shea C.R. Melanocytes, Melanocyte Stem Cells, and Melanoma Stem Cells. *Clin Dermatol* **2013**; *31*, 166–178. doi:10.1016/j.clindermatol.2012.08.014

[8] Nyström A., Velati D., Mittapalli V.R., et al. Collagen VII Plays a Dual Role in Wound Healing. *J Clin Invest* **2013**; *123*, 3498–3509. doi:10.1172/JCI68127DS1

[9] Tay S.S., Roediger B., Tong P.L., et al. The Skin-Resident Immune Network. *Curr Derm Rep* **2014**; *3*, 13–22. doi:10.1007/s13671-013-0063-9

[10] Suri H., Suri H., Nagda N., et al. Current Perspectives on the Human Skin Microbiome : Functional Insights and Strategies for Therapeutic Modulation. *Biomed & Pharmacotherapy* **2025**; *193*: 118655. doi:10.1016/j.biopha.2025.118655

[11] Grice E.A., Kong H.H., Conlan S., et al. Topographical and Temporal Diversity of the Human Skin Microbiome. *Science* **2009**; *324*, 1190–1192. doi:10.1126/science.1171700

[12] Adamczyk K., Garncarczyk A.A. The Microbiome of the Skin. *Dermatol Rev/Przegl Dermatol* **2018**, 2018; *105*, 285–297. doi:https://doi.org/10.5114/dr.2018.75584

[13] Schommer N.N., Gallo R.L. Structure and Function of the Human Skin Microbiome. *Trends Microbiol* **2013**; *21*, 660–668. doi:10.1016/j.tim.2013.10.001

[14] Chen H., Zhao Q., Zhong Q., et al. Skin Microbiome, Metabolome and Skin Phenome, from the Perspectives of Skin as an Ecosystem. *Phenomics* **2022**; *2*, 363–382. doi:10.1007/s43657-022-00073-y

[15] Byrd A.L., Belkaid Y., Segre J.A. The Human Skin Microbiome. *Nat Publ Gr* **2018**; *16*, 143–155. doi:10.1038/nrmicro.2017.157

[16] Chen Y., Yao Z., Ruan Z., et al. The Role and Prospects of Skin Microbiota in Dermatosis. *Ther Adv Infect Dis* **2025**; *12*, 1–13. doi:10.1177/20499361251333562

[17] Sanford J.A., Gallo R.L. Functions of the Skin Microbiota in Health and Disease. *Semin Immuno*l **2014**; *25*,370–377. doi:10.1016/j.smim.2013.09.005

[18] Gallo R.L., Hooper L.V. Epithelial Antimicrobial Defense of the Skin and Intestine. *Nat Rev Immunol* **2013**; *12,* 503–516. doi:10.1038/nri322

[19] Dorschner R.A., Pestonjamasp V.K., Tamakuwala S., et al. Cutaneous Injury Induces the Release of Cathelicidin Anti-Microbial Peptides Active Against Group A *Streptococcus*. *J Invest Dermatol* **2001**; *117*, 91–97. doi:10.1046/j.1523-1747.2001.01340.x

[20] Emelianov V.U., Bechara F.G., Glaser R., et al. Immunohistological Pointers to a Possible Role for Excessive Cathelicidin (LL- 37) Expression by Apocrine Sweat Glands in the Pathogenesis of *Hidradenitis suppurativa/Acne inversa*. *Br J Dermatol* **2012**; *166*, 1023–1035. doi:10.1111/j.1365-2133.2011.10765.x

[21] Nilsson M.F., Sandstedt B., Sørensen O.L.E., et al. The Human Cationic Antimicrobial Protein (hCAP18), a Peptide Antibiotic, Is Widely Expressed in Human Squamous Epithelia and Colocalizes with Interleukin-6. *Infect Immun* **1999**; *67*, 2561–2566. doi:10.1128/IAI.67.5.2561-2566.1999

[22] Frohm M., Agerberth B., Ahangari G., et al. The Expression of the Gene Coding for the Antibacterial Peptide LL-37 Is Induced in Human Keratinocytes during Inflammatory Disorders *. *J Biol Chem* **1997**; *272*, 15258–15263. doi:10.1074/jbc.272.24.15258

[23] Holland K.T., Greenman J. Growth of Cutaneous *Propionibacteria* on Synthetic Medium ; Growth Yields and Exoenzyme Production. *J of Ap Bact* **1979**; *47*, 383–394. doi:10.1111/j.1365-2672.1979.tb01198.x

[24] Ong J.S., Taylor T.D., Yong C.C., et al. *Lactobacillus plantarum* USM8613 Aids in Wound Healing and Suppresses *Staphylococcus aureus* Infection at Wound Sites. *Probiotics Antimicrob Proteins* **2020**; *12*, 125-137. doi:https://doi.org/10.1007/s12602-018-9505-9

[25] Yuki T., Yoshida H., Akazawa Y., et al. Activation of TLR2 Enhances Tight Junction Barrier in Epidermal Keratinocytes. *J Immunol* **2011**; *187,* 3230–3237.

doi:10.4049/jimmunol.1100058

[26] Lai Y., Cogen A.L., Radek K.A., et al. Activation of TLR2 by a Small Molecule Produced by *Staphylococcus epidermidis* Increases Antimicrobial Defense against Bacterial Skin Infections. *J Invest Dermatol* **2010**; *130*, 2211–2221. doi:10.1038/jid.2010.123.

[27] Liu Q., Ranallo R., Rios C., et al. Crosstalk Between Skin Microbiota and Immune System in Health and Disease. *Nat Immunol* **2023**; *24*, 895–898. doi:10.1038/s41590-023-01500-6.

[28] Yang Y., Qu L., Mijakovic I., et al. Advances in the Human Skin Microbiota and Its Roles in Cutaneous Diseases. *Microb Cell Fact* **2022**; *21*: 1–14. doi:10.1186/s12934-022-01901-6

[29] Khan A., Koh Y.-S. The Role of Skin Microbiome in Human Health and Diseases. *J Bacteriol Virol* **2024**; 54: 191–202. doi:10.4167/jbv.2024.54.3.191

[30] Boulesnane Y., Leloup J., Lerch T.Z., et al. Impact of sampling and DNA extraction methods on skin microbiota assessment. *J Microbiol Methods* **2020**; *171*: 105880. doi:10.1016/j.mimet.2020.105880

[31] Borrego-Ruiz A., Borrego J.J. Microbial Dysbiosis in the Skin Microbiome and Its Psychological Consequences. *Microorganisms* **2024**; *12*, 1908, 1–23. doi:10.3390/ microorganisms12091908

[32] Christou D., Stevanovic K., Evers S., et al. Evaluating the Impact of Laundry Detergents on the Skin Microbiome of Atopic Dermatitis Patients — A Clinical Study. *Health Sci Rep* **2024**; 1–6. doi:10.1002/hsr2.70261

[33] Bay L., Ring H.C. Human skin microbiota in health and disease: The cutaneous communities ' interplay in equilibrium and dysbiosis. *J Path Micro and Imm* **2021**; 706–718. doi:10.1111/apm.13201

[34] Koh L.F., Ong R.Y., Common J.E. Skin Microbiome of Atopic Dermatitis. *Allergol Int* **2022**; *71*, 31–39. doi:10.1016/j.alit.2021.11.001

[35] Edslev S., Agner T., Andersen P. Skin Microbiome in Atopic Dermatitis Sofie. *Acta Derm Venereol* **2020**; *100*, 358–366. doi:10.2340/00015555-3514

[36] Wollina U. Microbiome in Atopic Dermatitis. *Clin Cosmet Investig Dermatol* 2017; **10**, 51–56. doi:10.2147/CCID.S130013

[37] Nakatsuji T., Gallo R.L. The Role of the Skin Microbiome in Atopic Dermatitis. *Ann Allergy, Asthma Immunol* **2019**; *122*, 263–269. doi:10.1016/j.anai.2018.12.003

[38] Bjerre R.D.D., Bandier J., Skov L., et al. The Role of the Skin Microbiome in

Atopic Dermatitis : a Systematic Review *. *Br J Dermatol* **2017**; *77*, 1272–1278. doi:10.1111/bjd.15390

[39] Murillo N., Raoult D. Skin Microbiota: Overview and Role in the Skin Diseases Acne Vulgaris and Rosacea. *Future Microbiol* **2013**; *8*, 209–222. doi:10.2217/FMB.12.141

[40] Ramasamy S.D., Barnard E., Dawsom T.L., et al. The Role of the Skin Microbiota in Acne Pathophysiology. *Br J Dermatol* **2019**; *181*, 691–699. doi:10.1111/bjd.18230

[41] Dagnelie M.-A, Montassier E,. Khammari A., et al. Inflammatory Skin is Associated with Changes in the Skin Microbiota Composition on the Back of Severe Acne Patients. *Exp Dermatol* **2019**; *18*, 961–967. doi:10.1111/exd.13988

[42] Park S., Kim H.S., Lee S.H., et al. Characterization and Analysis of the Skin Microbiota in Acne : Impact of Systemic Antibiotics. *J Clin Med* **2020**; *9*, 1–14. doi:10.3390/jcm9010168

[43] Chien A.L., Tsai J., Leung S., et al. Association of Systemic Antibiotic Treatment of Acne With Skin Microbiota Characteristics. *JAMADermatol* **2019**; *155 (4)*, 425-434. doi:10.1001/jamadermatol.2018.5221

[44] Condr G., Guerini M., Castello M., et al. Acne Vulgaris, Atopic Dermatitis and Rosacea : The Role of the Skin Microbiota — A Review. *Biomedicines* **2022**; *10*, 1–18. doi:10.3390/biomedicines10102523

[45] Stehlikova Z., Kostovcik M., Kostovcikova K., et al. Dysbiosis of Skin Microbiota in Psoriatic Patients : Co-occurrence of Fungal and Bacterial Communities. Front Microbiol **2019**; 10, 1–13. doi:10.3389/fmicb.2019.00438

[46] Fahlen A., Engstrand L., Baker B., et al. Comparison of Bacterial Microbiota in Skin Biopsies from Normal and Psoriatic Skin. *Arch Dermatol Res* **2012**; *304*, 15–22. doi:10.1007/s00403-011-1189-x

[47] Gupta M., Weinberg J.M., Yamauchi P.S., et al. Psoriasis : Embarking a Dynamic Shift in the Skin Microbiota. *J Cosmet Dermatol* **2022**; *21*, 1402–1406. doi:10.1111/jocd.14273

[48] Benhadou F., Mintoff D., Schnebert B., et al. Psoriasis and Microbiota : A Systematic Review. *Diseases* **2018**; *6*, 1–9. doi:10.3390/diseases6020047

[49] Hsu D.K., Fung M.A., Chen H. Medicine in Microecology: Role of Skin and Gut Microbiota in the Pathogenesis of Psoriasis, an Inflammatory Skin Disease. Med *Microecol* **2020**; *4*, 1–8. doi:10.1016/j.medmic.2020.100016

[50] Rainer B.M., Thompson K.G., Antonescu C., et al. Characterization and Analysis

of the Skin Microbiota in Rosacea : A Case – Control Study. *Am J Clin Dermatol* **2020**; *21*, 139–147. doi:10.1007/s40257-019-00471-5

[51] Kim H.S. Microbiota in Rosacea. *Am J Clin Dermatol* **2020**; *21*, 25–35. doi:10.1007/s40257-020-00546-8

[52] Zhu W., Hamblin M.R., Wen X. Role of the Skin Microbiota and Intestinal Microbiome in Rosacea. *Front Microbiol* **2023**; *14*, 1–7. doi:10.3389/fmicb.2023.1108661

[53] Adalsteinsson J.A., Kaushik S., Muzumdar S., et al. An Update on the Microbiology, Immunology, and Genetics of Seborrheic Dermatitis. *Exp Dermatol* **2020**; *29*: 481–489. doi:10.1111/exd.14091

[54] Tao R., Li R., Wang R.. Skin Microbiome Alterations in Seborrheic Dermatitis and Dandruff : A Systematic Review. *Exp Dermatol* **2021**; *30*: 1546–1553. doi:10.1111/exd.14450

[55] Wikramanayake T.C., Borda L.J., Miteva N., Paus R. Seborrheic Dermatitis — Looking Beyond Malassezia. *Exp Dermatol* **2019**; *28*: 991–1001. doi:10.1111/exd.14006

[56] Paulino L.C. New Perspectives on Dandruff and Seborrheic Dermatitis: Lessons We Learned from Bacterial and Fungal Skin Microbiota. *Eur J Dermatol* **2017**; *27*: 4–7. doi:10.1684/ejd.2017.3038

[57] Sanders M., Nijsten T., Verlouw J., et al. Composition of Cutaneous Bacterial Microbiome in Seborrheic Dermatitis Patients : A Cross-Sectional Study. *PLoS ONE* **2021**; *16 (5)*: 1–16. doi:10.1371/journal.pone.0251136

[58] Ersanli C., Tzora A., Voidarou C., et al. Biodiversity of Skin Microbiota as an Important Biomarker for Wound Healing. *Biology (Basel)* **2023**; *12*, 1–16. doi:10.3390/biology12091187

[59] Yang H., Chen Y., Lan Y., et al. Skin Microbiota : A Novel Target for Promoting Burn Wound Healing. *Wound Repair Regen* **2025**; *33*, 1–15. doi:10.1111/wrr.70118

[60] Pawłowska A., Orzeł A., Sulej L., et al. Wound Microbiota and Its Impact on Wound Healing. *Int J Mol Sci* **2023**; *24*, 1–13. doi:10.3390/ijms242417318

[61] Tomic-Canic M., Burgess J.L., O'Neill K.E., et al. Skin Microbiota and Its Interplay with Wound Healing. *Am J Clin Dermatol* **2020**; *21*, 36–43. doi:10.1007/s40257-020-00536-w

[62] Xavier T.F., Kannan M., Auxilia A. Original Research Article: Traditional Medicinal Plants Used in the Treatment of Different Skin Diseases. *Int J Curr Microbiol Appl Sci* **2015**; *4*, 1043–1053

[63] Reddy K.V., Yachawad A.V., Pathan R.R., et al. Herbal Medicinal Plants Used In Treatment Of Skin Disorders : Overview. *GIS Sci J* **2022**; *9*, 2009–2025.

[64] Michalak M. Plant Extracts as Skin Care and Therapeutic Agents. *Int J Mol Sci* **2023**; *24*, 1–36. doi:10.3390/ ijms242015444

[65] Ahmed I.A., Dia K., Alhazmi A.A.L., et al. Medicinal Plants in Cosmeceuticals: Bridging Ethnopharmacology and Modern Dermatology. *Int J Biotechnol Biomed* **2025**; *2*, 45–58. doi:10.31674/ijbb.2025.v02i03.005

[66] Kim B.J., Kim J.H., Kim H.P., Heo M.Y. Biological Screening of 100 Plant Extracts for Cosmetic Use (II): Anti-oxidative Activity and Free Radical Scavenging Activity. *Int J Cosmet Sci* **1997**; *19*, 299–307. doi:10.1111/j.1467-2494.1997.tb00194.x

[67] Galvan A., Pellicciari C., Calderan L. Recreating Human Skin In Vitro : Should the Microbiota Be Taken into Account ?. *Int J Mol Sci* **2024**; *25*, 1165. doi: 10.3390/ijms25021165

[68] Piwowarski J.P., Granica S., Zwierzyńska M., et al. Role of Human Gut Microbiota Metabolism in the Anti-inflammatory Effect of Traditionally Used Ellagitannin-rich Plant Materials. *J Ethnopharmacol* **2014**; *155*, 801–809. doi:10.1016/j.jep.2014.06.032

[69] Piwowarski J.P., Granica S., Kiss A.K. Influence of Gut Microbiota-Derived Ellagitannins Metabolites, Urolithins, on Pro-inflammatory Activities of Human Neutrophils. *Planta Med* **2014**; *80*, 887–895. doi:10.1055/s-0034-1368615

[70] Piwowarski J.P., Kiss A.K., Granica S., et al. Urolithins, Gut Microbiota-Derived Metabolites of Ellagitannins, Inhibit LPS-induced Inflammation in RAW 264.7 Murine Macrophages. *Mol Nutr Food Res* **2015**; *59*, 2168–2177. doi:10.1002/mnfr.201500264

[71] Popowski D., Czerwińska M.E., Kruk A., et al. Gut Microbiota Metabolism and the Permeability of Natural Products Contained in Infusions from the Herb of European Goldenrod *Solidago virgaurea* L. *J Ethnopharmacol* **2021**; *273*, 1–12. doi:10.1016/j.jep.2021.113924

[72] Kruk A., Granica S., Popowski D., et al. *Tiliae flos* Metabolites and Their Beneficial Influence on Human Gut Microbiota Biodiversity *Ex Vivo*. *J Ethnopharmacol* **2022**; 294: 115355. doi:10.1016/j.jep.2022.115355

[73] Ferdaous M., Hasan M., Hasan Z., et al. The Dynamic Relationship Between Skin Microbiomes and Personal Care Products : A Comprehensive Review. *Heliyon* **2024**; *10*: e34549. doi:10.1016/j.heliyon.2024.e34549

Attachments

(reprint of publications and co-authors' statements)

International Journal of
Molecular Sciences

MDPI

Review

Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota

Natalia Melnyk [1], Inna Vlasova [1,2], Weronika Skowrońska [3], Agnieszka Bazylko [3], Jakub P. Piwowarski [1] and Sebastian Granica [1,*]

[1] Microbiota Lab, Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland
[2] Department of Pharmacognosy, National University of Pharmacy, 53 Pushkinska Str., 61002 Kharkiv, Ukraine
[3] Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland
* Correspondence: sgranica@wum.edu.pl; Tel.: +48-225-720-9053

Abstract: Skin disorders of different etiology, such as dermatitis, atopic dermatitis, eczema, psoriasis, wounds, burns, and others, are widely spread in the population. In severe cases, they require the topical application of drugs, such as antibiotics, steroids, and calcineurin inhibitors. With milder symptoms, which do not require acute pharmacological interventions, medications, dietary supplements, and cosmetic products of plant material origin are gaining greater popularity among professionals and patients. They are applied in various pharmaceutical forms, such as raw infusions, tinctures, creams, and ointments. Although plant-based formulations have been used by humankind since ancient times, it is often unclear what the mechanisms of the observed beneficial effects are. Recent advances in the contribution of the skin microbiota in maintaining skin homeostasis can shed new light on understanding the activity of topically applied plant-based products. Although the influence of various plants on skin-related ailments are well documented in vivo and in vitro, little is known about the interaction with the network of the skin microbial ecosystem. The review aims to summarize the hitherto scientific data on plant-based topical preparations used in Poland and Ukraine and indicate future directions of the studies respecting recent developments in understanding the etiology of skin diseases. The current knowledge on investigations of interactions of plant materials/extracts with skin microbiome was reviewed for the first time.

Keywords: dermatology; skin; microbiota; interaction; topical; skin diseases; plant materials; phytotherapy; keratinocytes; fibroblasts

Citation: Melnyk, N.; Vlasova, I.; Skowrońska, W.; Bazylko, A.; Piwowarski, J.P.; Granica, S. Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota. *Int. J. Mol. Sci.* **2022**, *23*, 9644. https://doi.org/10.3390/ijms23179644

Academic Editors: Geoffrey Brown, Enikö Kallay and Andrzej Kutner

Received: 25 July 2022
Accepted: 21 August 2022
Published: 25 August 2022

1. Introduction

More and more drugs, dietary supplements, and cosmetic products appear on the pharmaceutical and cosmetic market, which contain medicinal plant materials or substances of plant origin [1,2]. In recent years, phytotherapeutic preparations are gaining greater importance in solving many problems in dermatology and cosmetology [3]. Severe skin ailments require the application of antibiotics, steroids, or calcineurin inhibitors. However, in mild skin disorders, the topical application of plant-based remedies in various pharmaceutical forms, such as raw infusions, creams, ointments, balms, and tinctures can be effective and successfully prevent further disease development [4,5].

Human skin is a complex organ that accounts for about 15% of the total body weight of an adult and has a surface area of 1.5–2 m^2. This organ is responsible for many vital functions [6]. It protects against external factors; participates in thermoregulation, metabolism, the regulation of fluid balance, and body shape maintenance; and eliminates toxins from the body by sweat excretion [7]. It consists of several layers, such as the living tissue of the dermis, epidermis, and the outer-facing layer, which is called the stratum corneum [8].

On the microanatomic level, skin is composed of keratinocytes, Langerhans cells, fibroblasts, mast cells, macrophages, endothelial cells, and lymphocytes, which form a complicated and fine-tuned organization such as the skin immune system [9,10]. Likewise, the superficial layer of the skin is home to millions of bacteria, fungi, and viruses that compose the skin microbiota [8]. This microbial ecosystem supports many skin functions, including metabolism, vitamin synthesis, protection against pathogen invasion, immunity development, and regulation [11].

Currently, an increase in the incidence of skin diseases worldwide is observed, which is mostly linked to the adverse factors of modern civilization [12]. For instance, 42 of the 145 surveyed people in Poland reported past or present skin disorders [13]. In Ukraine, the morbidity rate of skin diseases increases every year. The currently reported problems mostly relate to dermatitis, atopic dermatitis, eczema, psoriasis, wounds, and others. Many factors influence skin well-being, with the highest contribution attributed to domestic detergents, cosmetics, and antiseptics.

The present review aims to summarize the current information on the plant materials that are contained in pharmaceutical and cosmetic preparations available in Poland and Ukraine and marketed as non-prescription medicines or medical products. The paper presents the current knowledge regarding the traditionally used plant raw materials related to their influence on the skin and skin microbiota. Medicinal plants used in treating wounds, burns, dermatitis, atopic dermatitis, eczema, and other skin inflammatory diseases have been included.

Based on the scientific database Scopus, a graphical representation of the publication trend in the field of the present review was generated. The trend in the number of papers focused on plant extracts or materials plants and skin shows a significant increase over the last 20 years. Around 190 reports were published in 2001 according to Scopus, and in 2021, it was over 1300 original papers and reviews. Similarly, a dynamic increase in the interest in research devoted to plant preparations and microbiota was observed (Figure 1) in 2001 when 4 papers matching chosen keywords were recorded compared to 377 in 2021. This analysis confirms that the general interest in the different aspects of the interaction of plant materials with skin increases, and the subject is worth further investigations. This trend can be explained by the fact that natural therapies are gaining much interest in the context of changes in the lifestyle of the population around the world.

Figure 1. Graphical representation of the publication trend in the field of using plants related to their influence on the skin and microbiota. A graph was created based on the Scopus database, keywords 1 (plant material or plant extract and skin) and keywords 2 (plant material or plant extract and microbiota) were used.

2. Methods Used in the Review

A literature search was conducted using scientific databases such as PubMed, Scopus, and Wiley Online Library for relevant studies with the keywords "microbiome", "microbiota", "commensal", "topical", "plant materials", and "phytotherapy", "dermatology", "skin", "skin disorders", "keratinocytes", "fibroblasts". All search terms were used in various combinations, and studies were screened for relevance based on their abstracts. Studies written in English, Polish, and Ukrainian languages were considered.

The selection of non-prescription medicines and medical products was based on the search of topically used remedies containing material of plant origin in the Ukrainian directory of drugs "Compendium" and on the Polish pharmacy websites. Moreover, the Ukrainian online service "Tabletki.ua", which provides information on the availability of medical preparations and other pharmaceutical products in pharmacies, was considered.

3. Human Microbiota in the Skin Inflammation Process

The skin is the habitat and a source of nutrients for various symbiotic, commensal, and pathogenic microorganisms described as skin microbiota [7]. Early studies showed that abundant bacterial genera on the surface layers of the human skin include *Staphylococcus*, *Propionibacterium*, *Micrococcus*, and *Corynebacterium* [14]. The composition of the human microbiome similarly varies quantitatively and qualitatively according to the body site on which it is located, depending on the distinctive characteristics (pH, moisture, salinity, and sebum content), and may also vary due to other factors (e.g., genotype, age, and sex) [5,12]. The recent surveys have a greatly advanced understanding of the host-symbiont and host-pathogen relationships and established that the skin microbiota plays a beneficial role, much like the gut microbiota, indicating that the bacteria present on our skin have similar functions in immune regulation and disease pathogenesis [11,13,14].

Commensal bacteria can passively occupy a similar ecological niche to a pathogenic microbe, thus impeding its skin colonization. Additionally, commensal bacteria on human skin can selectively induce antimicrobial peptides production and provide a protective effect in vivo when administered before the infectious challenge. The human microbiome may also modulate the immune system, directing it to eliminate the disease-causing factor [14,15].

4. Interaction of Plant Origin Material with Microbiota

The interaction of microorganisms with plants can be considered from two sides. On the one hand, there is the influence of plants on microbes' growth and metabolic functions, as medicinal plants contain extractable biochemical and bioactive compounds, which can target certain viruses, bacteria, or fungi [16]. On the other hand, a metabolically active microbial community can alter the chemical structure and composition of natural products applied to the skin.

To date, there are a lot of known natural products which act effectively against bacteria, fungi, viruses, or protozoa. For instance, phenolics have antifungal and antiviral properties, and it is established that their overall harmfulness to microorganisms is associated with the measure of hydroxyl bunches and their locations on the phenol bunch. Low dosages of phenols (0.032%, 320 g/mL) destroyed fast-developing cultures of *Staphylococci* and *Streptococci* [17]. Quinones have been found to frame irreversible edifices with nucleophilic amino acids in proteins, leading to protein inactivation and function loss resulting in antibacterial effects. Quinones may also make it difficult for bacteria to obtain substrates [18]. The antimicrobial mode of action of tannins is linked to their ability to inactivate microbial adhesins, enzymes, and cell envelope transport proteins [19].

Several in vivo and in vitro examinations demonstrated that different plant preparations repress bacterial species found in cutaneous diseases. Chamomile essential oil and bisabolol were found to have activity mostly against Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, and the fungus *Candida albicans*. Aqueous extracts from *Allium cepa*

showed antifungal activity against *Malassezia furfur*, *Candida albicans*, other *Candida* sp., and other dermatophyte species [17].

In terms of the influence of microbiota on plant materials, it should be mentioned that the literature review showed a lack of information about the skin microbiota metabolism. However, nowadays, many investigations on microbiota residing in the gut have been described. For instance, human and swine microbiota transformed natural products in the goldenrod infusion into smaller molecules, mainly phenylpropanoid acid derivatives [20].Twenty metabolites were detected and characterized after incubating the linden flower extract with human gut microbiota [21]. All changes in the chemical composition of the raw plant material caused by the interaction with microbiota can lead to potentially active compounds responsible for their bioactivity in vivo. This is also evidenced by experiments on the gut microbiota-derived metabolites of ellagitannins-urolithins. It was established that urolithins inhibit proinflammatory cytokines expression in RAW 264.7 macrophages, which has an important role in inflammatory bowel diseases [22].

5. Phytotherapy in Skin Diseases

Patients and physicians widely use phytotherapy throughout the whole world. This type of therapy is as old as humankind. Plant-derived drugs are in demand because of several advantages, such as often having fewer side effects and better patient tolerance. Apart from this, treatment with natural products is more affordable to patients than chemical medicines.

Clinical trials and in vitro and in vivo experiments were conducted for many plant materials, showing their effectiveness in inhibiting the formation of cytokines and eicosanoids, preventing the inflammatory reaction cascade. However, still, the use of most herbal medicines is based solely on their longstanding traditional use in folk medicine [23].

Over the years, plants that deserve special attention in treating skin diseases have been identified. Below is a list of plant materials that are the most popular in the traditional treatment of skin diseases in Poland and Ukraine (Table 1).

Table 1. Traditionally used plant material in skin disorders in Poland and Ukraine.

Botanical Name	Common Name	Part Used	Application Properties [24–26]	Traditional Use/Therapeutic Area [24–26]
Achillea millefolium L.	yarrow	herb	antimicrobial, anti-inflammatory, antioxidant, antiproliferative, and cytotoxic	small superficial wounds
Agrimonia eupatoria L.	agrimony	herb	anti-inflammatory and antioxidant	minor inflammation and small, superficial wounds
Allium cepa L.	onion	bulbs	anti-inflammatory, antioxidant, and antimicrobial	insect bites treatment, wounds, minor burns, boils, warts, and treatment of bruises
Aloe vera (L.) Burm.f.	aloes	leaves	antiproliferative	bedsores
Artemisia absinthium L.	wormwood	herb	antiseptic and anti-inflammatory	boils, wounds, and bruises
Arctium lappa L.	burdock	root	antibacterial, antiviral, antioxidant, anti-inflammatory, antiallergic, antimutagenic, and antiproliferative	seborrheic skin conditions
Arnica montana L.	arnica	flowers	anti-inflammatory, antimicrobial activity, and antioxidant, immunotoxic, cytotoxic, and anti-platelet	inflammation
Bidens tripartita L.	three-lobe beggartick	herb	anti-inflammatory, bactericidal, and hemostatic	diathesis
Calendula officinalis L.	calendula	flowers	wound healing, antiviral, antimicrobial, anti-inflammatory, antioxidant, photoprotective, repellent, and anti-irritative	sunburn, minor wounds, and minor inflammations
Chelidonium majus L.	greater celandine	herb	antiseptic and fungicidal	warts, callus, corns, pimples, shingles, eczema, and skin tumors
Equisetum arvense L.	field horsetail	herb	antibacterial, antioxidant, anti-inflammatory, and wound healing	superficial wounds

Table 1. *Cont.*

Botanical Name	Common Name	Part Used	Application Properties [24–26]	Traditional Use/Therapeutic Area [24–26]
Hamamelis virginiana L.	hamamelis	leaves, bark	antibacterial, anti-inflammatory, antiviral, and radical-scavenging	minor inflammation and dryness
Hippophae rhamnoides L.	sea-buckthorn	fruits	anti-inflammatory, bactericidal, analgesic, and epithelializing properties	rashes, eczema, burns, bedsores, frostbite, ulcers that do not heal well, and radiation skin diseases
Hypericum perforatum L.	st. John's wort	herb	anti-inflammatory wound healing	skin disorders and minor wounds
Linum usitatissimum L.	linseed	seeds	antiproliferative	trophic ulcers, burns, and radiation damage
Melissa officinalis L.	melissa	leaves	anti-inflammatory, antiviral, antimicrobial, antioxidant, and anti-inflammatory	external remedies for herpes
Matricaria chamomilla L.	chamomile	flowers	anti-inflammatory, antimicrobial, and wound healing	irritation, minor inflammation, sunburn, superficial wounds, and furuncles
Plantago lanceolata L.	ribwort plantain	leaves, herb	anti-inflammatory, antibacterial, antiviral, antioxidant, and analgesic	Boils, edema, and insect bites
Potentilla erecta L.	tormentil	rhizomes	antibacterial, antioxidant, and antitumor	minor inflammations
Quercus robur L.	common oak	bark	astringent and anti-inflammatory	minor inflammation
Salvia officinalis L.	sage	leaves	antimicrobial, antioxidant, anti-inflammatory, cytoprotective, and wound healing	minor inflammations
Sophora japonica L.	Japanese pagoda tree	fruits	hemostatic, antiseptic, and wound healing	wounds, trophic ulcers, and seborrheic dermatitis
Symphytum officinale L.	comfrey	roots	anti-inflammatory, wound healing, and antibacterial	pain and inflammation
Trigonella foenum-graecum L.	fenugreek	seeds	anti-inflammatory and antiulcer	minor inflammations
Urtica dioica L.	nettle	leaves, herb	anti-inflammatory, analgesic, and local anesthetic	seborrheic skin conditions
Viola tricolor L.	wild pansy	herb	antibacterial, antioxidant, cytotoxic activity against cancer cells, anti-nociceptive, and anti-inflammatory	skin disorders, minor wounds, and mild seborrheic skin conditions

Different herbal parts are used in treating skin ailments, but the most usable are the above-ground parts, such as herbs, leaves, or flowers. Among all plant effects are frequently noted anti-inflammatory, bactericidal, and wound healing effects. The mostly described plants are traditionally used for minor superficial wounds, burns, minor inflammations of different etiology, irritations, bedsores, and ulcers. However, for example, *Chelidonii herba* is applied to warts, corns, and pimples due to their antiseptic and fungicidal effects, and *Melissae folium*, like antiviral, antimicrobial external remedies for herpes.

Polish and Ukrainian pharmaceutical markets have been analyzed and preparations marketed as non-prescription medicines or medical products containing plant material from Table 1 as an active ingredient are presented in Table 2.

In Poland, as in Ukraine, the most popular plant material for treating skin diseases is marigold flowers (*Calendulae flos*). It is in pharmaceutical forms, such as ointments or tinctures, and is produced by different manufacturers. It is used externally in treating mild inflammatory conditions of the skin and ancillary in treating minor skin injuries. At Polish pharmacies, a large number of remedies from linseed, chamomile, and tormentil are found. Tormentil is presented mostly in ointments ("Tormentiol", "Tormentile Forte", and "Tormentillae unguentum compositum"), linseed in ointments and creams ("Linomag"), and chamomile in ointments, gels, and tinctures ("Kamagel" and "Azulan"). Ukrainian pharmacies have some distinguishing topical remedies of *Hippophae rhamnoides*, such as "Sea buckthorn ointment", "Olasol spray", and some balms.

The plant materials were selected for the literature surveys based on the above approach. The plants which are considered effective in the treatment of inflammatory skin diseases are discussed below. Additionally, to information on the chemical composition, activity studies, and traditional use, data were also searched for in studies of the effect of skin microbiota on natural products contained in the listed plant materials and/or the analyses of changes in the composition of the microbiome as a result of the use of preparations from the raw materials mentioned above.

5.1. *Allium cepa* L. (Onion Bulbs)

The plant substance of *Allii cepae bulbus* consists of thick and fleshy leaf sheaths and leaf approaches from *Allium cepa* L. (Amaryllidaceae). Biologically active compounds of onion bulbs are flavonoids and sulfur-containing compounds. In traditional medicine, onions have been used externally to treat insect bites, wounds, minor burns, boils, warts, and bruises [27]. Many studies have demonstrated the antioxidant and antimicrobial activity of the extracts [28]. The study using human fibroblasts showed that the onion extract inhibited their proliferation, induced apoptosis, and decreased expression of β1 integrin, which may be beneficial in treating keloid and hypertrophic scars [29]. However, data on the activity of onion extracts in treating hypertrophic scars are inconclusive.

The effect of Mederma (Merz Pharma, Frankfurt, Germany) gel, containing *Allium cepa* as the active ingredient, was investigated in a rabbit hypertrophic scar model. No significant effect of the gel on the reduction in scar hypertrophy, vascularization, or inflammation was determined, but an improvement in the organization of dermal collagen was observed [30]. The effectiveness of the same gel was investigated in patients with new surgical scars compared to petrolatum emollient. No significant differences were found in the activity of these formulations in the treatment of scars as assessed by redness, itching, burning, pain, thickness, or overall cosmetic appearance [31]. Another study comparing the activity of Mederna with a petroleum-based emollient assessed the appearance and symptomatology of postoperative scars. The onion extract gel was ineffective, no difference was found in the evaluation of the redness and itching of the scar after one month of use. In contrast, a reduction in redness has been observed in patients using the emollient [32].

Table 2. Selected preparations marketed as non-prescription medicines or medical products containing plant material as an active ingredient in Poland (PL) and Ukraine (UA).

Plant Material	Country Code	Marketed Product	Active Ingredient(s)	Manufacturer	Pharmaceutical Form	Registered Indications
***Allium cepa* L. (onion bulbs)**	PL	Cepan	in 100 g of cream: 20.0 g ethanolic extract of *Allium cepa*, 5.0 g extract of *Matricaria chamomilla*, 5000.0 IU sodium heparin, and 1.0 g allantoin	Unia	cream	scars and keloids after burns and surgery; treatment of contractures; treatment of scarring of the eyelids; treatment of scars from boils, ulcers, and acne
	PL	Contractubex	in 100 g of gel: 10.0 g extract of *Allium cepa*, 5000.0 IU sodium heparin, and 1.0 g allantoin	Merz Pharma	gel	scars restricting movement, enlarged (hypertrophic, swollen, and keloid-shaped), unaesthetic postoperative scars, amputation scars, burn and accident scars, contractures of, e.g., fingers (Dupuytren's contracture), tendon contractures caused by injuries, and scar shrinkage
***Arnica montana* L. (arnica flowers)**	PL	Arnithei	in 100 g of gel: 24.0 g arnica tincture	Dr. Theiss Naturwaren	gel	relieve bruises, sprains, and local muscle pain
	PL	Uzarin	in 100 g of gel: 1.0 g extract of *Arnica montana*, 1.0 g extract of *Calendula officinalis*, and 1.0 g aluminum acetate	Nes Pharma	gel	bruises, swellings, first-degree burns, and insect bites
***Calendula officinalis* L. (marigold flowers)**	UA	Marigold ointment	tincture of calendula flowers	Viola	ointment	burns, cuts, cracks in the skin, and purulent wounds
				DKP Pharmaceutical Factory		
				Lubnyfarm		
				Ternopharm		
				FITOPHARM		
	PL	Marigold ointment	ethanolic extract of *Calendula officinalis*	Elissa	ointment	mild inflammation of the skin, as an aid to the healing of minor wounds

Table 2. *Cont.*

Plant Material	Country Code	Marketed Product	Active Ingredient(s)	Manufacturer	Pharmaceutical Form	Registered Indications
	PL	Marigold ointment	extract of *Calendula officinalis* (extraction solvent: liquid paraffin)	Ziaja	ointment	symptomatic treatment of mild skin inflammations and as an auxiliary in the treatment of minor wounds (abrasions of the epidermis)
	UA	Marigold tincture	tincture of calendula flowers	Lubnypharm FITOPHARM Viola Vishpa	tincture	drugs that promote wound healing
	PL	Marigold tincture	tincture of marigold flowers	FITOPHARM	tincture	symptomatic treatment of mild skin inflammations (such as sunburn) and as an adjunct in the treatment of minor skin wounds and mild inflammation of the mouth and throat
***Chelidonium majus* L. (greater celandine herb)**	UA	CHYSTOTIL	celandine extract	Khimpharmzavod Chervona Zirka	cream	bactericidal and wound healing
	UA	CHYSTOTIL	oil extracts of flowers, leaves, and roots of celandine	NATURE LIFE	ointment	analgetic, anti-inflammatory, and bactericidal
***Hippophae rhamnoides* L. (sea-buckthorn fruits)**	UA	Sea buckthorn ointment	sea buckthorn oil	Fitolic	ointment	healing (scarring) of wounds
	UA	Olasol spray	sea buckthorn oil—5.40 g; chloramphenicol—1.62 g; benzocaine—1.62 g; and boric acid—0.27 g	STOMA	spray	infected wounds, including long-term non-healing burns, trophic ulcers, and skin grafts

Table 2. *Cont.*

Plant Material	Country Code	Marketed Product	Active Ingredient(s)	Manufacturer	Pharmaceutical Form	Registered Indications
	UA	Mintalon	propolis, mummy, sea buckthorn oil, wheat germ oil, natural honey, geranium oil, terpene oil, vaseline oil, lecithin, pine resin, birch tar, camphor, and vitamin E	MINTA	balm	wounds, mechanical damage to the body surface, bruises; thermal damage (burns and frostbite); inflammation and purulent processes; bedsores; bumps; animal and insect bites; dryness and cracks of the skin; skin irritation; prevention of negative effects in frost, sun, wind; and moisturizing and normalizing skin nutrition
	UA	Reskinol	terpene oil, propolis, mummy, lecithin, sea buckthorn oil, pine resin, wheat germ oil, birch tar, natural honey, camphor, geranium oil, and vitamin E	Botany	balm	from pain and inflammation
***Linum usitatissimum* L. (linseed)**	PL	Linomag	virgin linseed oil	Ziołolek	Ointment, cream, or liquid	eczema, blemishes, and nappy rash: in states of excessive dryness of the skin; and to relieve the symptoms of psoriasis
	PL	Poldermin Hydro	*Linum usitatissimum* seed extract, *Avena sativa* extract, xylitol, and β-glucan	Polfa Tarchomin	Cream	intensively moisturizes the skin, softens it, and eliminates itching. In addition, it creates a protective film on the skin surface, accelerates the healing process of irritations, and reduces skin peeling
	PL	Aquastop Radioterapia	linseed oil and allantoin	Ziołolek	Cream	for skin care during and after radiotherapy

Table 2. *Cont.*

Plant Material	Country Code	Marketed Product	Active Ingredient(s)	Manufacturer	Pharmaceutical Form	Registered Indications
***Matricaria chamomilla* L. (chamomile flowers)**	PL	Chamomile ointment	ethanolic extract of *Matricaria chamomilla*	Elissa	ointment	skin inflammation
	PL	Kamagel	glycolic extract of *Matricaria chamomilla* and aluminum acetate	KRKA	Gel	inflammatory symptoms in various inflammations of the skin
	PL	Azulan	ethanolic extract of *Matricaria chamomilla*	Herbapol	Tinctura	in inflammation of the skin and mucous membranes, e.g., for rinsing in inflammations of the mouth and throat and inflammation of the gums
***Potentilla erecta* L. (tormentil roots)**	PL	Tormentil complex ointment (Tormentillae unguentum compositum)	in 100 g of ointment: 3.0 g liquid extract of the tormentil and 2.0 g ammonium bituminosulphonate 20.0 g zinc oxide	Unia	Ointment	treatment of minor skin lesions, such as skin abrasions and scrapes
				Amara		
				Ziaja		
				Prolab		
	PL	Tormentile forte	in 100 g of ointment: 3.0 g liquid extract of the tormentil, 2.0 g ammonium bituminosulphonate, 20.0 g zinc oxide, and 1.0 g borax (sodium tetraborate decahydrate)	Farmina	Ointment	skin lesions such as eczema lesions, first-degree burns, and mild acne vulgaris
	PL	Tormentiol	in 100 g of ointment: 2.0 g liquid extract of the tormentil, 2.0 g ammonium bituminosulphonate, 20.0 g zinc oxide, and 1.0 g borax (sodium tetraborate decahydrate)	Omega pharma	Ointment	minor skin damage such as abrasions and scratches. Incidentally, in purulent lesions and skin inflammations
***Quercus robur* L. (common oak bark)**	UA	BIOFLORIN	oak bark extract, coriander essential oil, and jojoba oil	Khimpharmzavod Chervona Zirka	cream	anti-inflammatory and wound healing

Table 2. *Cont.*

Plant Material	Country Code	Marketed Product	Active Ingredient(s)	Manufacturer	Pharmaceutical Form	Registered Indications
***Salvia officinalis* L. (sage leaves)**	UA	Tinctura Salviae	tincture of leaves salviae	DKP Pharmaceutical Factory	tincture	inflammation of the mucous membranes of the mouth, gums (stomatitis, gingivitis, periodontitis), pharynx, tonsils (pharyngitis, sore throat), upper respiratory tract, and infected wounds, cuts, and skin burns
	PL	Sage ointment	ethanolic extract of *Salvia officinalis*	Elissa	ointment	skin inflammation
***Sophora japonica* (Japanese pagoda tree fruits)**	UA	Tinctura sophorae japonicae	tinctura of Japanese sophora fruit	FITOPHARM	tinctura	antiseptics and disinfectants; purulent inflammatory processes (wounds and trophic ulcers)
***Symphytum officinale* L. (comfrey roots)**	UA	Ointment Dr. Taissa with Comrey	tincture of comfrey and tocopherol acetate	Dr. Theiss Naturwaren GmbH	ointment	the anti-inflammatory, analgesic effect, and promote the formation of calluses
	UA	Bainvel Comfrey Dr. Theiss	tincture of comfrey	Dr. Theiss Naturwaren GmbH	cream	degenerative-dystrophic and inflammatory diseases of the joints, as well as for recovery after sports and excessive or prolonged physical exertion
	UA	Ointment with Comfrey	comfrey root tincture and tocopherol acetate (vitamin E)	DKP Pharmaceutical Factory	ointment	pain in the joints, back, lower back with radiculitis, osteochondrosis, and arthritis. Sports, domestic injuries and bruises, sprains, and closed bone fractures; and dryness and cracked skin
Another/Complex	UA	Wundehil ointment	propolis, calendula, gooseberry foxglove, Japanese sophora fruit, and yarrow herb	AEM	ointment	remedies for wounds and ulcers

Table 2. *Cont.*

Plant Material	Country Code	Marketed Product	Active Ingredient(s)	Manufacturer	Pharmaceutical Form	Registered Indications
	UA	TRAUMEEL® S	*Achillea millefolium* 0.09 g, *Aconitum napellus* D1 0.05 g, *Arnica montana* D3 1.5 g, *Atropa belladonna* D1 0.05 g, *Bellis perennis* 0.1 g, *Calendula officinalis* 0.45 g, *Echinacea* 0.15 g, *Echinacea purpurea* 0.15 g, *Hamamelis virginiana* 0.45 g, Hepar sulfuris D6 0.025 g, *Hypericum perforatum* D6 0.09 g, *Matricaria recutita* 0.15 g, Mercurius solubilis Hahnemanni D6 0.04 g, and *Symphytum officinale* D4 0.1 g	Biologische Heilmittel Heel GmbH	ointment	bedsores, burns, pityriasis, and trophic ulcers
	UA	Express BITE	*Chamomila recutita extract*, *Melaleuca alternifolia*, pantpenol, *Aloe arborescens extract*, *and Eugenia caryophyllus oil*	Georg BioSystems	cream	to eliminate skin itching
	UA	Express Burn	Chamomila extract Aloe extract Shea butter Tea tree oil Colloidal silver D-panthenol	Georg BioSystems	cream	treatment wounds and scars
	UA	UGRIN	*Millefolii herb*, *Menthae folia*, *Calendulae officinalis flores*, *Tanaceti flores*, *Lavandulae herba*, *Chelidoni herba*, and *Chamomillae recutitae flores*,	Khimpharmzavod Chervona Zirka	tincture	wound healing, anti-inflammatory, and antimicrobial action

The effectiveness of this plant material in treating scars after minor dermatological procedures has been proven for the occlusive overnight intensive patch medical device containing onion bulbs extract and allantoin. After 24 weeks, a clear improvement in the appearance of the scar was observed, assessed with the Patient and Observer Scar Assessment Scale and a Global Aesthetic Improvement Scale [33]. The beneficial effect in the treatment of scars has also been confirmed for other combined preparations containing *Allium cepa* extract. The gel enriched with allantoin and heparin (Contractubex, Merz Pharma, Frankfurt, Germany) improved vascularization, pigmentation, and the overall appearance of the scar according to the Vancouver Scar Scale [34]. The use of a gel containing *Allium cepa* extract, allantoin, and pentaglycan (Kaloidon gel, Laboratorio Farmacologico Milanese SRL, Caronno Pertusella, Italy) for 24 weeks successfully reduced neoangiogenesis in patients with hypertrophic scars and keloids, resulting in the clinical improvement of skin lesions [35]. Moreover, using a patch containing 10% *Allium cepa* extract, 1% allantoin, and 4% pentaglycan (Kaloidon patch, Laboratorio Farmacologico Milanese SRL, Caronno Pertusella, Italy), after 24 weeks, showed beneficial effects according to the Patient and Observer Scar Assessment Scale. In addition, significantly improved skin scar thickness and vascularization were observed after 12 weeks [36].

There are two medicinal products available on the Polish market, containing *Allium cepa* extract, allantoin, and heparin. It is a Contractubex (Merz Pharma, Frankfurt, Germany) gel and Cepan (Unia, Warsaw, Poland) cream, which additionally contains chamomile extract. In the literature, no reports on the interactions of extracts from onion bulbs with skin microbiota were found.

5.2. *Aloe vera* L. (Aloes Leaves)

Aloe vera is a plant that belongs to the Asphodelaceae family. *Aloe vera* leaf gel is mainly used in dermal ailments. This gel is rich in polysaccharides. Glucomannan, acetylated glucomannan, galactogalacturan, glucogalactomannan, and acemannan had been extracted and described from this *Aloe* species [37]. Moreover, such sterols as lupeol, campesterol, and β-sitosterol were also found [37].

In the past, the influence on skin cells was investigated. In vitro pharmacological studies determined that the effect of *Aloe vera* gel and its compounds on HaCaT cells lies in decreasing photodamage; maintaining membrane integrity; reducing the levels of TNF-α, IL-8, IL-12 and p65; and increasing IκB-α protein expression. *Aloe vera* gel, in turn, increases the wound healing ability, number of cells, keratinocyte proliferation and differentiation, and cell surface expression of adhesion molecules (β1-integrin, α6-integrin, β4-integrin, and E-cadherin) in HEKa [38]. In vivo pharmacological studies on *Aloe vera* were also conducted and showed an increasing amount of fibroblasts, TGF-β gene expression, wound closure and skin tensile strength, collagen deposition, and wound healing activity as re-epithelialization and angiogenesis [38]. Moreover, it was established that *Aloe* sterols reduce skin dryness, epidermal thickness, wrinkle formation, and pro-inflammatory cytokines levels, and lupeol, campesterol, and β-sitosterol are significantly anti-inflammatory in wounded mice [37]. Polysaccharides isolated from *A. vera* help to regulate the wound healing activity, inducing matrix metallopeptidase (MMP)-3 and metallopeptidase inhibitor-2 gene expression during the skin wound repair in rats [39].

In most cases, aloes occur in complex medical products as additional ingredients. On the Ukrainian market, it is presented in creams “Express BITE” and “Express Burn” (Georg BioSystems, Kirovograd, Ukraine).

5.3. *Arnica montana* L. (Arnica Flowers)

Arnica montana is a widely used therapeutic plant belonging to the Asteraceae family. This plant possesses numerous medicinal activities due to such constituents as flavonoids, sesquiterpene lactones (metacryl, isobutyryl, tygloyl, methacryloyl, and isovaleryl helenalin derivatives), acetylenes, hydroxycoumarines (umbelliferone and scopoletin), phenyl acrylic acids, essential oil components, and phenolic acids (chlorogenic and caffeic acid). It is also

known to contain pyrrolizidine alkaloids (tussilagin and isotussilagin) [40]. It has been used for centuries in dermatology as an antiphlogistic, antibiotic, and anti-inflammatory remedy [41]. The pharmaceutical form in traditional topical use is presented as herbal preparations in semi-solid and liquid dosage forms for cutaneous use [42].

Lyss et al. investigation shows that the main anti-inflammatory sesquiterpene lactone from arnica, helenalin, modifies the NF-κB/IκB complex, preventing the release of IκB [38,40]. Some results present that arnica reduced the UVB-induced inflammatory response as demonstrated by the inhibition of myeloperoxidase activation, a decrease in NF-κB levels, and a reduction in proinflammatory cytokines levels (IL-1β, IL-6, TNF-α, and IFN-γ) in in vivo studies [43]. In some in vitro experimental models, the production of IL-6, IL-8, and TNF-α pro-inflammatory cytokines was also measured. The secretion of IL-6, IL-8, and TNF-α in an H_2O_2-stressed fibroblast cell culture decreased, which indicates the cytoprotective effect against cell membrane oxidative damage and higher anti-inflammatory activity [42,44].

Arnica is a popular and characteristic plant material for the Polish market and two OTC medicines with extracts from this plant material are available at Polish pharmacies. "Arnithei" gel (Dr. Theiss Naturwaren, Homburg, Germany) contains an arnica tincture and is used for relieving bruises, sprains, and local muscle pain. "Uzarin" gel (Nes Pharma, Tarnów, Poland) contains the extract of *Arnica montana* and *Calendula officinalis* and is applied for bruises, swellings, first-degree burns, and after insect bites. In some sources, the beneficial effect of arnica extracts on the composition of skin microbiome is mentioned; however, there are no fine basic studies supporting these statements [45].

5.4. *Calendula officinalis L. (Marigold Flowers)*

Calendula officinalis (Asteraceae) is one of the most popular plants used clinically throughout the world [46]. This plant contains triterpene saponins (2–10%), mainly oleanolic acid glycosides; free and esterified triterpene alcohols, especially faradiol 3-mono- and diesters; carotenoids (up to 3%): α- and β-carotene, lutein, and rubixanthin; flavonoids (0.3–0.8%) based on quercetin, quercitrin and isorhamnetin; polysaccharides; sterols; sesquiterpenoids (aloaromadendrol and epicubebol); phenolcarbonic acids; fatty and amino acids; tocopherols; and essential oil (0.2–0.3%) with α-cadinol and β-cadinen as the major components [24,25,47,48]. Frequently, the infusion, tincture, and ointment of marigold are used as a wound healing remedy for inflammation of the skin and mucous membranes and externally in the treatment of long-healing wounds, cuts, boils, burns, and ulcers [25,48,49].

Pharmacological studies had confirmed that extracts from this plant exhibit a broad range of biological effects, such as antibacterial, antifungal [47,48,50,51], antioxidant [47,52], anti-inflammatory [47,48,51,52], spasmolytic, anticancer [24,53], anti-HIV, and hepatoprotective activities [51], and stimulate the proliferation and migration of fibroblasts in vitro [54]. In fact, most of the studies on marigold focus on its anti-inflammatory property. Some results present that *C. officinalis*, with other plants, reduced cutaneous inflammation at the price of the downregulation of inflammatory IL-1β, IL-6, and IL-8 and suppressed an increase in stratum corneum dehydration through the upregulation of AQP3 [55]. Marigold flowers extracts protect HaCaT skin cells against an oxidative stress challenge in the form of H_2O_2 [56]. Moreover, other scientists found that the *n*-hexane and the ethanolic extracts modulated the inflammatory phase of wound healing by activating the transcription factor NF-κB and increasing the amount of the chemokine IL-8 [57].

Nowadays, marigold flowers are one of the most famous plant materials among manufacturers and patients as they are presented in large amounts in Polish and Ukrainian pharmacies. For patients, it is available mainly in two forms (ointments and tincture) and is used in mild inflammations of the skin, minor wounds, burns, and cuts. One study on the potential influence of the marigold extract on the composition of skin microbiota was performed. The 90% hydroethanolic extract from marigold was shown to inhibit the

growth of *P. acens* and *S. epidermidis*. The results suggest that this plant material can be considered a skin prebiotic important in treating acne [58].

5.5. Chelidonium majus L. (Greater Celandine Herb)

Chelidonium majus L. is also known as greater celandine (family Papaveraceae). The herb of *Chelidonium majus* L. contains over 20 different alkaloids, including chelerythrine, chelidonine, sanguinarine, isochelidonine, and protoberberines (berberine, coptisine, dihydrocoptisine, and stylopine) protopine [59]. Several flavonoids were found in the aerial parts in low amounts. Among them are derivatives of kaempferol and quercetin. Moreover, other phenolic compounds such as hydroxycinnamic acids, hydroxybenzoic acids, and their derivatives were identified. Additionally, organic acids (chelidonic, malic, citric, and succinic acids), biogenic amines (histamine, methylamine, and tyramine), essential oil constituents, triterpenoids, saponins, vitamins A and C, and nicotinic acid were found in *C. majus* extracts [60].

N. Cordes et al.'s investigation shows that ukrain, an alkaloid thiophosphoric acid derivative of *C. majus*., demonstrates a protective effect in normal human fibroblasts in modulating radiation toxicity [61]. Vavrecková et al. determined the antiproliferative activity of the extract on human keratinocytes, showing IC_{50} was lowest for sanguinarine (2.26 μM), extract (as chelidonine) ca. 5.68 μM, chelidonine, and chelerythrine ca. 28 μM, and poor activity of berberine and hydrastinine. The lactate dehydrogenase assay showed the cytostatic activity of the *C. majus* extract rather than cytotoxic activity which can be considered beneficial in treating wards [62]. Some medical products with celandine extracts are available on the Ukrainian market and presented in such preparations as "CHYSTOTIL" cream (Khimpharmzavod Chervona Zirka, Ukraine) and "CHYSTOTIL" ointment (NATURE LIFE, Ukraine). Bactericidal, wound healing, anti-inflammatory, and analgesic properties are indicated.

5.6. Hamamelis virginiana L. (Witch Hazel Leaves, Bark)

Hamamelis virginiana L., also known as witch hazel, is a shrub that belongs to the Hamamelidaceae family [63,64]. Preparations from *Hamamelis* leaves, bark, and twigs, present in extracts, tinctures, creams, and salves, are utilized to treat dermatological (sunburn, irritated skin, and atopic eczema) and vascular disorders (hemorrhoids, varicose veins, and phlebitis), highlighting the fact that this plant has a wide range of biologically active substances [64–66].

Witch hazel bark contains up to 10% tannins (hamamelitannin and catechins), free gallic acid, and a small amount of flavonols, fats, and waxes. Leaves contain 3–10% of tannins (a mixture of gallotannins and condensed catechins–procyanidins); notably a small amount of hamamelitannin; phenolic acids (caffeic and gallic acids); flavonoids such as kaempferol, quercetin, quercitrin, and isoquercitrin; and essential oil [26,64,67–69]. Numerous in vitro and in vivo studies have shown that this plant has antitumoral, antioxidant, anti-inflammatory, antibacterial, antiviral, and antimutagenic activity [63,64,68–70]. For instance, the extract of witch hazel leaves and small twigs can decrease the amount of IL-8 produced by fibroblast cells [71]. The hazel extract and its component—hexagallloylglucose—regulated the inflammatory response via inhibiting NF-κB and PAR-2 pathways in human keratinocytes [72].

Witch hazel, one of the active ingredients, was found in the combined homeopathic ointment TRAUMEEL® S (Biologische Heilmittel Heel GmbH, Baden-Baden, Germany), which is available on the Ukrainian market and applied for the treatment of bedsores, burns, pityriasis, and trophic ulcers. No papers reporting the possible interactions of hazel extracts with human sin microbiome were found.

5.7. Hippophae rhamnoides L. (Sea-Buckthorn Fruits)

Hippophae rhamnoides L., (Elaeagnaceae family), is commonly known as sea buckthorn. Fruits and seeds contain fatty oil (about 8% in fruits and about 12% in seeds), which contains

a significant amount of carotene (up to 250 mg%), vitamins E, F, and K, phospholipids (up to 1%), and fatty acids (linoleic, oleic, palmitic, palmitoleic, and stearic). Fruits contain mono- and disaccharides, mucus, vitamins (C, B_1, B_2, B_6, B_9, P, and PP), organic acids (malic, tartaric, oxalic, and succinic), sulfur-containing substances, including betaine and choline, tannins, flavonoids (rutin, quercetin, kaempferol, and isorhamnetin), phenolic acids (chlorogenic and caffeic), and coumarin [25]. Patients have used sea buckthorn for a long time due to its rich composition, which provides a wound healing effect, modification of sebum characteristics, and improvement of atopic skin [73].

To date, many investigations have been conducted on seeds and their non-polar compounds. It was shown that the palmitic acid-enriched fraction supported cell proliferation properties on normal human keratinocytes (NHEK) and normal human dermal fibroblasts (HDFa). However, some fractions did not alter the cellular morphology of normal keratinocytes and did not influence the inflammatory response [73]. There are also studies confirming that sea buckthorn seed oil stimulated the proliferation of dysplastic cells, while it also impaired the ability of both normal and dysplastic cells to migrate over a denuded area [74]. 1,5-dimethyl citrate isolated from *Hippophae rhamnoides* was demonstrated to prevent LPS-induced NO production and inhibited the expression of IKK-α/β, IκB-α, NF-κB p65, iNOS, and COX-2 and the activities of IL-6 and TNF-α [75].

Sea buckthorn ointment (Fitolic, Ivano-Frankivsk, Ukraine) and Olasol spray (STOMA, Kharkiv, Ukraine) are drugs popular in Ukraine and contain sea buckthorn oil, used for healing infected wounds, including long-term non-healing burns, trophic ulcers, and skin grafts. Moreover, some other medical products consisting of *Hippophae rhamnoides* extract are available, such as the balms Mintalon (Minta, Kharkiv, Ukraine) and Reskinol (Botany, Kramatorsk, Ukraine). To the best of our knowledge, the interaction of sea buckthorn extracts with skin microbiota has never been investigated.

5.8. Linum usitatissimum L. (Linseed)

Linseed is usually defined as the ripe dried seeds of *Linum usitatissimum* L. (Linaceae), which contain 30–45% of fixed oil, 25% protein, 3–9% polysaccharides, and 0.1–1.5% cyanogenic glycosides. In addition, it also contains lignans, mainly secoisolariciresinol, and its glycosides [76]. It is a herbal medicinal product with well-established use in treating habitual constipation [77]. In traditional medicine, flax seeds have been used to relieve inflammation of the upper respiratory tract and gastrointestinal tract and externally in skin inflammation, eczema, ulcers, burns, chilblains, hard-to-heal wounds, skin drying, and cracking [78].

On the Polish market, some drugs contain virgin linseed oil, indicated in the treatment of skin diseases such as eczema and rash, as well as in conditions of excessive dryness and symptoms of psoriasis. In addition, there are preparations containing flax in medical products that intensely moisturize, soften, and nourish the skin during or after radiotherapy.

In vivo studies have shown that the external application of linseed oil has an anti-inflammatory effect. In rats with carrageenan-induced paw edema, a reduction in clinical signs of inflammation, infiltration of inflammatory cells, vascular congestion, and an improvement in biochemical parameters were observed [79].

The effectiveness of treating burns with linseed oil has been proven in animal models. After applying linseed oil to second-degree burns in rats, a higher wound closure rate was observed, and the performed biopsy showed better tissue regenerative properties and higher angiogenesis than the control [80]. In another study, also on the model of second-degree burns in rats, it was confirmed that after 21 days of linseed oil application, the severity of the inflammatory process decreased, and collagen synthesis, re-epithelialization, and angiogenesis increased [81]. In a burn model in rabbits, after 12 days of treatment with linseed oil, the degree of wound closure was significantly higher than in the control group; moreover, complete wound closure was 9 days earlier than in the control group. A histopathological study showed that in the group treated with linseed oil, the wound contained fewer inflammatory cells and had complete re-epithelialization with reduced

thickness compared to the non-treated control. In addition, an increased number of new capillaries, collagen fibers, and fibroblasts was observed [82]. The effectiveness in treating burns was also confirmed for a gel containing flax seed polysaccharides (composed of glucose, mannose, xylose, and arabinose in glycerol) in the rats' burns model. The group treated with the gel showed the best results. The skin was naturally colored, and a histological evaluation showed epidermal regeneration without inflammation, growth in connective tissue, and increased collagen production [83].

The beneficial effects of flax have also been confirmed in wound healing. In wound models made in rats, it was shown that the application of linseed oil accelerates wound closure, increases re-epithelialization, and reduces inflammation [84,85]. The histopathological examination also showed the increased synthesis of collagen fibers, vascularization, and hair follicles [86]. Linseed oil used on wounds made with a scalpel in rabbits increased the skin's elasticity and firmness and stimulated microcirculation and the influx of fibroblasts, as well as the growth of collagen fibers [87]. No information on the research focusing on interactions of flax extracts with skin microbiota was found in the literature.

5.9. *Matricaria chamomilla L. (Chamomile Flowers)*

Flowers from *Matricaria chamomilla* L. (Asteraceae) contain not less than 4 mL/kg of essential oil and 0.25% of apigenin-7-glucoside. In addition to essential oil and flavonoids, they contain coumarins, phenolic acids, and polysaccharides [88]. The indications for external use include the treatment of minor ulcers and inflammation of the mouth and throat, inflammation of the skin (sunburn), superficial wounds and small boils (furuncles), and as an adjunct to the treatment of skin and mucosa irritation around the anus and genital region [89].

The anti-inflammatory properties of the essential oil and water extract of chamomile flowers have been confirmed in animal models. In a model of rats with carrageenan-induced paw edema, they reduced swelling and decreased prostaglandin E_2 secretion and NO levels. In a mouse model with xylene-induced ear swelling, they reduced the swelling and lowered the allergic reaction. Additionally, the essential oil reduced the duration and frequency of scratching in mice with dextran-induced itching [90].

The acceleration of the second-degree burn regeneration in rats was observed for the chamomile flower oil extract, which significantly reduced the lesion area after 20 days [91]. In addition, another study showed that the same extract reduced the size of the incision made on the back of rats after just 5 days. Complete healing was observed after 11 days, while for olive oil alone, the healing process took 20 days [92].

The ability to regenerate wounds was also confirmed for ethanolic and methanolic extracts from *M. chamomilla* flowers. The daily use of the gel containing 5 or 10% ethanolic extract improved wound healing in diabetic rats by increasing fibroblast proliferation and revascularization. A higher wound closure ratio was noted after three days of treatment compared to the control group. No significant differences were observed between the treatment with the gel containing 5 and 10% of the extract [93]. The wound healing capacity of the methanolic extracts was tested in an excision wound model on the rats' dorsum, using concentrations of 2.5, 5, and 10%. Seven days after the injury, a difference in wound regeneration was observed between the test and control groups, the ointment containing 10% of the extract had the strongest healing effect. After 11 days, the differences in wound healing after the application of the 2.5, 5, and 10% ointments were insignificant, and still, all of them aided the healing more strongly than the control. Histopathological examinations carried out after 14 days showed that using the ointments with the chamomile flower extract increased the number of fibroblasts, basal epidermal cells, and the amount of collagen. In contrast, the number of neutrophils in the wound decreased [94]. The activity of the ethanolic extract from chamomile flowers was also tested in a model of wounds infected with *Staphylococcus aureus* in mice. After 14 days of daily use, the wound was completely healed, and the hair was present, without scarring, while scar tissue appeared in the gentamycin-treated group and severe inflammation in the control group. Moreover,

in the group treated with chamomile extract, an increase in granulation tissue production, fibroblast density, keratinization on the wound surface, and thickness of collagen fibers was observed [95].

The clinical efficacy of the *M. chamomilla* flower extract applied to the forearm and face of healthy volunteers was tested. Skin physiology was assessed after 2 h and 2 and 4 weeks of daily use. The use of the extract significantly increased the hydration of the corneum, and after prolonged use, it reduced the transepidermal water loss by 27% [96]. Another study looked at the activity of chamomile gel in preventing acute radiation dermatitis in head and neck cancer patients. The effect of a gel containing 8.35% of chamomile flower extract was compared with urea cream. The use of the gel delayed the onset of the inflammatory reaction to the radiation. In addition, its use was associated with a lesser incidence of itching, burning, and discoloration among patients, which were seen in the group using urea cream [97].

The activity of 3% of *M. chamomilla* essential oil was tested in a BALB/c mouse model in which atopic dermatitis was induced with dinitrochlorobenzene. The daily use of the oil for four weeks contributed to a decrease in the levels of Ig E and Ig G1 and histamine in the blood of the animals. In addition, it reduced the frequency of scratching [98]. Although several basic and clinical studies were performed with chamomile extract as a skin medicine, no research considering its influence on skin microbiota has been reported so far.

5.10. Potentilla erecta L. (Tormentil Root)

Tormentillae rhizoma is a whole or cut dried rhizome of *Potentilla erecta* (syn. *Potentilla tormentilla*, Rosaceae), containing not less than 7% of tannins expressed as pyrogallol. The composition is dominated by condensed tannins (up to 22%), but ellagitannins (including agrimoniin and pedunculagin) are also present. In addition, there are phenolic acids (coumaric, sinapic, caffeic, and gallic acids and their derivatives), flavonoids (kaempferol and quercetin and their derivatives), as well as triterpene saponins [99].

The indications for use exclusively based on longstanding use only include the symptomatic treatment of mild diarrhea and mild inflammation of the oral mucosa [100].

On the Polish market, ointments containing a liquid extract from the rhizome of common tormentil are available. It is used together with zinc oxide and ichthammol. The indication for the use of these preparations is the treatment of minor skin lesions, such as scratches or abrasions of the epidermis. In the case of ointments that additionally contain borax, the indications for use are extended to the treatment of purulent and acne lesions.

Studies on in vitro models have shown the astringent, antimicrobial, antioxidant, and anti-inflammatory effects of tormentil rhizomes [101].

The potent anti-inflammatory properties of the agrimoniin-rich fraction were confirmed in in vitro and in vivo models of UVB-induced inflammation. The investigation using HaCaT keratinocyte cells showed that the abovementioned fraction reduced the production of prostaglandin PGE_2 by inhibiting COX-2 [102]. In similar studies on the HaCaT cell model, it was demonstrated that the tormentil ethanolic extract inhibits the activation of NF-κB and, in addition to inhibiting PGE_2 production, also inhibits the production of IL-6 [103]. In the in vivo model of erythema induction on the skin of healthy volunteers, a significant reduction in inflammation and redness was observed after the use of the agrimoniin-rich fraction in the concentration of 100 mg/mL [102]. The effect of the methanolic extract from *P. erecta* rhizomes on the healing of diabetic wounds was investigated in Wistar rats with streptozocin-induced diabetes. Studies have shown that the extract significantly accelerates wound contraction compared to control and increases nitric oxide, glutathione, and collagen levels, while the thiobarbituric-acid reactive substances levels decreased [104]. No studies on the influence of the tormentil rhizome extract on skin microbiota or the metabolism of natural products contained in this plant material by skin microorganisms have been reported.

5.11. Quercus robur L. (Common Oak Bark)

Common oak belongs to the family Fagaceae and contains a highly variable amount of tannins (8–20%). *Quercus cortex* contains hydrolyzable tannins (gallotannins, ellagitannins, and flavonols-ellagitannins) and condensed tannins (proanthocyanidins). More than 20 compounds (catechins and low-molecular-mass, oligomeric, and polymeric proanthocyanidins) have been isolated from the bark [105,106]. Triterpenes, insoluble lipid polyesters, and volatile acids are also presented in oak bark. It is considered a traditional herbal medicinal product for the symptomatic treatment of minor inflammation of the oral mucosa or skin in pharmaceutical forms, such as infusion and decoction [105,106].

Ji-Ae Hong et al. found that *Quercus fruits* rescued UVB-induced cytotoxicity and substantially inhibited cellular ROS production in human keratinocytes [107]. Likewise, this study showed that *Quercus fruits* effectively prevent skin photoaging by enhancing collagen deposition and inhibiting MMP-1 via the ERK/AP-1 signaling pathway. *Quercus mongolica* and its isolated compounds have shown inhibitory activities toward inflammatory cytokines and chemokines. Potent activities against MCP-1, TARC, IL-6, IL-8, IL-10, and IL-13 in keratinocytes irradiated with UVB were determined [108]. Chang Seok Lee et al.'s study revealed that oak wood vinegar has anti-inflammatory and antiproliferative effects in a 2,4-dinitrochlorobenzene-induced contact dermatitis mice model. Furthermore, they showed that the mechanism by which oak wood vinegar most likely inhibits epithelial proliferation is through STAT3 inactivation [109].

In most cases, oak bark is used in decoctions by humankind, but in the Ukrainian market, this plant material was also found in a combined medical product as cream, "Bioflorin" (Khimpharmzavod Chervona Zirka, Kharkiv, Uktaine), which has anti-inflammatory and wound healing properties. No information on the interactions of oak bark extracts with sin microbiota was found in the available literature.

5.12. Salvia officinalis L. (Sage Leaf)

Salviae folium, obtained from *Salvia officinalis* L. (Lamiaceae), has a monograph in the European Pharmacopoeia (Ph. Eur. 10th Edition), European Medicines Agency (EMA), and European Scientific Cooperative on Phytotherapy (ESCOP). The medicinal plant material consists of whole or cut dried sage leaves containing not less than 12 or 10 mL/kg of essential oil, respectively. In addition to the essential oil containing monoterpenes and sesquiterpenes, the chemical composition includes diterpenoids, triterpenoids, flavonoids, hydroxycinnamic acid derivatives, and phenolic glycosides [110]. Traditionally, sage leaf can be used to relieve dyspeptic disorders such as heartburn and flatulence, reduce hyperhidrosis, relieve inflammation of the mouth and throat, and treat inflammation of the skin [111].

Medicinal products available on the Polish market are intended primarily for treating inflammations in the mouth and throat. These are concentrates for preparing rinse solutions (Dentosept, Salviasept, Tinctura Salviae, and Tymsal) and gels applied directly to the lesions within the oral cavity (Aperisan, Dentosept A, and Mucosit). In addition, sage leaf is available as a single herb for making infusions (Salviae folium), as well as in the form of herbal mixtures for making infusions for gargling (Septosan) and for use in mild inflammatory conditions of the female genitalia (Vagosan). The only medicinal product to be applied directly to the skin is sage ointment containing the ethanolic extract of *Salvia officinalis* leaves.

The traditional use of sage leaf has been partially supported by scientific research. Studies conducted on in vitro and in vivo models have confirmed its anti-inflammatory, antioxidant, and antimicrobial properties as well as the beneficial effects on wound healing [112].

Strong anti-inflammatory properties after topical application on a model of mouse ear edema induced by croton oil have been demonstrated for *n*-hexane and chloroform extracts from sage leaves. The component responsible for the anti-inflammatory activity was ursolic acid [113].

The essential oil of *S. officinalis* showed antifungal activity against clinical strains of dermatophytes isolated from skin and nails and also inhibited NO production by LPS-stimulated macrophages [114]. In in vitro studies, sage leaf oil showed stronger antibacterial properties against *S. aureus* and *P. aeruginosa* than penicillin and mupirocin. Studies on an in vivo model of infected wounds in BALB/c mice showed that the ointment containing 4% of the *S. officinalis* oil statistically reduced the number of bacteria compared to the control group and the mupirocin-treated group. Moreover, compared to the control group, there was a shortening of the inflammatory phase, acceleration of cell proliferation, increased collagen accumulation, revascularization, and re-epithelialization. The content of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) decreased, and the content of growth factors (FGF-2 and VEGF) increased [115].

In another in vivo study in a Wistar rat model, the wound healing capacity of an ointment containing 3 and 5% hydroethanolic extract of sage leaves was tested. The time of wound closure and re-epithelialization was significantly shortened compared to the control group. Moreover, the formation of new blood vessels and the number of fibroblasts increased in the wound, improving the proliferation phase of healing [116]. The methanolic extract of *S. officinalis* leaves inhibited hyaluronidase, elastase, and collagenase activity in vitro. In vivo studies on Swiss albino mice inhibited the formation of wrinkles induced by UV exposure [117]. In the prospective randomized, double-blind placebo-controlled study, an area of the skin of healthy volunteers was irradiated to induce local erythema. An ointment containing 2% sage extract has been shown to reduce local inflammation and erythema to a similar extent as 1% hydrocortisone ointment [118]. Although sage leaves extracts and essential oil are widely used for skin problems, no reports on the interactions of this plant material with skin microbiome were published.

5.13. *Sophora japonicum L. (Japanese Pagoda Tree Fruits)*

The Japanese pagoda tree is also named sophora and belongs to the *Fabaceae* family. At least 153 constituents, including flavonoids, isoflavonoids, triterpenoids, alkaloids, mineral elements, and amino acids, were identified and isolated from *S. japonica*. The most important and abundant components of the dried flower buds and ripe fruits are rutin and sophoricoside [119]. These substances have been used to control the quality of medicinal products and determine the high medicinal value of this plant material. Based on the chemical composition, this plant material has anti-inflammatory, antibacterial, antiviral, and antioxidant effects and is traditionally used in treating wounds and trophic ulcers.

Sophora's polysaccharides protect HaCaT keratinocytes from UVB irradiation-induced skin injuries and may involve the MAPK signaling pathway, which contributes to apoptotic cell death [120]. Sophoricoside ameliorates contact dermatitis due to the inhibition of the phosphorylation and degradation of IκB-α/β and the nuclear translocation of NF-κB p65 in B cells [121]. In turn, sophoricoside exhibited a potent inhibitory effect in the IL-5 bioassay in a dose-dependent manner [122]. This isoflavone glycoside inhibited the IL-6 bioactivity with an IC_{50} value of 6.1 μM. In contrast, it had no effects on IL-1β and TNF-α production and was established as a selective inhibitor of cyclooxygenase COX-2 activity [123].

In Ukraine, a tincture of Japanese sophora fruit (FITOPHARM, Kyiv, Ukraine) is very popular as an antiseptic and wound healing preparation and is used in purulent inflammatory processes (wounds and trophic ulcers).

5.14. *Symphytum officinale L. (Comfrey Root)*

Symphytum officinale is a plant belonging to the *Boraginaceae* family, rich in allantoin, phenolic acids (e.g., rosmarinic, *p*-hydroxybenzoic, caffeic, chlorogenic, and p-coumaric acids), pyrrolizidine alkaloids, triterpene saponins, tannins, amino acids, flavonoids, triterpenes, terpenoids, saponins, sterols, and mucopolysaccharides [124]. The comfrey plant material therapeutic properties include anti-inflammatory, analgesic, granulation-promoting, and anti-exudative effects [125,126].

The previous investigation determined the in vivo wound healing effects of *Symphytum officinale* L. leaves extract. The results showed that comfrey extract modulates the inflammatory process and stimulates collagen production [126]. Proliferative and antioxidant studies demonstrate a beneficial effect on human skin fibroblasts. It is non-toxic and simultaneously expresses the high ability to reduce ROS [125]. Several investigations with isolated compounds from comfrey were conducted, and it was established that crude comfrey polysaccharides possess the antioxidative activity and revealed that, by efficiency, it is superior to allantoin ointment in burn wound healing. Moreover, poly[3-(3,4-dihydroxyphenyl) glyceric acid from *S. asperum* and *S.caucasicum* roots inhibits the TNF-α production by human macrophages [127]. Furthermore, rosmarinic acid isolated from *Symphytum officinale* L. was shown to inhibit the formation of inflammation mediators of the arachidonic acid cascade in vitro [128]. Moreover, researchers conclude that comfrey root extract inhibits NF-κB by interfering with the activation pathway, at least in part at the level of IκB-α phosphorylation and possibly of IKK activation.

Mostly, medicines from comfrey root are presented in ointments ("Ointment Dr. Taissa with Comfrey", Dr. Theiss Naturwaren GmbH, Homburg, Germany, and "Ointment with Comfrey", DKP Pharmaceutical Factory, Zhytomyr, Ukraine) and used in the treatment of pain in the joints, back, lower back with radiculitis, osteochondrosis, arthritis, domestic injuries and bruises, sprains, dryness, and cracked skin. No research on the interaction of comfrey extracts with skin microbiome is available.

6. Conclusions

Plants have been used to prevent and treat skin diseases of various etiologies since ancient times. Due to their longstanding use, people have gained information regarding their effectiveness, active ingredients, as well as associated side effects. However, very often, it is not clear what mechanisms are responsible for the observed therapeutic effects. Moreover, recent advances in understanding the contribution of the skin microbiota in the maintenance of skin homeostasis can put new light on understanding the activity of topically applied plant-based products. Although the influence of various plants on skin-related ailments is well documented in vivo and in vitro, little is known about the interaction with the network of the skin microbial ecosystem, especially considering the prolonged treatment. It is also unclear whether skin microbiota can alter the chemical composition of herbal drugs applied directly on the skin surface. It was shown that some of the reported plant materials (e.g., sage leaves preparations) could have antimicrobial potential. However, available reports are strictly limited to investigating plant preparations influencing the growth of single strains of the chosen microorganisms. The analysis of the number of studies reported in Scopus between 2001 and 2021 using plant extract/material and skin microbiota as keywords showed that only 48 reports were found. Skin microbiota is a complex ecosystem that can certainly be modulated by plant extracts in many ways. Without solid basic studies involving human skin microbiota, the interaction of plant materials with the microbiome will remain unknown. Recently, there has been an arising interest in an investigation of the interaction between drugs and gut microbiota [129,130]. Based on the present review, it can be suspected that one of the major problems related to the lack of proper studies is the lack of well-described and reliable models that can be used for the investigation of the interactions between skin microbiome and plant extracts in vitro. More focus on this aspect of the problem is needed. For sure, future studies devoted to the investigation of skin microbiota with topically used plant materials or extracts are essential for the complex understating of the mechanism of action of those natural drugs in the prevention and treatment of various skin diseases.

Author Contributions: Conceptualization, S.G.; data writing—original draft preparation, N.M., W.S. and I.V.; writing—review and editing, N.M., W.S., S.G., A.B. and J.P.P.; supervision, S.G. All authors have read and agreed to the published version of the manuscript.

Funding: The presented research was financially supported by the NCN research grant Preludium Bis 2 No. 2020/39/O/NZ7/01109.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bilia, A.R. Herbal Medicinal Products *versus* Botanical-Food Supplements in the European market: State of Art and Perspectives. *Nat. Prod. Commun.* **2015**, *10*, 125–131. [CrossRef]
2. Aberer, W. Contact Allergy and Medicinal Herbs. *JDDG J. Ger. Soc. Dermatol.* **2007**, *6*, 15–24. [CrossRef]
3. Gurib-Fakim, A. Medicinal Plants: Traditions of Yesterday and Drugs of Tomorrow. *Mol. Asp. Med.* **2006**, *27*, 1–93. [CrossRef] [PubMed]
4. Shedoeva, A.; Leavesley, D.; Upton, Z.; Fan, C. Wound Healing and the Use of Medicinal Plants. *Evid. Based Complement. Altern. Med.* **2019**, *2019*, 2684108. [CrossRef]
5. Wyszkowska-Kolatko, M.; Koczurkiewicz, P.; Wójcik, K.; Pękala, E. Rośliny Lecznicze w Terapii Chorób Skóry. *Postępy Fitoter.* **2011**, *12*, 191–196.
6. Bos, J.D.; Kapsenberg, M.L. The Skin Immune System Its Cellular Constituents and their Interactions. *Immunol. Today* **1986**, *7*, 235–240. [CrossRef]
7. Bos, J.D.; Kapsenberg, M.L. The Skin Immune System: Progress in Cutaneous Biology. *Immunol. Today* **1993**, *14*, 75–78. [CrossRef]
8. Byrd, A.L.; Belkaid, Y.; Segre, J.A. The Human Skin Microbiome. *Nat. Rev. Microbiol.* **2018**, *16*, 143–155. [CrossRef]
9. Lai-Cheong, J.E.; McGrath, J.A. Structure and Function of Skin, Hair and Nails. *Medicine* **2021**, *49*, 337–342. [CrossRef]
10. Атопічний дерматит. Available online: https://www.dec.gov.ua/mtd/atopichnyj-dermatyt/ (accessed on 18 June 2022). (In Ukrainian)
11. Spiewak, R. Częstość Występowania Chorób Skóry w Losowej Grupie Dorosłych Polaków. *Estetologia Med. I Kosmetol.* **2012**, 50–53. [CrossRef]
12. Christensen, G.J.M.; Brüggemann, H. Bacterial Skin Commensals and their Role as Host Guardians. *Benef. Microbes* **2014**, *5*, 201–215. [CrossRef] [PubMed]
13. Zeeuwen, P.; Kleerebezem, M.; Timmerman, H.M.; Schalkwijk, J. Microbiome and Skin Diseases. *Curr. Opin. Allergy Clin. Immunol.* **2013**, *13*, 514–520. [CrossRef] [PubMed]
14. Belkaid, Y.; Segre, J.A. Dialogue Between Skin Microbiota and Immunity. *Science* **2014**, *346*, 954–959. [CrossRef]
15. Yu, Y.; Dunaway, S.; Champer, J.; Kim, J.; Alikhan, A. Changing Our Microbiome: Probiotics in Dermatology. *Br. J. Dermatol.* **2019**, *182*, 39–46. [CrossRef]
16. Dhama, K.; Karthik, K.; Khandia, R.; Munjal, A.; Tiwari, R.; Rana, R.; Khurana, S.K.; Ullah, S.; Khan, R.U.; Alagawany, M.; et al. Medicinal and Therapeutic Potential of Herbs and Plant Metabolites/Extracts Countering Viral Pathogens-Current Knowledge and Future Prospects. *Curr. Drug Metab.* **2018**, *19*, 236–263. [CrossRef]
17. Rahman, M.; Rahaman, S.; Islam, R.; Hossain, E.; Mithi, F.M.; Ahmed, M.; Saldías, M.; Akkol, E.K.; Sobarzo-Sánchez, E. Multifunctional Therapeutic Potential of Phytocomplexes and Natural Extracts for Antimicrobial Properties. *Antibiotics* **2021**, *10*, 1076. [CrossRef]
18. El-Najjar, N.; Gali-Muhtasib, H.; Ketola, R.A.; Vuorela, P.; Urtti, A.; Vuorela, H. The Chemical and Biological Activities of Quinones: Overview and Implications in Analytical Detection. *Phytochem. Rev.* **2011**, *10*, 353–370. [CrossRef]
19. Farha, A.K.; Yang, Q.-Q.; Kim, G.; Li, H.-B.; Zhu, F.; Liu, H.-Y.; Gan, R.-Y.; Corke, H. Tannins as an Alternative to Antibiotics. *Food Biosci.* **2020**, *38*, 100751. [CrossRef]
20. Popowski, D.; Czerwińska, M.E.; Kruk, A.; Pawłowska, K.A.; Zentek, J.; Melzig, M.F.; Piwowarski, J.P.; Granica, S. Gut Microbiota Metabolism and the Permeability of Natural Products Contained in Infusions from Herb of European Goldenrod *Solidago virgaurea* L. *J. Ethnopharmacol.* **2021**, *273*, 113924. [CrossRef]
21. Kruk, A.; Granica, S.; Popowski, D.; Malinowska, N.; Piwowarski, J.P. Tiliae Flos Metabolites and their Beneficial Influence on Human Gut Microbiota Biodiversity ex vivo. *J. Ethnopharmacol.* **2022**, *294*, 115355. [CrossRef]
22. 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.* **2015**, *59*, 2168–2177. [CrossRef]
23. Dawid-Pac, R. Medicinal Plants Used in Treatment of Inflammatory Skin Diseases. *Adv. Dermatol. Allergol.* **2013**, *3*, 170–177. [CrossRef]
24. ESCOP. *ESCOP Monographs: The Scientific Foundation for Herbal Medicinal Products*, 2nd ed.; European Scientific Cooperative on Phytotherapy: Exeter, UK, 2019. Available online: https://escop.com/ (accessed on 17 August 2022).
25. Кисличенко, В.С.; Журавель, І.О.; Марчишин, С.М.; Мінарченко, В.М.; Хворост, О.П. *фармакогнозія, Частина 1*; Національний фармацевтичний Університет: Харків, Україна, 2015; pp. 48–548. ISBN 978-966-400-365-7. (In Ukrainian)

26. Кисличенко, В.С.; Журавель, І.О.; Марчишин, С.М.; Мінарченко, В.М.; Хворост, О.П. *фармакогнозія Частина 2*; Національний фармацевтичний Університет: Харків, Україна, 2015; pp. 549–669. ISBN 978-966-400-365-7. (In Ukrainian)
27. HMPC. *Assessment Report on Allium cepa L., Bulbus*; EMA/HMPC/347195/2011; European Medicines Agency: London, UK, 2011. Available online: https://www.ema.europa.eu/en/documents/herbal-report/draft-assessment-report-allium-cepa-l-bulbus_en.pdf (accessed on 17 August 2022).
28. Teshika, J.D.; Zakariyyah, A.M.; Zaynab, T.; Zengin, G.; Rengasamy, K.R.; Pandian, S.K.; Fawzi, M.M. Traditional and Modern Uses of Onion Bulb (*Allium cepa* L.): A Systematic Review. *Crit. Rev. Food Sci. Nutr.* **2018**, *59*, S39–S70. [CrossRef]
29. Pikuła, M.; Żebrowska, M.E.; Poblocka-Olech, L.; Krauze-Baranowska, M.; Sznitowska, M.; Trzonkowski, P. Effect of Enoxaparin and Onion Extract on Human Skin Fibroblast Cell Line—Therapeutic Implications for the Treatment of Keloids. *Pharm. Biol.* **2013**, *52*, 262–267. [CrossRef]
30. Saulis, A.S.; Mogford, J.H.; Mustoe, T.A. Effect of Mederma on Hypertrophic Scarring in the Rabbit Ear Model. *Plast. Reconstr. Surg.* **2002**, *110*, 177–183. [CrossRef]
31. Chung, V.Q.; Kelley, L.; Marra, D.; Jiang, S.B. Onion Extract Gel Versus Petrolatum Emollient on New Surgical Scars: A Prospective Double-Blinded Study. *Dermatol. Surg.* **2006**, *32*, 193–197. [CrossRef]
32. Jackson, B.A.; Shelton, A.J. Pilot Study Evaluating Topical Onion Extract as Treatment for Postsurgical Scars. *Dermatol. Surg.* **1999**, *25*, 267–269. [CrossRef]
33. Prager, W.; Gauglitz, G.G. Effectiveness and Safety of an Overnight Patch Containing Allium cepa Extract and Allantoin for Post-Dermatologic Surgery Scars. *Aesthetic Plast. Surg.* **2018**, *42*, 1144–1150. [CrossRef]
34. Güngör, E.S.; Güzel, D.; Zebitay, A.G.; Ilhan, G.; Verit, F.F. The Efficacy of Onion Extract in the Management of Subsequent Abdominal Hypertrophic Scar Formation. *J. Wound Care* **2020**, *29*, 612–616. [CrossRef]
35. Campanati, A.; Savelli, A.; Sandroni, L.; Marconi, B.; Giuliano, A.; Giuliodori, K.; Ganzetti, G.; Offidani, A. Effect of Allium Cepa-Allantoin-Pentaglycan Gel on Skin Hypertrophic Scars: Clinical and Video-Capillaroscopic Results of an Open-Label, Controlled, Nonrandomized Clinical Trial. *Dermatol. Surg.* **2010**, *36*, 1439–1444. [CrossRef]
36. Campanati, A.; Ceccarelli, G.; Brisigotti, V.; Molinelli, E.; Martina, E.; Talevi, D.; Marconi, B.; Giannoni, M.; Markantoni, V.; Gregoriou, S.; et al. Effects of in Vivo Application of an Overnight Patch Containing *Allium Cepa*, Allantoin, and Pentaglycan on Hypertrophic Scars and Keloids: Clinical, Videocapillaroscopic, and Ultrasonographic Study. *Dermatol. Ther.* **2020**, *34*, e14665. [CrossRef] [PubMed]
37. Reynolds, T.; Dweck, A. Aloe vera leaf gel: A review update. *J. Ethnopharmacol.* **1999**, *68*, 3–37. [CrossRef]
38. Sánchez, M.; González-Burgos, E.; Iglesias, I.; Gómez-Serranillos, M.P. Pharmacological Update Properties of Aloe Vera and its Major Active Constituents. *Molecules* **2020**, *25*, 1324. [CrossRef] [PubMed]
39. Radha, M.H.; Laxmipriya, N.P. Evaluation of Biological Properties and Clinical Effectiveness of Aloe Vera: A Systematic Review. *J. Tradit. Complement. Med.* **2014**, *5*, 21–26. [CrossRef] [PubMed]
40. Kriplani, P.; Guarve, K.; Baghael, U.S. *Arnica montana* L.—A Plant of Healing: Review. *J. Pharm. Pharmacol.* **2017**, *69*, 925–945. [CrossRef]
41. Oberbaum, M.; Galoyan, N.; Lerner-Geva, L.; Singer, S.R.; Grisaru, S.; Shashar, D.; Samueloff, A. The Effect of the Homeopathic Remedies Arnica Montana and Bellis Perennis on Mild Postpartum Bleeding—A Randomized, Double-Blind, Placebo-Controlled Study—Preliminary Results. *Complement. Ther. Med.* **2005**, *13*, 87–90. [CrossRef]
42. HMPC. *Assessment Report on Arnica montana L., Flos*; EMA/HMPC/198794/2014; European Medicines Agency: London, UK, 2011. Available online: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-arnica-montana-l-flos_en.pdf (accessed on 17 August 2022).
43. Prade, J.D.S.; Bálsamo, E.C.; Machado, F.R.; Poetini, M.R.; Bortolotto, V.C.; Araújo, S.M.; Londero, L.; Boeira, S.P.; Sehn, C.P.; de Gomes, M.G.; et al. Anti-inflammatory effect of Arnica montana in a UVB radiation-induced skin-burn model in mice. *Cutan. Ocul. Toxicol.* **2020**, *39*, 126–133. [CrossRef]
44. Huber, R.; Bross, F.; Schempp, C.; Gründemann, C. Arnica and stinging nettle for treating burns—A self-experiment. *Complement. Ther. Med.* **2011**, *19*, 276–280. [CrossRef]
45. Hillebrand, G.; Ike, R.B.; Dimitriu, P.; Monh, W. Method and Topical Composition for Modification of Skin Microbiome. Patent PCT/US2018/0629387, 29 November 2018. Available online: https://patents.google.com/patent/US20190160117A1 (accessed on 17 August 2022).
46. Arora, D.; Rani, A.; Sharma, A. A Review on Phytochemistry and Ethnopharmacological Aspects of Genus Calendula. *Pharmacogn. Rev.* **2013**, *7*, 179–187. [CrossRef]
47. Muley, B.P.; Khadabadi, S.S.; Banarase, N. Phytochemical Constituents and Pharmacological Activities of Calendula officinalis Linn (Asteraceae): A Review. *Trop. J. Pharm. Res.* **2009**, *8*, 48090. [CrossRef]
48. Fialová, S.B.; Rendeková, K.; Mučaji, P.; Nagy, M.; Slobodníková, L. Antibacterial Activity of Medicinal Plants and Their Constituents in the Context of Skin and Wound Infections, Considering European Legislation and Folk Medicine—A Review. *Int. J. Mol. Sci.* **2021**, *22*, 10746. [CrossRef] [PubMed]
49. HMPC. *Assessment Report on Calendula officinalis L., Flos*; EMA/HMPC/603409/2017; European Medicines Agency: London, UK, 2018. Available online: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-calendula-officinalis-l-flos-revision-1_en.pdf (accessed on 17 August 2022).

50. Parente, L.M.L.; Júnior, R.D.S.L.; Tresvenzol, L.M.F.; Vinaud, M.C.; de Paula, J.R.; Paulo, N.M. Wound Healing and Anti-Inflammatory Effect in Animal Models of *Calendula officinalis* L. Growing in Brazil. *Evid. Based Complement. Altern. Med.* **2012**, *2012*, 375671. [CrossRef] [PubMed]
51. Tresch, M.; Mevissen, M.; Ayrle, H.; Melzig, M.; Roosje, P.; Walkenhorst, M. Medicinal Plants as Therapeutic Options for Topical Treatment in Canine Dermatology? A Systematic Review. *BMC Veter Res.* **2019**, *15*, 174. [CrossRef] [PubMed]
52. Bernatoniene, J.; Masteikova, R.; Davalgiene, J.; Peciura, R.; Gauryliene, R.; Bernatoniene, R.; Majiene, D.; Lazauskas, R.; Civinskiene, G.; Velziene, S.; et al. Topical Application of *Calendula officinalis* (L.): Formulation and Evaluation of Hydrophilic Cream with Antioxidant Activity. *J. Med. Plants Res.* **2011**, *5*, 868–877. [CrossRef]
53. Hu, J.J.; Cui, T.; Rodriguez-Gil, J.L.; Allen, G.O.; Li, J.; Takita, C.; Lally, B.E. Complementary and Alternative Medicine in Reducing Radiation-Induced Skin Toxicity. *Radiat. Environ. Biophys.* **2014**, *53*, 621–626. [CrossRef]
54. Serra, M.B.; Barroso, W.A.; Da Silva, N.N.; Silva, S.D.N.; Borges, A.C.R.; Abreu, I.C.; Borges, M.O.D.R. From Inflammation to Current and Alternative Therapies Involved in Wound Healing. *Int. J. Inflamm.* **2017**, *2017*, 3406215. [CrossRef]
55. Wu, X.X.; Siu, W.S.; Wat, C.L.; Chan, C.L.; Koon, C.M.; Li, X.; Cheng, W.; Ma, H.; Tsang, M.S.M.; Lam, C.W.-K.; et al. Effects of Topical Application of a Tri-Herb Formula on Inflammatory Dry-Skin Condition in Mice with Oxazolone-Induced Atopic Dermatitis. *Phytomedicine* **2021**, *91*, 153691. [CrossRef]
56. Alnuqaydan, A.M.; Lenehan, C.E.; Hughes, R.R.; Sanderson, B.J. Extracts from *Calendula officinalis* Offer in Vitro Protection Against H_2O_2Induced Oxidative Stress Cell Killing of Human Skin Cells. *Phytotherapy Res.* **2014**, *29*, 120–124. [CrossRef]
57. Nicolaus, C.; Junghanns, S.; Hartmann, A.; Murillo, R.; Ganzera, M.; Merfort, I. In Vitro Studies to Evaluate the Wound Healing Properties of Calendula Officinalis Extracts. *J. Ethnopharmacol.* **2017**, *196*, 94–103. [CrossRef]
58. Pelletier-ahmed, L. Selective Inhibition of Propionibacterium Acnes by Calendula Officinalis: A Potential Role in Acne Treatment through Modulation of the Skin Microbiome. Master's. Thesis, Middlesex University, London, UK, 2016. [CrossRef]
59. HMPC. *Public Statement on Chelidonium majus L., Herba*; EMA/HMPC/743927/2010; European Medicines Agency: London, UK, 2011. Available online: https://www.ema.europa.eu/en/documents/public-statement/final-public-statement-chelidonium-majus-l-herba_en.pdf (accessed on 17 August 2022).
60. Zielińska, S.; Jezierska-Domaradzka, A.; Wójciak-Kosior, M.; Sowa, I.; Junka, A.; Matkowski, A.M. Greater Celandine's Ups and Downs−21 Centuries of Medicinal Uses of Chelidonium majus From the Viewpoint of Today's Pharmacology. *Front. Pharmacol.* **2018**, *9*, 299. [CrossRef]
61. Cordes, N.; Plasswilm, L.; Bamberg, M.; Rodemann, H.P. Ukrain ®, an Alkaloid Thiophosphoric Acid Derivative of Chelidonium Majus L. Protects Human Fibroblasts but not Human Tumour Cells in Vitro Against Ionizing Radiation. *Int. J. Radiat. Biol.* **2002**, *78*, 17–27. [CrossRef] [PubMed]
62. Vavrečková, C.; Gawlik, I.; Müller, K. Benzophenanthridine Alkaloids ofChelidonium majus; II. Potent Inhibitory Action Against the Growth of Human Keratinocytes. *Planta Medica* **1996**, *62*, 491–494. [CrossRef] [PubMed]
63. Raposo, A.; Saraiva, A.; Ramos, F.; Carrascosa, C.; Raheem, D.; Bárbara, R.; Silva, H. The Role of Food Supplementation in Microcirculation—A Comprehensive Review. *Biology* **2021**, *10*, 616. [CrossRef] [PubMed]
64. HMPC. *Assessment Report on Hamamelis virginiana L., Cortex, Hamamelis virginiana L. Folium, Hamamelis virginiana L., Folium et Cortex Aut Ramunculus Destillatum*; EMA/HMPC/114585/2008; European Medicines Agency: London, UK, 2009. Available online: https://www.ema.europa.eu/en/documents/herbal-report/assessment-report-hamamelis-virginiana-l-cortex-hamamelis-virginiana-l-folium-hamamelis-virginiana-l_en.pdf (accessed on 17 August 2022).
65. Natella, F.; Guantario, B.; Ambra, R.; Ranaldi, G.; Intorre, F.; Burki, C.; Canali, R. Human Metabolites of Hamaforton™ (*Hamamelis virginiana* L. Extract) Modulates Fibroblast Extracellular Matrix Components in Response to UV-A Irradiation. *Front. Pharmacol.* **2021**, *12*, 747638. [CrossRef] [PubMed]
66. Smeriglio, A.; Barreca, D.; Bellocco, E.; Trombetta, D. Proanthocyanidins and Hydrolysable Tannins: Occurrence, Dietary Intake and Pharmacological Effects. *Br. J. Pharmacol.* **2017**, *174*, 1244–1262. [CrossRef]
67. Wang, H.; Provan, G.J.; Helliwell, K. Determination of Hamamelitannin, Catechins and Gallic Acid in Witch Hazel Bark, Twig and Leaf by HPLC. *J. Pharm. Biomed. Anal.* **2003**, *33*, 539–544. [CrossRef]
68. Duckstein, S.M.; Stintzing, F.C. Investigation on the Phenolic Constituents in Hamamelis Virginiana Leaves by HPLC-DAD and LC-MS/MS. *Anal. Bioanal. Chem.* **2011**, *401*, 677–688. [CrossRef]
69. Masaki, H.; Atsumi, T.; Sakurai, H. Hamamelitannin as a New Potent Active Oxygen Scavenger. *Phytochemistry* **1994**, *37*, 337–343. [CrossRef]
70. Theisen, L.L.; Erdelmeier, C.A.J.; Spoden, G.A.; Boukhallouk, F.; Sausy, A.; Florin, L.; Muller, C.P. Tannins from Hamamelis virginiana Bark Extract: Characterization and Improvement of the Antiviral Efficacy against Influenza A Virus and Human Papillomavirus. *PLoS ONE* **2014**, *9*, e88062. [CrossRef]
71. Thring, T.S.; Hili, P.; Naughton, D.P. Antioxidant and Potential Anti-inflammatory Activity of Extracts and Formulations of White Tea, Rose, and Witch Hazel on Primary Human Dermal Fibroblast Cells. *J. Inflamm.* **2011**, *8*, 27. [CrossRef]
72. Choi, J.; Yang, D.; Moon, M.Y.; Han, G.Y.; Chang, M.S.; Cha, J. The Protective Effect of *Hamamelis virginiana* Stem and Leaf Extract on Fine Dust-Induced Damage on Human Keratinocytes. *Cosmetics* **2021**, *8*, 119. [CrossRef]
73. Dudau, M.; Codrici, E.; Tarcomnicu, I.; Mihai, S.; Popescu, I.D.; Albulescu, L.; Constantin, N.; Cucolea, I.; Costache, T.; Rambu, D.; et al. A Fatty Acid Fraction Purified From Sea Buckthorn Seed Oil Has Regenerative Properties on Normal Skin Cells. *Front. Pharmacol.* **2021**, *12*, 737571. [CrossRef] [PubMed]

74. Dudau, M.; Vilceanu, A.; Codrici, E.; Mihai, S.; Popescu, I.; Albulescu, L.; Tarcomnicu, I.; Moise, G.; Ceafalan, L.; Hinescu, M.; et al. Sea-Buckthorn Seed Oil Induces Proliferation of both Normal and Dysplastic Keratinocytes in Basal Conditions and under UVA Irradiation. *J. Pers. Med.* **2021**, *11*, 278. [CrossRef] [PubMed]
75. Balkrishna, A.; Sakat, S.S.; Joshi, K.; Joshi, K.; Sharma, V.; Ranjan, R.; Bhattacharya, K.; Varshney, A. Cytokines Driven Anti-Inflammatory and Anti-Psoriasis Like Efficacies of Nutraceutical Sea Buckthorn (*Hippophae rhamnoides*) Oil. *Front. Pharmacol.* **2019**, *10*, 1186. [CrossRef]
76. ESCOP. Lini Semen (Linseed). Available online: https://escop.com/lini-semen-linseed-online/ (accessed on 17 August 2022).
77. HMPC. *Assessment report on Linum usitatissimum L., Semen*; EMA/HMPC/377674/2014; European Medicines Agency: London, UK, 2015. Available online: https://www.ema.europa.eu/en/documents/herbal-report/assessment-report-linum-usitatissimum-l-semen_en.pdf (accessed on 17 August 2022).
78. Sederski, M.E. *Prawie Wszystko o Ziołach i Ziołolecznictwie*; Mateusz, E., Ed.; Senderski: Wroclaw, Poland, 2017; p. 383. ISBN 978-83-945-5401-9.
79. Bardaa, S.; Turki, M.; Ben Khedir, S.; Mzid, M.; Rebai, T.; Ayadi, F.; Sahnoun, Z. The Effect of Prickly Pear, Pumpkin, and Linseed Oils on Biological Mediators of Acute Inflammation and Oxidative Stress Markers. *BioMed Res. Int.* **2020**, *2020*, 5643465. [CrossRef]
80. Bardaa, S.; Moalla, D.; Ben Khedir, S.; Rebai, T.; Sahnoun, Z. The Evaluation of the Healing Proprieties of Pumpkin and Linseed Oils on Deep Second-Degree Burns in Rats. *Pharm. Biol.* **2015**, *54*, 581–587. [CrossRef]
81. Naseri, S.; Golpich, M.; Roshancheshm, T.; Joobeni, M.G.; Khodayari, M.; Noori, S.; Zahed, S.A.; Razzaghi, S.; Shirzad, M.; Salavat, F.S.; et al. The Effect of Henna and Linseed Herbal Ointment Blend on Wound Healing in Rats with Second-Degree Burns. *Burns* **2020**, *47*, 1442–1450. [CrossRef]
82. Beroual, K.; Agabou, A.; Abdeldjelil, M.-C.; Boutaghane, N.; Haouam, S.; Hamdi-Pacha, Y. Evaluation of Crude Flaxseed (*Linum usitatissimum* L.) Oil in Burn Wound Healenig New Zeland Rabbits. *Afr. J. Tradit. Complement. Altern. Med.* **2017**, *14*, 280–286. [CrossRef]
83. Trabelsi, I.; Ben Slima, S.; Ktari, N.; Bardaa, S.; Elkaroui, K.; Abdeslam, A.; Ben Salah, R. Purification, Composition and Biological Activities of a Novel Heteropolysaccharide Extracted from *Linum usitatissimum* L. Seeds on Laser Burn Wound. *Int. J. Biol. Macromol.* **2019**, *144*, 781–790. [CrossRef]
84. Franco, E.D.S.; de Aquino, C.M.F.; de Medeiros, P.L.; Evêncio, L.B.; Góes, A.J.D.S.; Maia, M.B.D.S. Effect of a Semisolid Formulation of *Linum usitatissimum* L. (Linseed) Oil on the Repair of Skin Wounds. *Evid.-Based Complement. Altern. Med.* **2011**, *2012*, 270752. [CrossRef]
85. Farahpour, M.R. Wound Healing Activity of Flaxseed *Linum usitatissimum* L. in Rats. *Afr. J. Pharm. Pharmacol.* **2011**, *5*, 258. [CrossRef]
86. Rafiee, S.; Nekouyian, N.; Sarabandi, F.; Chavoshi-Nejad, M.; Mohsenikia, M.; Yadollah-Damavandi, S.; Seifaee, A.; Jangholi, E.; Eghtedari, D.; Najafi, H.; et al. Effect of Topical Linum usitatissimum on Full Thickness Excisional Skin Wounds. *Trauma Mon.* **2016**, *22*, 39045. [CrossRef]
87. Abdul Jabbar, O.; Kashmoola, M.A.; Mustafa Al-Ahmad, B.E.; Mokhtar, K.I.; Muhammad, N.; Abdul Rahim, R.; Qouta, L.A. The Effect of Flaxseed Extract on Skin Elasticity of The Healing Wound In Rabbits. *IIUM Med. J. Malays.* **2019**, *18*, 230. [CrossRef]
88. ESCOP. Matricariae flos (Matricaria flower). Available online: https://escop.com/downloads/matricariae-flos-matricaria-flower/ (accessed on 17 August 2022).
89. HMPC. *Assessment Report on Matricaria recutita L., Flos*; EMA/HMPC/55837/2011; European Medicines Agency: London, UK, 2015. Available online: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-matricaria-recutita-l-flos-matricaria-recutita-l-aetheroleum-first-version_en.pdf (accessed on 17 August 2022).
90. Wu, Y.-N.; Xu, Y.; Yao, L. Anti-Inflammatory and Anti-allergic Effects of German Chamomile (*Matricaria chamomilla* L.). *J. Essent. Oil Bear. Plants* **2011**, *14*, 549–558. [CrossRef]
91. Jarrahi, M. An Experimental Study of the Effects of *Matricaria Chamomilla* Extract on Cutaneous Burn Wound Healing in Albino Rats. *Nat. Prod. Res.* **2008**, *22*, 422–427. [CrossRef]
92. Jarrahi, M.; Vafaei, A.A.; Taherian, A.A.; Miladi, H.; Pour, A.R. Evaluation of Topical *Matricaria Chamomilla* Extract Activity on Linear Incisional Wound Healing in Albino Rats. *Nat. Prod. Res.* **2010**, *24*, 697–702. [CrossRef]
93. Nematollahi, P.; Aref, N.M.; Meimandi, F.Z.; Rozei, S.L.; Zareé, H.; Mirlohi, S.M.J.; Rafiee, S.; Mohsenikia, M.; Soleymani, A.; Ashkani-Esfahani, S.; et al. Matricaria Chamomilla Extract Improves Diabetic Wound Healing in Rat Models. *Trauma Mon.* 2019; *in press*. [CrossRef]
94. Niknam, S.; Tofighi, Z.; Faramarzi, M.A.; Abdollahifar, M.A.; Sajadi, E.; Dinarvand, R.; Toliyat, T. Polyherbal Combination for Wound Healing: *Matricaria chamomilla* L. and *Punica granatum* L. *DARU J. Pharm. Sci.* **2021**, *29*, 133–145. [CrossRef]
95. Khashan, A.A.; Hamad, M.A.; Jadaan, M.S. In Vivo Antimicrobial Activity of Matricaria Chamomilla Extract against Pathogenic Bacteria Induced Skin Infections in Mice. *Syst. Rev. Pharm.* **2020**, *11*, 672–676. [CrossRef]
96. Nobrega, A.T.; Wagemaker, T.A.L.; Campos, P.M.B.G.M. Antioxidant Activity of *Matricaria chamomilla* L. Extract and Clinical Efficacy of Cosmetic Formulations Containing This Extract and Its Isolated Compounds. *J. Biomed. Biopharm. Res.* **2013**, *10*, 249–261. [CrossRef]

97. Ferreira, E.B.; Ciol, M.A.; De Meneses, A.G.; Bontempo, P.D.S.M.; Hoffman, J.M.; Dos Reis, P.E.D. Chamomile Gel versus Urea Cream to Prevent Acute Radiation Dermatitis in Head and Neck Cancer Patients: Results from a Preliminary Clinical Trial. *Integr. Cancer Ther.* **2020**, *19*, 1534735420962174. [CrossRef] [PubMed]
98. Lee, S.-H.; Heo, Y.; Kim, Y.-C. Effect of German Chamomile Oil Application on Alleviating Atopic Dermatitis-Like Immune Alterations in Mice. *J. Veter Sci.* **2010**, *11*, 35–41. [CrossRef] [PubMed]
99. ESCOP. Tormentillae Rhizoma. Available online: https://escop.com/wp-content/uploads/edd/2015/09/Tormentil.pdf (accessed on 17 August 2022).
100. HMPC. *Assessment Report on Potentilla erecta (L.) Raeusch., Rhizoma*; EMA/HMPC/5511/2010; European Medicines Agency: London, UK, 2010. Available online: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-potentilla-erecta-l-raeusch-rhizoma_en.pdf (accessed on 17 August 2022).
101. Melzig, M.F.; Böttger, S. Tormentillae rhizoma—Review for an Underestimated European Herbal Drug. *Planta Medica* **2020**, *86*, 1050–1057. [CrossRef] [PubMed]
102. Hoffmann, J.; Casetti, F.; Bullerkotte, U.; Haarhaus, B.; Vagedes, J.; Schempp, C.M.; Wölfle, U. Anti-Inflammatory Effects of Agrimoniin-Enriched Fractions of Potentilla erecta. *Molecules* **2016**, *21*, 792. [CrossRef]
103. Wölfle, U.; Hoffmann, J.; Haarhaus, B.; Mittapalli, V.R.; Schempp, C.M. Anti-Inflammatory and Vasoconstrictive Properties of Potentilla Erecta—A Traditional Medicinal Plant From the Northern Hemisphere. *J. Ethnopharmacol.* **2017**, *204*, 86–94. [CrossRef]
104. Kaltalioglu, K.; Balabanli, B.; Coskun-Cevher, S. Phenolic, Antioxidant, Antimicrobial, and In-vivo Wound Healing Properties of *Potentilla erecta* L. *Root Extract in Diabetic Rats.* **2020**, *19*, 264–274. [CrossRef]
105. HMPC. *Assessment Report on Quercus robur L., Quercus petraea (Matt.) Liebl., Quercus pubescens Willd, Cortex*; EMA/HMPC/3206/2009; European Medicines Agency: London, UK, 2010. Available online: https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-quercus-robur-l-quercus-petraea-matt-liebl-quercus-pubescens-willd-cortex_en.pdf (accessed on 17 August 2022).
106. HMPC. *Community Herbal Monograph on Quercus robur L., Quercus petraea.(Matt.) Liebl., Quercus pubescens Willd, Cortex*; EMA/HMPC/3203/2009; European Medicines Agency: London, UK, 2010. Available online: https://www.ema.europa.eu/en/documents/herbal-monograph/final-community-herbal-monograph-quercus-robur-l-quercus-petraea-matt-liebl-quercus-pubescens-willd_en.pdf (accessed on 17 August 2022).
107. Hong, J.-A.; Bae, D.; Oh, K.-N.; Oh, D.-R.; Kim, Y.; Kim, Y.; Im, S.J.; Choi, E.-J.; Lee, S.-G.; Kim, M.; et al. Protective Effects of Quercus Acuta Thunb. Fruit Extract Against UVB-induced Photoaging through ERK/AP-1 Signaling Modulation in Human Keratinocytes. *BMC Complement. Med. Ther.* **2022**, *22*, 6. [CrossRef]
108. Yin, J.; Kim, H.H.; Hwang, I.H.; Kim, D.H.; Lee, M.W. Anti-Inflammatory Effects of Phenolic Compounds Isolated from Quercus Mongolica Fisch. ex Ledeb. on UVB-Irradiated Human Skin Cells. *Molecules* **2019**, *24*, 3094. [CrossRef]
109. Lee, C.S.; Yi, E.H.; Kim, H.-R.; Huh, S.-R.; Sung, S.-H.; Chung, M.-H.; Ye, S.-K. Anti-dermatitis Effects of Oak Wood Vinegar on the DNCB-induced Contact Hypersensitivity via STAT3 Suppression. *J. Ethnopharmacol.* **2011**, *135*, 747–753. [CrossRef]
110. ESCOP. Salviae Folium (Sage Leaf). Available online: https://escop.com/downloads/salviae-folium-sage-leaf/ (accessed on 17 August 2022).
111. HMPC. *European Union Herbal Monograph on Salvia officinalis L., Folium*; EMA/HMPC/277152/2015; European Medicines Agency: London, UK, 2016. Available online: https://www.ema.europa.eu/en/documents/herbal-monograph/final-european-union-herbal-monograph-salvia-officinalis-l-folium-revision-1_en.pdf (accessed on 17 August 2022).
112. Grdiša, M.; Jug-Dujaković, M.; Lončarić, M.; Carović-Stanko, K.; Ninčević, T.; Liber, Z.; Radosavljević, I.; Šatović, Z. Dalmatian Sage (*Salvia officinalis* L.): A Review of Biochemical Contents, Medical Properties and Genetic Diversity. *Agric. Conspec. Sci.* **2015**, *80*, 69–78.
113. Baricevic, D.; Sosa, S.; Della Loggia, R.; Tubaro, A.; Simonovska, B.; Krasna, A.; Zupancic, A. Topical Anti-inflammatory Activity of *Salvia officinalis* L. Leaves: The Relevance of Ursolic Acid. *J. Ethnopharmacol.* **2001**, *75*, 125–132. [CrossRef]
114. Abu-Darwish, M.S.; Cabral, C.; Ferreira, I.V.; Gonçalves, M.J.; Cavaleiro, C.; Cruz, M.T.; Al-Bdour, T.H.; Salgueiro, L. Essential Oil of Common Sage (*Salvia officinalis* L.) from Jordan: Assessment of Safety in Mammalian Cells and Its Antifungal and Anti-Inflammatory Potential. *BioMed Res. Int.* **2013**, *2013*, 538940. [CrossRef] [PubMed]
115. Farahpour, M.R.; Pirkhezr, E.; Ashrafian, A.; Sonboli, A. Accelerated Healing by Topical Administration of Salvia Officinalis Essential Oil on Pseudomonas Aeruginosa and Staphylococcus Aureus Infected Wound Model. *Biomed. Pharmacother.* **2020**, *128*, 110120. [CrossRef] [PubMed]
116. Karimzadeh, S.; Farahpour, M.R. Topical Application of Salvia Officinalis Hydroethanolic Leaf Extract Improves Wound Healing Process. *Indian J. Exp. Biol.* **2017**, *55*, 98–106.
117. Khare, R.; Upmanyu, N.; Jha, M. Exploring the Potential Effect of Methanolic Extract of Salvia officinalis Against UV Exposed Skin Aging: In vivo and In vitro Model. *Curr. Aging Sci.* **2021**, *14*, 46–55. [CrossRef]
118. Reuter, J.; Jocher, A.; Hornstein, S.; Mönting, J.S.; Schempp, C.M. Sage Extract Rich in Phenolic Diterpenes Inhibits Ultraviolet-Induced Erythema in Vivo. *Planta Medica* **2007**, *73*, 1190–1191. [CrossRef]
119. He, X.; Bai, Y.; Zhao, Z.; Wang, X.; Fang, J.; Huang, L.; Zeng, M.; Zhang, Q.; Zhang, Y.; Zheng, X. Local and Traditional Uses, Phytochemistry, and Pharmacology of *Sophora japonica* L.: A Review. *J. Ethnopharmacol.* **2016**, *187*, 160–182. [CrossRef]

120. Li, L.; Huang, T.; Lan, C.; Ding, H.; Yan, C.; Dou, Y. Protective Effect of Polysaccharide from Sophora Japonica L. Flower Buds Against UVB Radiation in a Human Keratinocyte Cell Line (HaCaT Cells). *J. Photochem. Photobiol. B Biol.* **2018**, *191*, 135–142. [CrossRef]
121. Lee, H.K.; Kim, H.S.; Kim, Y.J.; Kim, J.S.; Park, Y.S.; Kang, J.S.; Yuk, D.Y.; Hong, J.T.; Kim, Y.; Han, S.-B. Sophoricoside isolated from Sophora japonica ameliorates contact dermatitis by inhibiting NF-κB signaling in B cells. *Int. Immunopharmacol.* **2013**, *15*, 467–473. [CrossRef]
122. Min, B.; Oh, S.R.; Lee, H.-K.; Takatsu, K.; Chang, I.-M.; Min, K.R.; Kim, Y. Sophoricoside Analogs as the IL-5 Inhibitors from Sophora japonica. *Planta Medica* **1999**, *65*, 408–412. [CrossRef]
123. Kim, B.H.; Chung, E.Y.; Ryu, J.-C.; Jung, S.-H.; Min, K.R.; Kim, Y. Anti-inflammatory Mode of Isoflavone Glycoside Sophoricoside by Inhibition of lnterleukin-6 and Cyclooxygenase-2 in Inflammatory Response. *Arch. Pharmacal Res.* **2003**, *26*, 306–311. [CrossRef] [PubMed]
124. Salehi, B.; Sharopov, F.; Tumer, T.B.; Ozleyen, A.; Rodríguez-Pérez, C.; Ezzat, S.M.; Azzini, E.; Hosseinabadi, T.; Butnariu, M.; Sarac, I.; et al. Symphytum Species: A Comprehensive Review on Chemical Composition, Food Applications and Phytopharmacology. *Molecules* **2019**, *24*, 2272. [CrossRef] [PubMed]
125. Sowa, I.; Paduch, R.; Strzemski, M.; Zielińska, S.; Rydzik-Strzemska, E.; Sawicki, J.; Kocjan, R.; Polkowski, J.; Matkowski, A.; Latalski, M.; et al. Proliferative and Antioxidant Activity of *Symphytum Officinale* Root Extract. *Nat. Prod. Res.* **2017**, *32*, 605–609. [CrossRef] [PubMed]
126. Silva-Barcellos, N.M.; Araujo, L.U.; Reis, P.G. In Vivo Wound Healing Effects of *Symphytum officinale* L. Leaves extract in different topical formulations. *Die Pharm. Int. J. Pharm. Sci.* **2012**, *67*, 355–360. [CrossRef]
127. Mulkijanyan, K.; Barbakadze, V.; Novikova, Z.; Sulakvelidze, M.; Gogilashvili, L.; Amiranashvili, L.; Merlani, M. Burn Healing Compositions from Caucasian Species of Comfrey (*Symphytum* L.). *Chemistry* **2009**, *3*, 2–5.
128. Matta, K.L.; Ochrymowycz, A.; Neplyuev, V.M.; Sinenko, A.; Pel, S.; Miiller, C.; Gracza, L.; Koch, H.; Löffler, E. Isolierung von Rosmarinsaure Aus Symphytum Officinale Und Ihre Anti-Inflammatorische Wirksamkeit in Einem In-Vitro-Model. *Arch. Pharm.* **1985**, *318*, 1090–1095.
129. Piwowarski, J.P.; Granica, S.; Kiss, A.K. Influence of Gut Microbiota-Derived Ellagitannins' Metabolites Urolithins on Pro-Inflammatory Activities of Human Neutrophils. *Planta Medica* **2014**, *80*, 887–895. [CrossRef]
130. Piwowarski, J.P.; Granica, S.; Zwierzyńska, M.; Stefańska, J.; Schopohl, P.; Melzig, M.F.; Kiss, A.K. Role of Human Gut Microbiota Metabolism in the Anti-inflammatory Effect of Traditionally Used Ellagitannin-Rich Plant Materials. *J. Ethnopharmacol.* **2014**, *155*, 801–809. [CrossRef]

Manuscript co-author statement

As a one of co-authors of the manuscript: "**Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota**" published in *International Journal of Molecular Sciences* (Volume 23, 25 August 2022, 9644, DOI: 10.3390/ijms23179644) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: „**Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota** " opublikowanego w *International Journal of Molecular Sciences* (tom 23, 25 sierpnia 2022, 9644, DOI: 10.3390/ijms23179644) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Conceptualization the review, writing and compilation the main body of the manuscript, integration contributions from all co-authors, preparation all tables and figures, and finalization the manuscript for submission. Conduction the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie koncepcji przeglądu, przygotowanie głównej części manuskryptu, wszystkich tabel i rysunków, integracja wkładu współautorów oraz finalizacja tekstu do publikacji. Analiza ukraińskiego rynku farmaceutycznego w Tabeli 2.
Inna Vlasova	Writing sections 5.6, assistance in the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.6, udział w analizie ukraińskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Weronika Skowrońska	Writing sections 5.1, 5.8, 5.9, 5.10, and 5.12. Conduction the analysis of the Polish pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.1, 5.8, 5.9, 5.10 i 5.12. Przeprowadzenie analizy polskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Agnieszka Bazylko	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Jakub P. Piwowarski	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Sebastian Granica	Conceptualization the review; reviewing and editing the final version; project supervision.
	Koncepcja przeglądu; sprawdzanie i redagowanie ostatecznej wersji; nadzór nad projektem.

26.01.2026

Date / Data

Natalia Melnyk

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: **"Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota"** published in *International Journal of Molecular Sciences* (Volume 23, 25 August 2022, 9644, DOI: 10.3390/ijms23179644) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **„Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota "** opublikowanego w *International Journal of Molecular Sciences* (tom 23, 25 sierpnia 2022, 9644, DOI: 10.3390/ijms23179644) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name	Scope of contribution
Imię i nazwisko współautora	**Zakres wkładu**
Natalia Melnyk	Conceptualization the review, writing and compilation the main body of the manuscript, integration contributions from all co-authors, preparation all tables and figures, and finalization the manuscript for submission. Conduction the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie koncepcji przeglądu, przygotowanie głównej części manuskryptu, wszystkich tabel i rysunków, integracja wkładu współautorów oraz finalizacja tekstu do publikacji. Analiza ukraińskiego rynku farmaceutycznego w Tabeli 2.
Inna Vlasova	Writing sections 5.6, assistance in the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.6, udział w analizie ukraińskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Weronika Skowrońska	Writing sections 5.1, 5.8, 5.9, 5.10, and 5.12. Conduction the analysis of the Polish pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.1, 5.8, 5.9, 5.10 i 5.12. Przeprowadzenie analizy polskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Agnieszka Bazylko	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Jakub P. Piwowarski	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Sebastian Granica	Conceptualization the review; reviewing and editing the final version; project supervision.
	Koncepcja przeglądu; sprawdzanie i redagowanie ostatecznej wersji; nadzór nad projektem.

19.01.2026

Date / Data

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: "**Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota**" published in *International Journal of Molecular Sciences* (Volume 23, 25 August 2022, 9644, DOI: 10.3390/ijms23179644) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: „**Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota** " opublikowanego w *International Journal of Molecular Sciences* (tom 23, 25 sierpnia 2022, 9644, DOI: 10.3390/ijms23179644) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name **Imię i nazwisko współautora**	**Scope of contribution** **Zakres wkładu**
Natalia Melnyk	Conceptualization the review, writing and compilation the main body of the manuscript, integration contributions from all co-authors, preparation all tables and figures, and finalization the manuscript for submission. Conduction the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie koncepcji przeglądu, przygotowanie głównej części manuskryptu, wszystkich tabel i rysunków, integracja wkładu współautorów oraz finalizacja tekstu do publikacji. Analiza ukraińskiego rynku farmaceutycznego w Tabeli 2.
Inna Vlasova	Writing sections 5.6, assistance in the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.6, udział w analizie ukraińskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Weronika Skowrońska	Writing sections 5.1, 5.8, 5.9, 5.10, and 5.12. Conduction the analysis of the Polish pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.1, 5.8, 5.9, 5.10 i 5.12. Przeprowadzenie analizy polskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Agnieszka Bazylko	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Jakub P. Piwowarski	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Sebastian Granica	Conceptualization the review; reviewing and editing the final version; project supervision.
	Koncepcja przeglądu; sprawdzanie i redagowanie ostatecznej wersji; nadzór nad projektem.

21.01.2026 ..... Weronika Skowrońska .....

Date / Data

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: "**Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota**" published in *International Journal of Molecular Sciences* (Volume 23, 25 August 2022, 9644, DOI: 10.3390/ijms23179644) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **„Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota "** opublikowanego w *International Journal of Molecular Sciences* (tom 23, 25 sierpnia 2022, 9644, DOI: 10.3390/ijms23179644) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Conceptualization the review, writing and compilation the main body of the manuscript, integration contributions from all co-authors, preparation all tables and figures, and finalization the manuscript for submission. Conduction the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie koncepcji przeglądu, przygotowanie głównej części manuskryptu, wszystkich tabel i rysunków, integracja wkładu współautorów oraz finalizacja tekstu do publikacji. Analiza ukraińskiego rynku farmaceutycznego w Tabeli 2.
Inna Vlasova	Writing sections 5.6, assistance in the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.6, udział w analizie ukraińskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Weronika Skowrońska	Writing sections 5.1, 5.8, 5.9, 5.10, and 5.12. Conduction the analysis of the Polish pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.1, 5.8, 5.9, 5.10 i 5.12. Przeprowadzenie analizy polskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Agnieszka Bazylko	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Jakub P. Piwowarski	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Sebastian Granica	Conceptualization the review; reviewing and editing the final version; project supervision.
	Koncepcja przeglądu; sprawdzanie i redagowanie ostatecznej wersji; nadzór nad projektem.

27.01.2026

Date / Data

Agnieszka Bazylko

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: "**Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota**" published in *International Journal of Molecular Sciences* (Volume 23, 25 August 2022, 9644, DOI: 10.3390/ijms23179644) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **„Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota "** opublikowanego w *International Journal of Molecular Sciences* (tom 23, 25 sierpnia 2022, 9644, DOI: 10.3390/ijms23179644) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Conceptualization the review, writing and compilation the main body of the manuscript, integration contributions from all co-authors, preparation all tables and figures, and finalization the manuscript for submission. Conduction the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie koncepcji przeglądu, przygotowanie głównej części manuskryptu, wszystkich tabel i rysunków, integracja wkładu współautorów oraz finalizacja tekstu do publikacji. Analiza ukraińskiego rynku farmaceutycznego w Tabeli 2.
Inna Vlasova	Writing sections 5.6, assistance in the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.6, udział w analizie ukraińskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Weronika Skowrońska	Writing sections 5.1, 5.8, 5.9, 5.10, and 5.12. Conduction the analysis of the Polish pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.1, 5.8, 5.9, 5.10 i 5.12. Przeprowadzenie analizy polskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Agnieszka Bazylko	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Jakub P. Piwowarski	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Sebastian Granica	Conceptualization the review; reviewing and editing the final version; project supervision.
	Koncepcja przeglądu; sprawdzanie i redagowanie ostatecznej wersji; nadzór nad projektem.

Kierownik
Zakład Mikrobiologii Farmaceutycznej i Bioanalizy
Wydział Farmaceutyczny
Warszawski Uniwersytet Medyczny

21.01.2026
Date / Data

prof. dr hab. n med. i n. o zdr. Jakub Piwowarski
Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: **"Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota"** published in *International Journal of Molecular Sciences* (Volume 23, 25 August 2022, 9644, DOI: 10.3390/ijms23179644) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

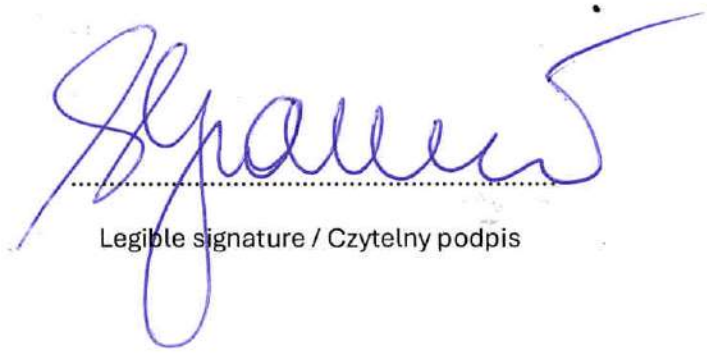
Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **„Current Knowledge on Interactions of Plant Materials Traditionally Used in Skin Diseases in Poland and Ukraine with Human Skin Microbiota "** opublikowanego w *International Journal of Molecular Sciences* (tom 23, 25 sierpnia 2022, 9644, DOI: 10.3390/ijms23179644) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Conceptualization the review, writing and compilation the main body of the manuscript, integration contributions from all co-authors, preparation all tables and figures, and finalization the manuscript for submission. Conduction the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie koncepcji przeglądu, przygotowanie głównej części manuskryptu, wszystkich tabel i rysunków, integracja wkładu współautorów oraz finalizacja tekstu do publikacji. Analiza ukraińskiego rynku farmaceutycznego w Tabeli 2.
Inna Vlasova	Writing sections 5.6, assistance in the analysis of the Ukrainian pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.6, udział w analizie ukraińskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Weronika Skowrońska	Writing sections 5.1, 5.8, 5.9, 5.10, and 5.12. Conduction the analysis of the Polish pharmaceutical market summarized in Table 2.
	Opracowanie sekcji 5.1, 5.8, 5.9, 5.10 i 5.12. Przeprowadzenie analizy polskiego rynku farmaceutycznego podsumowanej w Tabeli 2.
Agnieszka Bazylko	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Jakub P. Piwowarski	Reviewing and editing the final version.
	Sprawdzanie i redagowanie ostatecznej wersji.
Sebastian Granica	Conceptualization the review; reviewing and editing the final version; project supervision.
	Koncepcja przeglądu; sprawdzanie i redagowanie ostatecznej wersji; nadzór nad projektem.



.....

Date / Data

.....

Legible signature / Czytelny podpis

Journal of Ethnopharmacology 318 (2024) 116968

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)

Natalia Melnyk [a], Dominik Popowski [a,c], Jakub W. Strawa [b], Klaudia Przygodzińska [a], Michał Tomczyk [b], Jakub P. Piwowarski [a], Sebastian Granica [a,*]

[a] Microbiota Lab, Department of Pharmaceutical Biology, Faculty of Pharmacy, Medical University of Warsaw, ul. Banacha 1, 02-097, Warsaw, Poland
[b] Department of Pharmacognosy, Faculty of Pharmacy with the Division of Laboratory Medicine, Medical University of Bialystok, ul. Mickiewicza 2a, 15-230, Białystok, Poland
[c] Department of Food Safety and Chemical Analysis, Waclaw Dabrowski Institute of Agricultural and Food Biotechnology-State Research Institute, ul. Rakowiecka 36, 02-532, Warsaw, Poland

ARTICLE INFO

Handling Editor: Alexander N. Shikov

Keywords:
Symphytum officinale
Comfrey
Skin microbiota
Pyrrolizidine alkaloids
Metabolism

ABSTRACT

Ethnopharmacological relevance: Comfrey root (*Symphytum officinale* L., Boraginaceae) has been used in folk medicine for a long time to treat different diseases. It is recommended for swellings, phlebitis, contusions, gastroduodenal ulcers, respiratory diseases, and metrorrhagia. Currently, preparations from *S. officinale* are only topically used due to its wound-healing effects, and for reducing inflammation and the treatment of broken bones, tendon damage, painful joints and muscles. Although it is a widespread plant material, little is known about the interaction of externally applied preparations of comfrey with the human skin microbiome.
Aim of the study: The study aims to determine the interaction between human skin microbiota and the comfrey root extracts, by monitoring the biotransformation of the constituents present in the extract and evaluating changes in the population of the skin microbiota in an *ex vivo* setting.
Material and methods: The comfrey root extract was incubated with the human skin microbiota from ten healthy donors. The UHPLC-DAD-MS^n analysis determined the composition of the raw extract and the microbial metabolites. Bacterial genomic DNA was extracted and examined by amplification sequencing of the 16S rDNA to determine changes in the bacterial composition.
Results: The hydroethanolic extract of comfrey root primarily consists of phenolic acids, pyrrolizidine alkaloids, and their derivatives, and lignans. The natural products present in the extract underwent biodegradation by the skin microbiota, leading to the formation of smaller molecules. It was observed that the skin microbial metabolism primarily focused on modifying the derivatives of pyrrolizidine alkaloids. It resulted in the production of deacetylated and deesterificated compounds. However, it did not lead to the conversion of these compounds into free alkaloids.
Conclusions: The microbiota-triggered biotransformation of the comfrey root extract was observed. A few N-oxides were metabolized to deacetylated and deesterificated forms in *ex vivo* conditions. It suggests that the intermittent external applications of comfrey preparations perchance are unlikely to pose a substantial risk. While it even may serve as a potential factor influencing the extract activity in treating skin diseases.

1. Introduction

Symphytum officinale L. is commonly known as comfrey and belongs to the Boraginaceae family (Committee on Herbal Medicinal Products (HMPC), 2015). It is a perennial herbaceous plant with pale purple flowers and long rough leaves (Кисличенко, В.С. et al., 2015). In the past 2000 years, people around the world have used comfrey to heal their ailments. The use of comfrey was first documented by the ancient Romans and Greeks. In the Middle Ages, comfrey in the form of an outer poultice became popular for curing broken bones (Cupp, 2000). *S. officinale* was used as a medicinal herb in the 16th and 17th centuries

* Corresponding author. Microbiota Lab, Department of Pharmaceutical Biology, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097, Warsaw, Poland.
E-mail addresses: nmelnyk@wum.edu.pl (N. Melnyk), dpopowski@wum.edu.pl (D. Popowski), jakub.strawa@umb.edu.pl (J.W. Strawa), klaudia.przygodzinska@onet.pl (K. Przygodzińska), michal.tomczyk@umb.edu.pl (M. Tomczyk), jpiwowarski@wum.edu.pl (J.P. Piwowarski), sgranica@wum.edu.pl (S. Granica).

https://doi.org/10.1016/j.jep.2023.116968
Received 17 March 2023; Received in revised form 19 July 2023; Accepted 25 July 2023
Available online 26 July 2023

List of abbreviations:

BHI	brain heart infusion
BLAST	basic local alignment search tool
D 1-10	donor number
DNA	deoxyribonucleic acid
EIC	extracted ion chromatogram
LC–DAD–ESI–MS/TOF	liquid chromatography (LC) coupled to diode array detection (DAD), electrospray ionization (ESI), and time of flight mass spectrometry (TOF/MS)
MW	molecular weight
m/z	mass to charge ratio
OTU	operational taxonomic unit
PCR	polymerase chain reaction
QIME	quantitative insights into microbial ecology
SorE	*Symphytum officinale* root extract
TLC	thin layer-chromatography
UCHIME	algorithm for detecting chimeric sequences
UHPLC-DAD-MS^n	ultra-high performance liquid chromatography (UHPLC) coupled to diode array detection (DAD) and multi-stage mass spectrometry (MS)
UPW	ultra-pure water
UV	ultraviolet–visible
v/v	volume-to-volume ratio
VSEARCH	versatile open-source tool for metagenomics
rpm	revolutions per minute

for painful joints (Adams et al., 2009). In Austria's folk medicine, *Symphyti* radix was consumed as a tea or tincture and externally as an ointment, compress, or alcoholic supplement for the locomotor system and gastrointestinal tract (Vogl et al., 2013). Italian ethnopharmacology investigations showed the frequent use of comfrey root as an anti-diarrhoea decoction (De Natale and Pollio, 2007). Likewise, comfrey usage has been mentioned in the records of Latvian folklore archives. It has been described as a raw root applied for abscesses (Sile et al., 2020). At present, there are numerous reports on the ethnopharmacological usage of leaves, herbs, and roots. Moreover, it was included in European Scientific Cooperative on Phytotherapy (ESCOP) monographs as well as listed by the European Medicines Agency's Committee (EMA) on Herbal Medicinal Products. Comfrey is widespread across Europe, with an important place in medicine due to its wound-healing effects and its ability to reduce inflammations and treat broken bones, tendon damage, painful joints and muscles (Jarić et al., 2018; Nastić et al., 2020; Sowa et al., 2018). After oral administration, a raw material of comfrey root and its extracts accelerate the regeneration of the mucous membranes of the stomach and duodenum, reduce bleeding, and facilitate scarring in peptic ulcer disease (Błach-Olszeska et al., 2007; Ożarowski and Jaroniewski, 1989). It inhibits the atrophic process of the gastrointestinal tract in the elderly and also has a coating and anti-inflammatory effect on the mucous membranes of the upper respiratory tract (Błach-Olszeska et al., 2007). The root has a long-standing tradition and continues to be applied externally in various preparations. However, its usage is limited to external applications only due to the pyrrolizidine alkaloids, which possess hepatotoxic properties following oral intake (He et al., 2021). It is primarily used topically for inflammatory disorders of joints, wounds, gout, bone fractures, distortions, hematomas, and thrombophlebitis; it finds application in the forms of liquid extract of pure ethanol or creams, ointments, and poultices with 60–65% ethanolic extracts (Alerico et al., 2015; Committee on Herbal Medicinal Products (HMPC), 2015; Melnyk et al., 2022). Compresses with a decoction of comfrey roots can be used for minor burns, frostbite, varicose leg ulcers, and after injuries. Furthermore, the genus name comes from the Greek words *symphyo*, meaning to grow together, and *phyton*, as the plant was believed to help heal wounds (Trifan et al., 2018). The common name of comfrey reportedly comes from *confirma* (Latin: meaning with strength), in reference to its reputation for healing wounds and broken bones.

Previous studies on the phytochemical composition of *Symphyti radix* revealed the constituents including allantoin, mucilage polysaccharides with fructose and glucose units, phenolic acids (such as rosmarinic acid, chlorogenic acid, caffeic acid), glycopeptides and amino acids, triterpene saponins in the form of monodesmosidic and bidesmosidic glycosides based on aglycones hederagenin (e.g., symphytoxide A), oleanolic acid, and lithospermic acid (Kimel and Krauze-Baranowska, 2021; Salehi et al., 2019; Savić et al., 2015). Moreover, comfrey root is also reported to be a source of pyrrolizidine alkaloids, such as intermedine, 7-acetylintermedine, 7-acetyllycopsamine, lycopsamine, symphytine, and symlidine, as well as their N-oxides, and because of their presence, after oral administration, hepatotoxic and carcinogenic effects were observed (Trifan et al., 2018, 2021). The mechanisms underlying toxicity and mutagenicity are not yet fully elucidated. However, it is believed that these effects are associated with the toxic mechanism involving the biotransformation of alkaloids by hepatic microsomal enzymes (Stickel and Seitz, 2000). Rosmarinic acid is considered to play a significant role in the anti-inflammatory, analgesic, and astringent effects of comfrey root (European scientific cooperative on phytotherapy (ESCOP), 2012). The stimulation of tissue proliferation and regeneration are also likely mediated through allantoin. Other constituents, such as triterpenoid saponins, have antibacterial properties, and symphytoxide A was shown to lower blood pressure in rats through an anti-cholinergic effect (Stickel and Seitz, 2000). In addition, rabdosiin and globoidnans A and B have already been claimed as possessing antioxidant, neuroprotective, or anti-HIV activities (Trifan et al., 2021).

The human skin is the largest and an important organ responsible for many activities of the body. It is a protective shell, as well as an excretory and secretory organ, a heat regulator, and a sensory receptor (Melnyk et al., 2022). It also takes an active part in numerous metabolic processes. Additionally, it is a home to trillions of microorganisms, known as the skin microbiome, and includes bacteria, fungi, and viruses, which cover the whole skin surface and reside in appendages (hair follicle, sebaceous glands, and sweat glands) (Belkaid and Segre, 2014). It was shown that bacteria are not uniformly and the highest bacterial density is found in the surface layers, whereas the stratum corneum adjacent to the stratum granulosum (the first living cell layer) contains very few bacteria (Zeeuwen et al., 2013). The balance of the microorganisms' composition is essential to maintain healthy skin, and its disturbance can predispose the host to cutaneous infectious and inflammatory conditions (Cogen et al., 2008). In recent years, considerable attention has been paid to the role of microbiota, mainly from the gut, in the biotransformation of ingested substances (Dadi et al., 2020). Nowadays, scientific databases are rich in research on the biotransformation by gut microbiota (Kruk et al., 2022; Popowski et al., 2021), but little is known about the influence of the skin microbiome on the chemical composition of externally applied preparations. As the mechanisms behind the pharmacological effects of extracts are not well understood, there is an expectation that the interaction between the extract's chemical composition and the human microbiota may play a role in their therapeutic effects towards dermatologic conditions. Thus, the study aimed to evaluate for the first time in *ex vivo* conditions the interaction between a comfrey extract and the microorganisms residing on the human skin.

2. Materials and methods

2.1. Plant material

Symphyti radix (batch number 01082023, expiration date: August 2023) was purchased from Dary Natury (Grodzisk, Poland). A voucher

specimen was deposited in the Herbarium of the Department of Pharmaceutical Biology (Medical University of Warsaw, Poland) under the number 2023SO0108. The plant material was examined by Prof. Sebastian Granica using macroscopic and microscopic examinations of the material (Deryng, 1961), and TLC analysis was according to the works of (Kimel et al., 2019; Wagner et al., 1981). The results confirmed the plant material's identity as comfrey root and were in accordance with the producer's specifications.

2.2. General chemicals used

BHI broth was purchased from bioMerieux SA (Craponne, France). NaCl 0.9% was prepared in-house. Formic acid, ethanol, and acetonitrile, methanol were obtained from Avantor (Gliwice, Poland). Ultrapure water was produced with the Merck Millipore Simplicity UV system. The acetonitrile Optima™ purchased from Fisher Scientific (Loughborough, UK) was used in the analysis with a TOF/MS detector. OmniGene-Skin kit for skin microbiota collection was purchased from DNA Genotek Inc. (Ottawa, Canada).

2.3. Preparation of the SorE from an investigated sample

A total of 100 g of dry comfrey root was extracted 3 times with 500 mL of 70% ethanol solution (7:3, *v/v*) at the room temperature, mixing from time to time. The ultrasonic bath was used for 15 min at each extraction step to increase the yields of the compounds contained in the root. The final volume (1400 mL) was filtered and evaporated under reduced pressure with a LABORANTA 4000 WB vacuum (<45 °C, Heidolph, Schwabach, Germany), frozen (−20 °C, 24 h), and freeze-dried with Cryodos apparatus (Telstar, Terrassa, Spain). The obtained lyophilizate (7.302 g) was stored in a tightly closed container at a temperature of 4 °C and used for further study investigations. The dry extract (SorE) was dissolved in H_2O to prepare a 10 mg/mL solution for the UHPLC-DAD-MS^n analysis and a 12 mg/mL stock solution for skin microbiota metabolism.

2.4. Chromatographic analysis of the SorE and metabolites

The UHPLC-DAD-MS^n analysis of SorE (10 mg/mL H_2O) and the samples from metabolism experiments were performed on a UHPLC-3000 RS system (Dionex, Leipzig, Germany), equipped with a DAD detector and splitless connection to an AmaZon SL ion trap mass spectrometer with an ESI interface (Bruker Daltonik GmbH, Bremen, Germany). UV spectra were recorded in the wavelength range 200–450 nm. The parameters of the MS unit were as follows: nebulizer pressure: 40 psi; drying gas flow rate: 9.0 L/min; nitrogen gas temperature 300 °C; and capillary voltage: 4.5 kV. The mass spectra were registered by scanning from m/z 70 to 2200. A Kinetex XB-C18 chromatography column was used (Phenomenex, Torrance, CA, 150 mm; 2.1 mm; 1.7 μm). The mobile phase (A): was UPW: HCOOH (99.9:0.1, *v/v*), and the mobile phase (B) was ACN: HCOOH (99.9:0.1, *v/v*). The gradient program was 0–60 min 1–26% B (0.416% B/min), 60–120 min 26–95% B (1.15% B/min), and the flow rate was 0.3 mL/min. The injection volume was 4.0 μL (40 μg/μL) both for the SorE and samples after incubation with microbiota after filtration through a 0.45 μm syringe filter.

In order, to establish the exact molecular weight, an LC–DAD–ESI–MS/TOF analysis was performed on a 1260 Infinity chromatography system hyphenated to a 6230 TOF mass spectrometer with Dual Agilent Jet Stream ESI (Agilent Technologies, Santa Clara, CA, USA). The MS conditions were as follows: electrospray ionization (ESI) source in both the positive and negative ionization mode, a gas flow of 11 L/min, a gas temperature of 350 °C, a nebulizer pressure of 60 psi, and capillary voltages of 4500 and 2500 V for the positive and negative ion modes, respectively. The mobile phases, gradient, and column as described in UHPLC-DAD-MS^n conditions.

2.5. Ex vivo experiments on human skin microbiota

Human skin microbiota samples were obtained from ten healthy volunteers and collected from the forearms by wetting swabs with sterile 0.9% NaCl (x10 from each forearm). Then samples were incubated in 30 mL of the BHI with shaking (120–140 rpm, 37 °C, 24 h). The donors did not take a shower on the day of the experiment and did not apply any medicines or cosmetics to their forearms at least 24 h before the experiment. The study conformed to the principles of the Declaration of Helsinki and the experiments were performed by the guidelines and regulations of the Ethics Committee of the Medical University of Warsaw (AKBE/151/2021), which allow skin microbiota collection from healthy human volunteers for use in *ex vivo* studies.

2.5.1. Skin microbiota metabolism of the extract

After the 24 h incubation of collected microbiota, the extract in volume 1 mL was added to 5 mL of each donor sample to obtain a 2 mg/mL concentration in the culture (D_x + SorE). Prior to that, the extract was sterilized through the sterile syringe filter with 0.45 μm cellulose acetate. As a control, incubations of extract without microbiota (BHI + SorE) and the method blank – microbiota without extract in the medium (BHI + D_x) were performed. BHI + SorE was prepared by adding 1 mL of extract to 5 mL of BHI, and in the case of method blank, 1 mL of sterile water was added to 5 mL of donor sample. The samples were incubated at 37 °C to maintain the temperature conditions of the human body and with shaking at 120–140 rpm to provide aeration using incubator mini Galaxy A (RS Biotech, Nunc GmbH&Co, Wiesbaden, Germany). Then, after 24, 48, and 72 h, each sample was transferred into the centrifuge tubes in amounts of 0.5 mL and mixed with MeOH: 0.1% HCOOH (1:1, *v/v*). Next, the tube contents were centrifuged for 3 min at 10000 rpm to separate the microbial sediment from the supernatant. The supernatant was transferred to the vials and analyzed as described in Section 2.4.

2.5.2. Skin microbiota sequencing

The amplification sequencing of 16S rDNA followed by bioinformatic analysis was performed on the experimental samples after 24 h of incubation with the addition of the tested extract. Each sample from every donor was collected using an OmniGene-Skin kit and tested separately. DNA was extracted and the amplicon was sequenced using a two-stage PCR protocol and an Illumina sequencer. Using a universal primer set, the 341F-785R V3–V4 region of the 16S rDNA was targeted and sequenced, resulting in 2.4 million sequence read pairs. The average length of the combined records was 409 nucleotides. Chimeric reads were detected and suppressed depending on the UCHIME de novo algorithm (Edgar et al., 2011) with the VSEARCH package (Rognes et al., 2016). The remainder of the high-quality readings were treated using minimum entropy decomposition (Eren et al., 2015). To assign taxonomic information to each OTU, sequences representing clusters were aligned to the discontinuous Mega BLAST sequence database (https://blast.ncbi.nlm.nih.gov/). The QIIME software (version 1.9.1) was applied to process OTUs and taxonomic tasks. Abundances were standardized with lineage-specific copy numbers of the relevant marker genes to improve estimates. The alpha diversity indexes were calculated according to (Chao and Chiu, 2016; Thukral, 2017). Box plots were generated using ggplot2 and ggplot packages in R.

3. Results and discussions

3.1. The UHPLC-DAD-MS^n profile of comfrey root

Using the UHPLC-DAD-MS^n technique, a method for the evaluation of the chemical composition of the hydroethanolic extract from *Symphyti radix* was established (Table 1, Fig. 1).

During the analysis, 29 compounds were detected and characterized, assigned to the groups such as phenolic acids and their derivatives, pyrrolizidine alkaloids and their derivatives, and lignans. The

Table 1
UHPLC-DAD-MS data of detected compounds in the 70% ethanolic extract from the comfrey root.

N°	Compound name	Rt [min]	UV–Vis max [nm]	$[M+H]^+$ *m/z*	MS^2 ions (+)	$[M-H]^-$ *m/z*	MS^2 ions (−)	Formula	MW	ΔM [ppm]	Ref.
1	allantoin	1.5	–	159	–	157	–	$C_4\ H_6\ N_4\ O_3$	158.04393	−0.38	(D'urso et al., 2020; Savić et al., 2015)
2	citric acid	2.4	–	193	–	191	173, 111b	$C_6\ H_7\ O_7$	192.02696	−0.23	Nastić et al. (2020)
3	hydroxybenzoic acid hexoside	7.9	248	301	283, 139b	299	137b	$C_{13}H_{16}O_8$	300.0845	−0.14	D'urso et al. (2020)
4	intermedine	10.1	–	300	138b, 120, 94	–	–	$C_{15}H_{25}NO_5$	299.1733	−0.17	Trifan et al. (2021)
5	lycopsamine	10.9	–	300	138b, 120, 94	–	–	$C_{15}H_{25}NO_5$	299.1733	−0.17	Trifan et al. (2021)
6	unknown compound	11.3	278	205	188b	203	159, 116	$C_{11}H_{12}N_2O_2$	204.0893	−1.12	–
7	intermedine N-oxide	13.9	–	316	172b	314	270, 170b, 152	$C_{15}H_{25}NO_6$	315.1682	0.11	Trifan et al. (2021)
8	lycopsamine N-oxide	14.8	–	316	172, 138b	360a	270, 242, 170b, 152, 117	$C_{15}H_{25}NO_6$	315.1682	0.11	Trifan et al. (2021)
9	dihydrointermedine N-oxide/ dihydrolycopsamine N-oxide	15.8	–	318	227, 174b	–	–	$C_{15}H_{27}NO_6$	317.18379	−0.14	Trifan et al. (2021)
10	unknown compound	19.7	–	325	307, 249, 211, 166	323	179, 161, 143, 131, 113, 101b, 89	$C_{15}H_{20}N_2O_6$	324.1321	−2.69	–
11	7-acetylintermedine N-oxide	21.3	–	358	268, 242, 214b	402a	356, 342, 258, 188, 161b	$C_{17}H_{27}NO_7$	357.1788	−0.08	Trifan et al. (2021)
12	7-acetyllycopsamine N-oxide	22.2	–	358	268, 242, 214b	402a	356b, 203, 161b, 152, 141	$C_{17}H_{27}NO_7$	357.1788	−0.08	Trifan et al. (2021)
13	7-saricinyl-9-trachelanthyl retronecin N-oxide	28.5	–	414	370, 298, 270b, 236, 218, 204	–	–	$C_{20}H_{31}NO_8$	413.2044	−0.21	Trifan et al. (2021)
14	unknown compound	29.6	–	372	363b			$C_{18}H_{31}NO_7$	372.2095	−0.54	–
15	isomer of compound 14	30.3	–	372	298, 228b, 212, 194, 136	–	–	$C_{18}H_{31}NO_7$	372.2095	−0.30	–
16	echimidine N-oxide	32.4	–	414	396, 352, 338, 254b, 154	–	–	$C_{20}H_{31}NO_8$	413.205	−0.11	Trifan et al. (2021)
17	globoidnan B	40.6	253, 343		–	537	493, 401, 339b, 267, 229	$C_{27}H_{22}O_{12}$	538.1111	−0.09	(Trifan et al., 2020, 2021)
18	symphytine (or stereoisomer)	40.8	–	382	220, 120b	–	–	$C_{20}H_{31}NO_6$	381.2146	−0.32	Trifan et al. (2021)
19	symphytine N-oxide	41.1	–	398	380, 354, 308, 254b, 192, 174, 136	442a	396b, 325, 252, 161	$C_{20}H_{31}NO_7$	397.210	−0.07	Trifan et al. (2021)
20	symladine (or stereoisomer)	42.1	–	382	300, 220, 138, 120b	–	–	$C_{20}H_{31}NO_6$	381.2146	−0.24	Trifan et al. (2021)
21	symladine N-oxide	42.6	–	398	354, 308, 254b, 220, 174, 136	442a	396b, 352, 314, 252, 181, 161	$C_{20}H_{31}NO_7$	397.210	−0.07	Trifan et al. (2021)
22	rabdosiin	48.9	348		–	717	519b, 475, 435, 366, 339, 197	$C_{36}H_{30}O_{16}$	718.1534	−0.01	Trifan et al. (2020)
23	rosmarinic acid	50.0	329	361	163b, 145	359	249, 223, 197b, 179, 161	$C_{18}H_{16}O_8$	360.0845	−0.11	Trifan et al. (2020)
24	dihydrorabdosiin	50.0	329	–	–	719	520b, 476, 359, 353, 243, 197	$C_{36}H_{32}O_{16}$	720.1685	−4.95	Trifan et al. (2021)
25	salvianic acid A (Danshensu)	50.0	329	–	–	197	–	$C_9H_{10}O_5$	198.0528	−0.24	Trifan et al. (2018)
26	3-acetylsymphytine-N-oxide (or stereoisomer)	51.7	–	440	422, 398, 380b, 362, 254	–	–	$C_{22}H_{33}NO_8$	439.2201	−0.11	Trifan et al. (2021)
27	3-acetylsymlandine-N-oxide (or stereoisomer)	53.0	–	440	422, 398, 380b, 362, 254, 192	–	–	$C_{22}H_{33}NO_8$	439.2201	−0.04	Trifan et al. (2021)
28	dihydrogloboidnan A	54.4	219	–	–	493	313, 295b, 203	$C_{26}H_{22}O_{10}$	492.1056	2.19	Trifan et al. (2021)

(*continued on next page*)

Table 1 (*continued*)

N°	Compound name	Rt [min]	UV–Vis max [nm]	$[M+H]^+$ *m/z*	MS^2 ions (+)	$[M-H]^-$ *m/z*	MS^2 ions (−)	Formula	MW	ΔM [ppm]	Ref.
29	globoidnan A	61.0	261,319	493	312b, 295	491	311b, 267, 223	$C_{26}H_{20}O_{10}$	492.1056	0.06	(D'urso et al., 2020; Trifan et al., 2020)

Rt, retention time; MS, mass spectra; MW, molecular weight; **Δ**M, mass error; a, [M + HCOOH–H]⁻; b, basic peak.

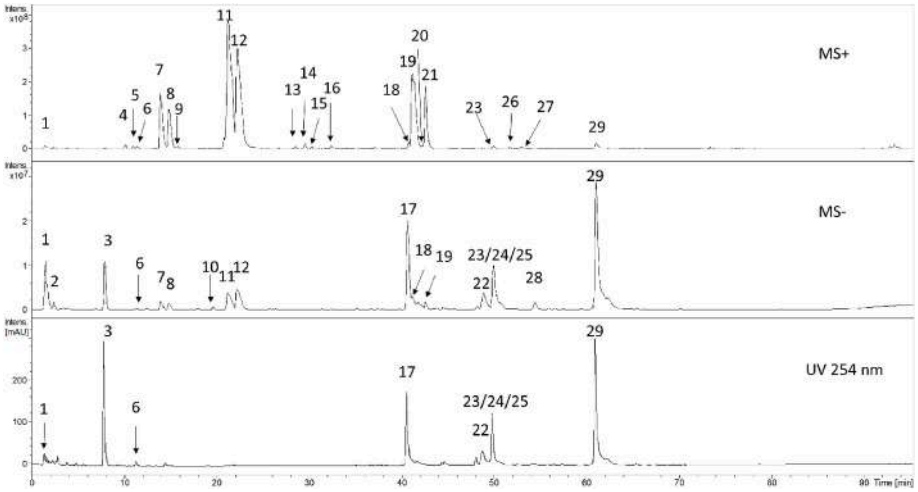


Fig. 1. UHPLC-DAD-MS data of the 70% ethanolic extract of comfrey root were recorded in the mode of positive ions (MS^+), negative ions (MS^-), and UV of 254 nm.

identification of compounds was performed based on the UV–vis maxima recorded with the DAD device and TOF/MS and MS^n spectra recorded in the positive and negative ion modes. The data were compared to the respective literature reports of the chromatographic analysis of comfrey extracts. The first eluted compound (**1**) at a retention time of 1.5 min peaked with major ion in the positive ion mode at *m/z* 159 without any fragmentation. Due to elution in the dead time the compound did not meet the intensity requirements for fragmentation; hence, other ions were fragmented instead. It was identified as allantoin, a urea derivative (Savić et al., 2015). Peak **2** was identified as citric acid and had the $[M-H]^-$ signal at *m/z* 191, followed by fragmentation to *m/z* 173, 111b (Nastić et al., 2020). The pseudomolecular ion in the positive mode at 301 *m/z* and its ion fragments at 283 and 139 indicated that peak **3** at a retention time of 7.9 min was hydroxybenzoic acid hexoside (D'urso et al., 2020).

Furthermore, peaks **4**, **5**, **7**–**9**, **11**–**13**, **16**, **18**–**21**, **26**, and **27** were tentatively assigned to pyrrolizidine alkaloids and their derivatives, annotated by comparison with Trifan et al.(Trifan et al., 2020, 2021). Peaks **4** and **5** with $[M+H]^+$ at *m/z* 300 were intermedine and lycopsamine, respectively, which differed in retention time (10.1 and 10.9 min, respectively). The compounds intermedine N-oxide (**7**) and lycopsamine N-oxide (**8**) ($[M+H]^+$ at *m/z* 316) had similar ion fragments with a mass of 172 that resulted in the loss of trachelanthyl and viridifloryl moieties. Peak **9** was a dihydrated form of the previous compound with an MS peak at 318 *m/z* and assigned as dihydrointermedin N-oxide or dihydrolycopsamine N-oxide. This compound produced a similar peak fragmentation as the previously detected alkaloid N-oxides, differing only by 2 atomic weight units (174 *m/z*). The main pyrrolizidine alkaloids derivatives **11**–**12** had a pseodomolecular ion $[M+H]^+$ at 358 *m/z* and, after MS fragmentation, three ion fragments at 268, 242, and 214 *m/z* were present. Based on the results obtained from the MS fragmentation, it can be concluded that these compounds are 7-acetylintermedine N-oxide and 7-acetyllycopsamine N-oxide, respectively. Pseudomolecular ion $[M+H]^+$ in the positive mode at 414 *m/z* and its ion fragments at 370, 298, 270, 236, and 218 *m/z* indicated that peak **13** is a derivative of retronecin N-oxide and named 7-saricinyl-9-trachelanthyl retronecin N-oxide. Echimidine N-oxide was identified as peak **16** and showed an MS fragmentation of a pseudomoecular ion at 414 *m/z*, with five ion fragments at 396, 352, 338, 254, and 154 *m/z*. Symphytine (**18**) and symladine (**20**) were eluted at 40.8 and 42.1 min, respectively, and had a pseudomolecular ion $[M+H]^+$ in the positive mode at 382 *m/z* with fragmentation at 220 and 120 *m/z*, respectively. Symphytine N-oxide (**19**) and symladine N-oxide (**21**) peaks had major ions at 398 *m/z* and fragmentations at 354, 308, 254, 192, 174, and 136 *m/z* with different retention times (41.1 and 42.6 min, respectively). Peaks **26** and **27** had the same value of $[M+H]^+$ at 440 *m/z* and fragmentations at 398, 380, 254 *m/z*. Based on the comparison of the mass spectrometry data with symphytine/symlandine-N-oxides, an additional acetyl group was suggested to be present. Therefore, compounds **26** and **27** were tentatively assigned as 3-acetylsymphytine-N-oxide and 3-acetylsymlandine-N-oxide, respectively.

Next, the compounds **17**, **22**, **24**, **28**, and **29** were visible in the negative mode and UV 254 nm, and belong to the chemical group of lignans. Compound **17** exhibited an elution time of 40.6 min. UV–Vis peaks were observed at wavelengths of 253 and 343 nm and $[M-H]^-$ was detected at 537 m/z. Further fragmentation of the compound occurred, resulting in ions at 493, 401, 339, 267, and 229 m/z. This peak was identified as globoidnan B (D'urso et al., 2020; Trifan et al., 2021). Rabdosiin (**22**) ($[M-H]^-$ at 717 *m/z*) had an ion fragmentation with masses 519, 475, 435, 366, 339, and 197, which is typical of this compound (Trifan et al., 2020, 2021). Peak **24** is a dihydrated form of the previous compound with an MS peak at 719 *m/z* (dihydrorabdosiin). Peak **28** with $[M-H]^-$ at 493 *m/z* was identified as dihydrogloboidnan A and showed MS fragmentation at 313, 295, and 203 *m/z*. The pseudomolecular ion in the negative mode at 491 *m/z* and its ion fragments at 311, 267, and 223 indicated that peak **29** was globoidnan A (D'urso et al., 2020; Trifan et al., 2021). Rosmarinic acid was also identified as peak **23** at a retention time of 50.0 min. The MS fragmentation of pseudomolecular ion $[M-H]^-$ in the negative mode at 359 *m/z* showed

five ion fragments at 249, 223, 197, 179, and 161 *m/z*. $[M+H]^+$ in the positive mode at 361 *m/z* had ion fragmentation at 163 and 145 *m/z*. Peak **25** was tentatively identified as salvianolic acid A (Danshensu) and showed a pseudomolecular ion $[M-H]^-$ at 197 *m/z* with UV–Vis of 329 nm (Trifan et al., 2018). Compounds **6**, **10**, **14**, and **15** were also present in the hydroethanolic extract of comfrey root could not be identified. They were characterized based on UV–Vis and MS data recorded during analysis.

3.2. Metabolism by human skin microbiota

The chromatograms of the tested samples, controls, and method blanks were carefully analyzed and compared. In order to provide a more precise evaluation of peak intensity and facilitate comparisons across different donors, specific signals that were identified as potential metabolites were selected. These selected signals were then presented as extracted ion chromatograms for a total of five donors. This approach allows for a focused examination of the metabolite peaks and facilitates the assessment of any variations or similarities in their abundance among the different donors.

The results indicated that, after incubation with the skin microbiota two metabolites with a molecular weight of 315 (peaks **7** and **8**) were identified in all samples, except the donor 4. These compounds were initially present in the raw extract, but their signal intensity increased after incubation with the microbiota. On the other hand, signals corresponding to compounds with [M+H]+ at 398 (peaks **19** and **21**) and 358 m/z (peaks **11** and **12**) decreased in intensity compared to the controls. (Fig. 2). No significant changes in the chemical composition of the extract were observed in the sample incubated with the microbiota from donor 4. Furthermore, all other constituents determined in the original extract remained stable over time, with no observed alterationss in their structures and abundance across all donors.

The study noted that the skin microbial metabolism predominantly focused on the modification of pyrrolizidine alkaloid derivatives. It was established that deacetylation of 7-acetylintermidine N-oxide (**11**) and 7-acetyllycopsamine N-oxide (**12**), and deestarification of symphytine N-oxide (**19**) and symladine N-oxide (**21**) take play in biotransformation of the extract's original composition. (Fig. 3). It is significant to point out that these four N-oxides underwent modifications and were transformed into intermidine N-oxide (**7**) and lycopsamine N-oxide (**8**). Importantly, pyrrolizidine alkaloid derivatives were not reduced to free alkaloids, a process typically observed during interaction with the intestinal microbiota (Yang et al., 2019).

The distinctive skin microbial metabolism of SorE observed in this context, coupled with the limited dermal absorption observed by (Brauchli et al., 1982), increases the likelihood that intermittent external use of comfrey preparations is unlikely to pose a significant hazard. Moreover, there is an expactation that metabolized extract constituents will play a role in their bioactivity. This expectation is supported by studies on gut microbiota, which have demonstrated that the microbiota has the capability to transform native natural products, resulting in increased anti-inflammatory activity (Piwowarski et al., 2014a, 2014b, 2015). However, further studies and research are needed.

3.3. Composition of the skin microbiota cultures

The study findings indicated that the addition of a comfrey root extract did not have a detrimental effect on the alpha-diversity of the skin microbiota. Furthermore, it did not induce dysbiosis, as assessed after a 24-h incubation period (Fig. 4).

While the observed changes in the microbiota composition were not statistically significant, some qualitative differences were noted between the extract and control groups. Specifically, variations in the abundance of specific microbial families were observed between the two groups (Fig. 5).

In general, the identified bacteria orders were *Micrococcales*, *Bacillales*, *Lactobacillales*, *Niesseriales,* *Enterobacterales*, and *Corynebacteriales*, and there were some unidentified bacteria. The microbiota present on the skin of each donor demonstrates interpersonal variation and specific relative abundance, which are influenced by distinct physiological characteristics such as pH, moisture, salinity, and sebum content. Moreover, factors including genetics, environmental conditions, and lifestyle contribute to the observed diversities in the composition of the skin microbiota among individuals (Zeeuwen et al., 2013). However, there are mutual microbial species that are found on the skin of different donors. These shared microorganisms contribute to the concept of the "core microbiota" of the skin, representing a subset of species that are consistently present across the population (Sharon et al., 2022).

In the samples both with and without extract, the dominating bacteria family were *Staphylococcaceae*, *Enterococcaceae*, *Bacillaceae*, and unidentified *Bacillales* and *Enterobacterales.* After the incubation of the microbiota with the extract, in all samples from different donors apart from D1, D4, D6, D8 an increase was observed for *Staphylococcaceae*, in samples from D4 for *Enterococcaceae* and *Micrococcaceae*, and in samples from D3 for *Bacillaceae*. The increase of *Bacillaceae* was detected for D1, D4, D6, D8. On the other hand, a decrease in bacteria abundance was detected in all samples, except D6 and D8, for the unidentified *Bacillales* as well as in a sample from D4 for *Lactobacillaceae, Leuconostocaceae*. *Neisseriaceae* from D3 and D4, and *Corynebacteriaceae* from D2 and D3 disappeared after incubation with the comfrey root extract in the test sample. Unidentified *Enterobacterales* were identified in D8, and after 24 h incubation extract reduced the amount of this bacteria the same way extract influenced on unidentified *Bacilli* from the D5.

The dissimilar microbiota composition observed in the samples from D4 could potentially explain the variations in metabolism observations as outlined in Section 3.2. Specifically, the presence of *Lactobacillales* (*Enterococcaceae, Lactobacillaceae, Leuconostocaceae*) and *Micrococcales* (*Micrococcaceae*) orders was exclusively identified in the microbiota of this particular donor. Consequently, this observation implies that the constituents from the extract may have remained unaltered due to the influence of families such as *Enterococcaceae, Lactobacillaceae, Leuconostocaceae* and *Micrococcaceae*. However, additional investigations are necessary to comprehensively elucidate the precise mechanisms underlying these observations.

4. Conclusions

Based on the provided data, it was determined that the hydroethanolic extract of comfrey root mainly primarily contains phenolic acids, pyrrolizidine alkaloids, their derivatives, and lignans. The biodegradation of these extract constituents by the human skin microbiota results in the production of metabolites at a mass of 315 atomic mass units (amu). Consequently, as present preparations with comfrey root are used only externally, they come into direct contact with the skin microbiota. Therefore, it is expected that the bioactivity of comfrey extract is not only attributed to the direct effects of its natural products but also to the metabolites formed as a result of interactions with microorganisms residing on the skin surface. Moreover, it should be emphasized that this metabolism did not result in the conversion of pyrrolizidine alkaloid derivatives into free alkaloids, which can be one of the confirmations of the relative safety of topical application. Additionally, it was observed that the comfrey root extract slightly altered the composition of the microbiota of the human skin in *ex vivo* cultures, without causing any imbalance. This findings suggests that comfrey root holds promise as an alternative approach for preventing and treating skin problems. Furthermore, it is important to note that the composition of the skin microbiota varies among individuals, leading to differences in the biodegradation of extract constituents after exposure to microbiota.

In conclusion, the skin microbiota-mediated biodegradation of comfrey root extract is expected to contribute to its traditionally

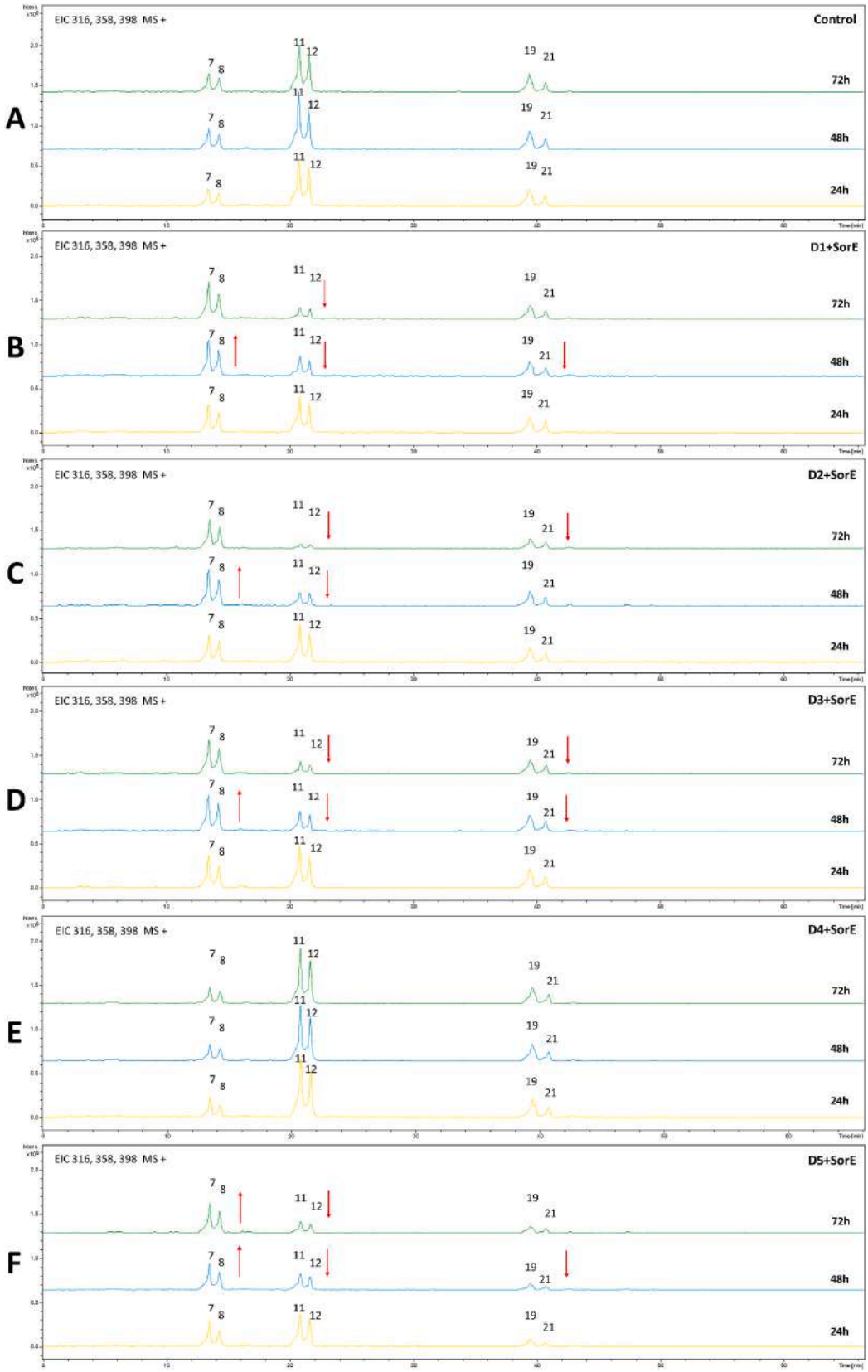


Fig. 2. UHPLC-DAD-MS^n extracted ion chromatograms (EIC) of the representative samples of comfrey root extract after 24, 48, and 72 h incubation with human skin microbiota. EIC for 316, 398, and 358 *m/z*. A, control; B, D1+SorE, C, D2+SorE; D, D3+SorE; E, D4+SorE; F, D5+SorE. SorE, *Symphytum officinale* root extract. D1-5, microbiota donor number. Yellow, chromatogram after 24 h incubation; Blue, after 48 h; Green, after 72 h. The red arrows indicate the changes in signal intensity (increasing or decreasing). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

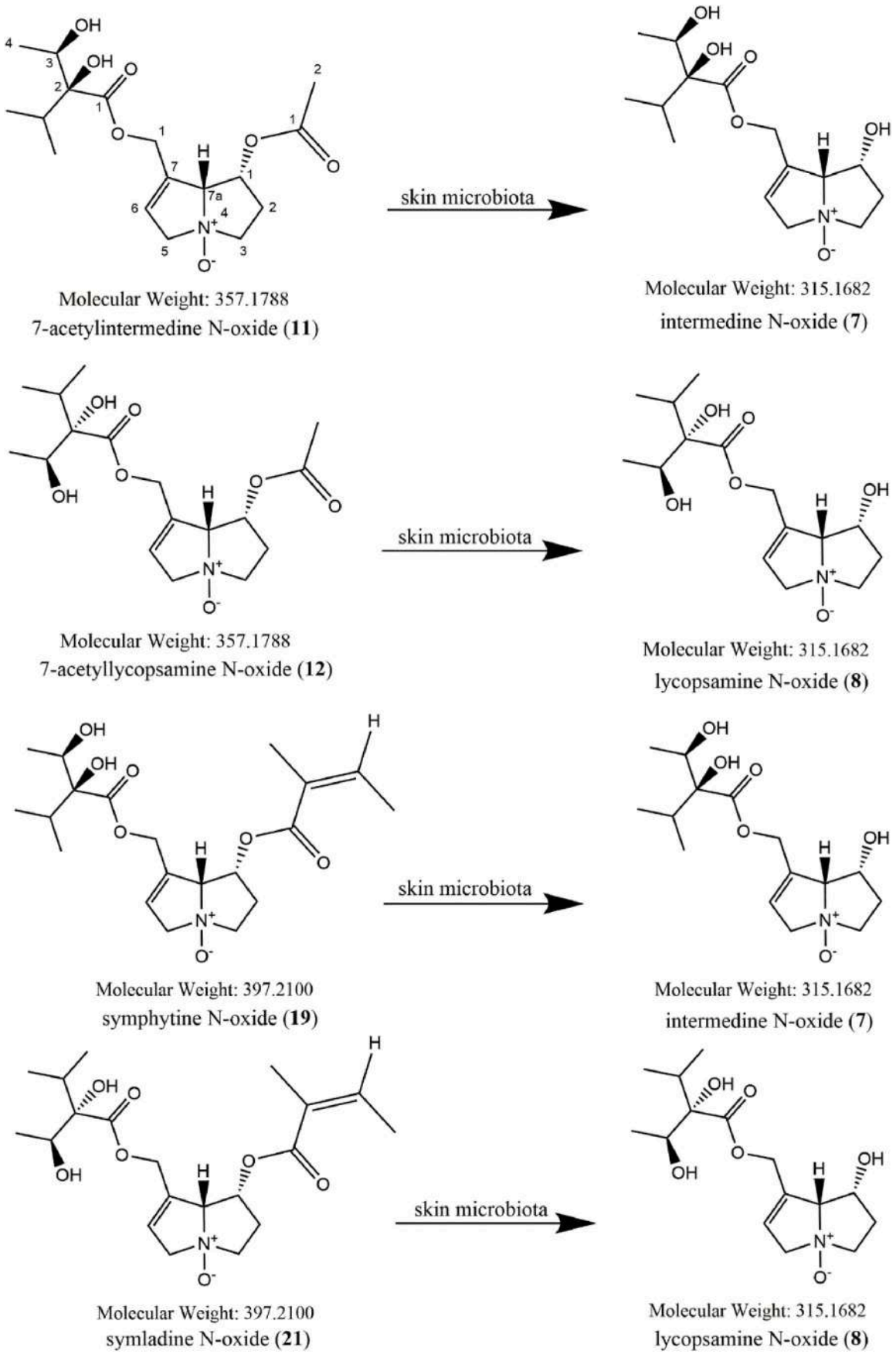


Fig. 3. Biotransformation of comfrey root extract constituents by the human skin microbiota. 7, 8, 11, 12, 19, and 21 – peak numbers on the chromatograms.

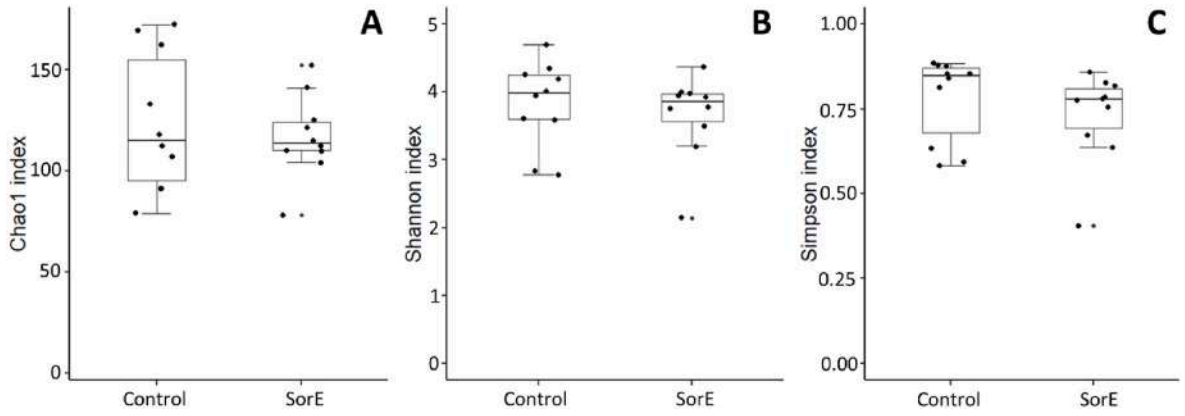


Fig. 4. Boxplot of alpha-diversity: Chao 1 (A), Shannon (B), Simpson (C) indices. SorE, *Symphytum officinale* root extract.

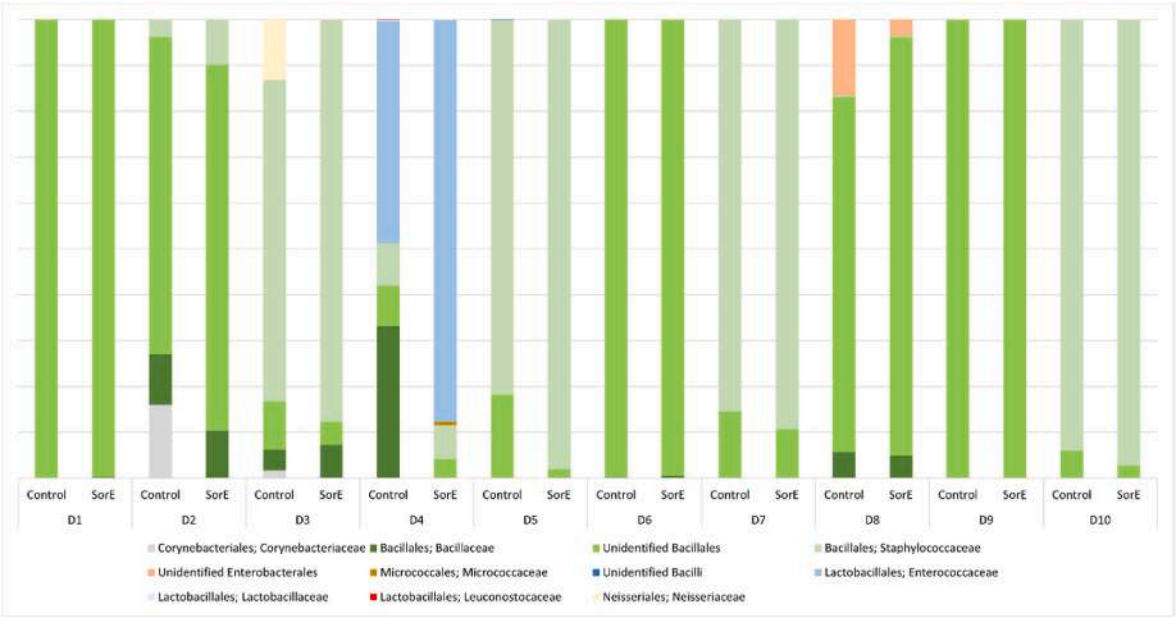


Fig. 5. The relative order and family abundance in samples after 24 h incubation. SorE, *Symphytum officinale* root extract, D1-10, donor number.

recognized effectiveness in treating skin diseases. As well, this metabolism can potentially serve as probable evidence of relative safety regarding potential hazards associated with alkaloids. However, it is important to note that further investigations are necessary to comprehensively comprehend the precise mechanisms involved in these processes.

CRediT authorship contribution statement

Natalia Melnyk: Conceptualization, Investigation, Visualization, Writing – review & editing. **Dominik Popowski:** Investigation, Visualization, Writing – review & editing. **Jakub W. Strawa:** Investigation, Writing – review & editing. **Klaudia Przygodzińska:** Investigation. **Michał Tomczyk:** Investigation, Writing – review & editing. **Jakub P. Piwowarski:** Conceptualization, Methodology, Resources, Writing – review & editing. **Sebastian Granica:** Conceptualization, Methodology, Resources, Writing – review & editing.

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.

Data availability

Data will be made available on request.

Acknowledgments

The present research was financially supported by the National Science Centre Poland grant Preludium Bis 2 No. 2020/39/O/NZ7/01109.

References

Adams, M., Berset, C., Kessler, M., Hamburger, M., 2009. Medicinal herbs for the treatment of rheumatic disorders-A survey of European herbals from the 16th and 17th century. J. Ethnopharmacol. 121, 343–359. https://doi.org/10.1016/j.jep.2008.11.010.

Alerico, G.C., Beckenkamp, A., Vignoli-Silva, M., Buffon, A., Von Poser, G.L., 2015. Proliferative effect of plants used for wound healing in Rio Grande do Sul state, Brazil. J. Ethnopharmacol. 176, 305–310. https://doi.org/10.1016/j.jep.2015.11.001.

Belkaid, Y., Segre, J.A., 2014. Dialogue between skin microbiota and immunity. Science 346, 954–959. https://doi.org/10.1126/science.1260144, 80-.

Błach-Olszeska, Z., Długosz, A., Kowal-Gierczak, B., Lamer-Zarawska, E., Niedworok, J., 2007. Fitoterapia I Leki Roślinne, Pierwsza. Wydawnictwo Lekarskie PZWL, Warszawa.

Brauchli, J., Lüthy, J., Zweifel, U., Schlatter, C., 1982. Pyrrolizidine alkaloids from *Symphytum officinale* L. and their percutaneous absorption in rats. Experientia 38, 1085–1087. https://doi.org/10.1007/BF01955382.

Chao, A., Chiu, C., 2016. Species Richness: Estimation and Comparison. Wiley StatsRef Stat. Ref. https://doi.org/10.1002/9781118445112.stat03432.pub2. Online 1–26.

Cogen, A.L., Nizet, V., Gallo, R.L., 2008. Skin microbiota: a source of disease or defence? Br. J. Dermatol. 158, 442–455. https://doi.org/10.1111/j.1365-2133.2008.08437.x.

Committee on Herbal Medicinal Products (HMPC), 2015. Assessment Report on *Symphytum officinale* L., Radix. https://www.ema.europa.eu/en/documents/herbal-report/final-assessment-report-symphytum-officinale-l-radix_en.pdf. (Accessed 16 February 2023).

Cupp, M.J., 2000. Toxicology and Clinical Pharmacology of Herbal Products, first. ed. Springer Science+Business Media, New York.

Dadi, T.H., Vahjen, W., Zentek, J., Melzig, M.F., Granica, S., Piwowarski, J.P., 2020. *Lythrum salicaria* L. herb and gut microbiota of healthy post-weaning piglets. Focus on prebiotic properties and formation of postbiotic metabolites in *ex vivo* cultures. J. Ethnopharmacol. 261, 113073 https://doi.org/10.1016/j.jep.2020.113073.

De Natale, A., Pollio, A., 2007. Plants species in the folk medicine of Montecorvino Rovella (inland Campania, Italy). J. Ethnopharmacol. 109, 295–303. https://doi.org/10.1016/j.jep.2006.07.038.

Deryng, J., 1961. Atlas Sproszkowanych Roślinnych Surowców Leczniczych, first. ed. (Warszawa).

D'urso, G., Masullo, M., Seigner, J., Holper-Schichl, Y.M., Martin, R. de, Plaza, A., Piacente, S., 2020. LC–ESI–FT–MSn metabolite profiling of *Symphytum officinale* L. Roots leads to isolation of comfreyn A, an unusual arylnaphthalene lignan. Int. J. Mol. Sci. 21, 1–12. https://doi.org/10.3390/ijms21134671.

Edgar, R.C., Haas, B.J., Clemente, J.C., Quince, C., Knight, R., 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics 27, 2194–2200. https://doi.org/10.1093/bioinformatics/btr381.

Eren, A.M., Morrison, H.G., Lescault, P.J., Reveillaud, J., Vineis, J.H., Sogin, M.L., 2015. Minimum entropy decomposition: unsupervised oligotyping for sensitive partitioning of high-throughput marker gene sequences. ISME J. 9, 968–979. https://doi.org/10.1038/ismej.2014.195.

European scientific cooperative on phytotherapy (ESCOP), 2012. Monograph, *Symphyti radix*, comfrey root. https://escop.com/wp-content/uploads/edd/2015/09/Symphytum.pdf. (Accessed 16 February 2023).

He, Y., Zhu, L., Ma, J., Lin, G., 2021. Metabolism-mediated cytotoxicity and genotoxicity of pyrrolizidine alkaloids. Arch. Toxicol. 95, 1917–1942. https://doi.org/10.1007/s00204-021-03060-w.

Jarić, S., Kostić, O., Mataruga, Z., Pavlović, D., Pavlović, M., Mitrović, M., Pavlović, P., 2018. Traditional wound-healing plants used in the Balkan region (Southeast Europe). J. Ethnopharmacol. 211, 311–328. https://doi.org/10.1016/j.jep.2017.09.018.

Kimel, K., Krauze-Baranowska, M., 2021. Skuteczność i bezpieczeństwo stosowania korzenia *Symphytum officinale* L. – przegląd danych literaturowych. Postępy Fitoter. 22, 23–31. https://doi.org/10.25121/pf.2021.22.1.23.

Kimel, K., Zienkiewicz, M., Sparzak-Stefanowska, B., Krauze-Baranowska, M., 2019. TLC-densitometric analysis of allantoin in *Symphytum officinale* L. roots. Acta Pharm. 70, 101–110. https://doi.org/10.2478/acph-2020-0014.

Kruk, A., Granica, S., Popowski, D., Malinowska, N., Piwowarski, J.P., 2022. *Tiliae flos* metabolites and their beneficial influence on human gut microbiota biodiversity ex vivo. J. Ethnopharmacol. 294, 115355 https://doi.org/10.1016/j.jep.2022.115355.

Melnyk, N., Vlasova, I., Skowrońska, W., Bazylko, A., Piwowarski, J.P., Granica, S., 2022. Current knowledge on interactions of plant materials traditionally used in skin diseases in Poland and Ukraine with human skin microbiota. Int. J. Mol. Sci. 28, 1–28. https://doi.org/10.3390/ijms23179644.

Nastić, N., Borrás-Linares, I., Lozano-Sánchez, J., Švarc-Gajić, J., Segura-Carretero, A., 2020. Comparative assessment of phytochemical profiles of comfrey (*Symphytum officinale* L.) poot extracts obtained by different extraction techniques. Molecules 17, 1–17. https://doi.org/10.3390/molecules25040837.

Ożarowski, A., Jaroniewski, W., 1989. Rośliny Lecznicze I Ich Praktyczne Zastosowania. Instytut Wydawniczy Związków Zawodowych, Warszawa.

Piwowarski, J.P., Granica, S., Kiss, A.K., 2014a. Influence of gut microbiota-derived ellagitannins metabolites urolithins on pro-inflammatory activities of human neutrophils. Planta Med. 80, 887–895. https://doi.org/10.1055/s-0034-1368615.

Piwowarski, J.P., Granica, S., Zwierzyńska, M., Stefańska, J., Schopohl, P., Melzig, M.F., Kiss, A.K., 2014b. Role of human gut microbiota metabolism in the anti-inflammatory effect of traditionally used ellagitannin-rich plant materials. J. Ethnopharmacol. 155, 801–809. https://doi.org/10.1016/j.jep.2014.06.032.

Piwowarski, J.P., Kiss, A.K., Granica, S., Moeslinger, T., 2015. Urolithins, gut microbiota-derived metabolites of ellagitannins, inhibit LPS-induced inflammation in RAW 264.7 murine macrophages. Mol. Nutr. Food Res. 59, 2168–2177. https://doi.org/10.1002/mnfr.201500264.

Popowski, D., Czerwińska, M.E., Kruk, A., Pawłowska, K.A., Zentek, J., Melzig, M.F., Piwowarski, J.P., Granica, S., 2021. Gut microbiota metabolism and the permeability of natural products contained in infusions from herb of European goldenrod *Solidago virgaurea* L. J. Ethnopharmacol. 273, 1–12. https://doi.org/10.1016/j.jep.2021.113924.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 1–22. https://doi.org/10.7717/peerj.2584, 2016.

Salehi, B., Sharopov, Farukh, BoyunegmezTumer, T., 2019. *Symphytum* species: a comprehensive review on chemical composition, food applications and phytopharmacology. Molecules 24, 1–33. https://doi.org/10.3390/molecules24122272.

Savić, V.L., Savić, S.R., Nikolić, V.D., Nikolić, L.B., Najman, S.J., Lazarević, J.S., Đorđević, A.S., 2015. The identification and quantification of bioactive compounds from the aqueous extract of comfrey root by UHPLC–DAD–HESI–MS method and its microbial activity. Hem. Ind. 69, 1–8. https://doi.org/10.2298/HEMIND131202013S.

Sharon, I., Quijada, N.M., Pasolli, E., Fabbrini, M., Vitali, F., Agamennone, V., Dötsch, A., Selberherr, E., Grau, J.H., Meixner, M., Liere, K., Ercolini, D., de Filippo, C., Caderni, G., Brigidi, P., Turroni, S., 2022. The core human microbiome: does it exist and how can we find it? A critical review of the concept. Nutrients 14, 1–25. https://doi.org/10.3390/nu14142872.

Sile, I., Romane, E., Reinsone, S., Maurina, B., Tirzite, D., Dambrova, M., 2020. Medicinal plants and their uses recorded in the Archives of Latvian Folklore from the 19th century. J. Ethnopharmacol. 249, 112378 https://doi.org/10.1016/j.jep.2019.112378.

Sowa, I., Paduch, R., Strzemski, M., Zielińska, S., Rydzik-Strzemska, E., Sawicki, J., Kocjan, R., Polkowski, J., Matkowski, A., Latalski, M., Wójciak-Kosior, M., 2018. Proliferative and antioxidant activity of *Symphytum officinale* root extract. Nat. Prod. Res. 32, 605–609. https://doi.org/10.1080/14786419.2017.1326492.

Stickel, F., Seitz, H., 2000. The efficacy and safety of comfrey. Publ. Health Nutr. 45, 501–508. https://doi.org/10.5543/tkda.2017.88459.

Thukral, A.K., 2017. A review on measurement of Alpha diversity in biology. Agric. Res. J. 54, 1. https://doi.org/10.5958/2395-146x.2017.00001.1.

Trifan, A., Opitz, S.E.W., Josuran, R., Grubelnik, A., Esslinger, N., Peter, S., Bräm, S., Meier, N., Wolfram, E., 2018. Is comfrey root more than toxic pyrrolizidine alkaloids? Salvianolic acids among antioxidant polyphenols in comfrey (*Symphytum officinale* L.) roots. Food Chem. Toxicol. 112, 178–187. https://doi.org/10.1016/j.fct.2017.12.051.

Trifan, A., Skalicka-Woźniak, K., Granica, S., Czerwińska, M.E., Kruk, A., Marcourt, L., Wolfender, J.L., Wolfram, E., Esslinger, N., Grubelnik, A., Luca, S.V., 2020. *Symphytum officinale* L.: liquid-liquid chromatography isolation of caffeic acid oligomers and evaluation of their influence on pro-inflammatory cytokine release in LPS-stimulated neutrophils. J. Ethnopharmacol. 262 https://doi.org/10.1016/j.jep.2020.113169.

Trifan, A., Wolfram, E., Esslinger, N., Grubelnik, A., Skalicka-Woźniak, K., Minceva, M., Luca, S.V., 2021. Globoidnan A, rabdosiin and globoidnan B as new phenolic markers in European-sourced comfrey (*Symphytum officinale* L.) root samples. Phytochem. Anal. 32, 482–494. https://doi.org/10.1002/pca.2996.

Vogl, S., Picker, P., Mihaly-Bison, J., Fakhrudin, N., Atanasov, A.G., Heiss, E.H., Wawrosch, C., Reznicek, G., Dirsch, V.M., Saukel, J., Kopp, B., 2013. Ethnopharmacological in vitro studies on Austria's folk medicine - an unexplored lore in vitro anti-inflammatory activities of 71 Austrian traditional herbal drugs. J. Ethnopharmacol. 149, 750–771. https://doi.org/10.1016/j.jep.2013.06.007.

Wagner, H., Neidhardt, U., Tittel, G., 1981. DC- und HPLC-analyse der pyrrolizidin-N-oxid- alkaloide von *Symphyti radix*. Planta Med. 41, 232–239. https://doi.org/10.1055/s-2007-971708.

Yang, M., Ma, J., Ruan, J., Ye, Y., Fu, P.P.C., Lin, G., 2019. Intestinal and hepatic biotransformation of pyrrolizidine alkaloid N-oxides to toxic pyrrolizidine alkaloids. Arch. Toxicol. 93, 2197–2209. https://doi.org/10.1007/s00204-019-02499-2.

Zeeuwen, P.L.J.M., Kleerebezem, M., Timmerman, H.M., Schalkwijk, J., 2013. Microbiome and skin diseases. Curr. Opin. Allergy Clin. Immunol. 13, 514–520. https://doi.org/10.1097/ACI.0b013e328364ebeb.

Кисличенко, В.С., Журавель, І.О., Марчишин, С.М., Мінарченко, В.М., Хворост, О.П., 2015. ФарМакогнозія Частина 1, перше. НФаУ, Харків.

Manuscript co-author statement

As a one of co-authors of the manuscript: **"Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)"** published in *Journal of Ehtnopharmacology* (Volume 318, 26 July 2023, 116968, DOI: 10.1016/j.jep.2023.116968) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **„Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)"** opublikowanego w *Journal of Ehtnopharmacology* (tom 318, 26 czerwca 2023, 116968, DOI: 10.1016/j.jep.2023.116968) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Preparation of extract, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktu, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz jego metodologia i analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Dominik Popowski	Assistance in the phytochemical and microbiota analysis, and their visualization.
	Pomoc w analizie fitochemicznej i mikrobiotycznej oraz ich wizualizacji.
Jakub Strawa	Methodology of the LC–DAD–ESI–MS/TOF analysis and assistance in its performance, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF i pomoc w jej przeprowadzeniu, redakcja manuskryptu.
Klaudia Przygodzińska	Assistance with the preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota.
	Pomoc w przygotowywaniu wyciągów, analizie fitochemicznej oraz przeprowadzaniu eksperymentów *ex vivo* dotyczących mikrobioty skóry.
Michał Tomczyk	Methodology of the LC–DAD–ESI–MS/TOF analysis, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF, redakcja manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research, and manuscript editing.
	Koncepcja projektu, metodologia badań nad mikrobiotą oraz redakcja manuskryptu.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript editing, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, redakcja manuskryptu oraz nadzór nad projektem.

26.01.2026

Date / Data

Natalia Melnyk

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: "**Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)** " published in *Journal of Ehtnopharmacology* (Volume 318, 26 July 2023, 116968, DOI: 10.1016/j.jep.2023.116968) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: „**Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)** " opublikowanego w *Journal of Ehtnopharmacology* (tom 318, 26 czerwca 2023, 116968, DOI: 10.1016/j.jep.2023.116968) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Preparation of extract, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktu, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz jego metodologia i analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Dominik Popowski	Assistance in the phytochemical and microbiota analysis, and their visualization.
	Pomoc w analizie fitochemicznej i mikrobiotycznej oraz ich wizualizacji.
Jakub Strawa	Methodology of the LC–DAD–ESI–MS/TOF analysis and assistance in its performance, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF i pomoc w jej przeprowadzeniu, redakcja manuskryptu.
Klaudia Przygodzińska	Assistance with the preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota.
	Pomoc w przygotowywaniu wyciągów, analizie fitochemicznej oraz przeprowadzaniu eksperymentów *ex vivo* dotyczących mikrobioty skóry.
Michał Tomczyk	Methodology of the LC–DAD–ESI–MS/TOF analysis, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF, redakcja manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research, and manuscript editing.
	Koncepcja projektu, metodologia badań nad mikrobiotą oraz redakcja manuskryptu.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript editing, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, redakcja manuskryptu oraz nadzór nad projektem.

22/12/2025

Date / Data

Dominik Popowski

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: "**Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)** " published in *Journal of Ehtnopharmacology* (Volume 318, 26 July 2023, 116968, DOI: 10.1016/j.jep.2023.116968) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: „**Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)** " opublikowanego w *Journal of Ehtnopharmacology* (tom 318, 26 czerwca 2023, 116968, DOI: 10.1016/j.jep.2023.116968) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Preparation of extract, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktu, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz jego metodologia i analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Dominik Popowski	Assistance in the phytochemical and microbiota analysis, and their visualization.
	Pomoc w analizie fitochemicznej i mikrobiotycznej oraz ich wizualizacji.
Jakub Strawa	Methodology of the LC–DAD–ESI–MS/TOF analysis and assistance in its performance, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF i pomoc w jej przeprowadzeniu, redakcja manuskryptu.
Klaudia Przygodzińska	Assistance with the preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota.
	Pomoc w przygotowywaniu wyciągów, analizie fitochemicznej oraz przeprowadzaniu eksperymentów *ex vivo* dotyczących mikrobioty skóry.
Michał Tomczyk	Methodology of the LC–DAD–ESI–MS/TOF analysis, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF, redakcja manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research, and manuscript editing.
	Koncepcja projektu, metodologia badań nad mikrobiotą oraz redakcja manuskryptu.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript editing, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, redakcja manuskryptu oraz nadzór nad projektem.

23.10.2025 r.

Date / Data

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: "**Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)** " published in *Journal of Ehtnopharmacology* (Volume 318, 26 July 2023, 116968, DOI: 10.1016/j.jep.2023.116968) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: „**Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)** " opublikowanego w *Journal of Ehtnopharmacology* (tom 318, 26 czerwca 2023, 116968, DOI: 10.1016/j.jep.2023.116968) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Preparation of extract, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktu, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz jego metodologia i analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Dominik Popowski	Assistance in the phytochemical and microbiota analysis, and their visualization.
	Pomoc w analizie fitochemicznej i mikrobiotycznej oraz ich wizualizacji.
Jakub Strawa	Methodology of the LC–DAD–ESI–MS/TOF analysis and assistance in its performance, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF i pomoc w jej przeprowadzeniu, redakcja manuskryptu.
Klaudia Przygodzińska	Assistance with the preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota.
	Pomoc w przygotowywaniu wyciągów, analizie fitochemicznej oraz przeprowadzaniu eksperymentów *ex vivo* dotyczących mikrobioty skóry.
Michał Tomczyk	Methodology of the LC–DAD–ESI–MS/TOF analysis, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF, redakcja manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research, and manuscript editing.
	Koncepcja projektu, metodologia badań nad mikrobiotą oraz redakcja manuskryptu.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript editing, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, redakcja manuskryptu oraz nadzór nad projektem.

25. 11. 2025 ..... Klaudia Przygodzińska .....

Date / Data

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: **"Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)** " published in *Journal of Ehtnopharmacology* (Volume 318, 26 July 2023, 116968, DOI: 10.1016/j.jep.2023.116968) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **„Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)** " opublikowanego w *Journal of Ehtnopharmacology* (tom 318, 26 czerwca 2023, 116968, DOI: 10.1016/j.jep.2023.116968) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Preparation of extract, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktu, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz jego metodologia i analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Dominik Popowski	Assistance in the phytochemical and microbiota analysis, and their visualization.
	Pomoc w analizie fitochemicznej i mikrobiotycznej oraz ich wizualizacji.
Jakub Strawa	Methodology of the LC–DAD–ESI–MS/TOF analysis and assistance in its performance, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF i pomoc w jej przeprowadzeniu, redakcja manuskryptu.
Klaudia Przygodzińska	Assistance with the preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota.
	Pomoc w przygotowywaniu wyciągów, analizie fitochemicznej oraz przeprowadzaniu eksperymentów *ex vivo* dotyczących mikrobioty skóry.
Michał Tomczyk	Methodology of the LC–DAD–ESI–MS/TOF analysis, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF, redakcja manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research, and manuscript editing.
	Koncepcja projektu, metodologia badań nad mikrobiotą oraz redakcja manuskryptu.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript editing, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, redakcja manuskryptu oraz nadzór nad projektem.

29 X 2023 r.

Date / Data

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: **"Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)** " published in *Journal of Ehtnopharmacology* (Volume 318, 26 July 2023, 116968, DOI: 10.1016/j.jep.2023.116968) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **„Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)** " opublikowanego w *Journal of Ehtnopharmacology* (tom 318, 26 czerwca 2023, 116968, DOI: 10.1016/j.jep.2023.116968) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Preparation of extract, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktu, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz jego metodologia i analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Dominik Popowski	Assistance in the phytochemical and microbiota analysis, and their visualization.
	Pomoc w analizie fitochemicznej i mikrobiotycznej oraz ich wizualizacji.
Jakub Strawa	Methodology of the LC–DAD–ESI–MS/TOF analysis and assistance in its performance, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF i pomoc w jej przeprowadzeniu, redakcja manuskryptu.
Klaudia Przygodzińska	Assistance with the preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota.
	Pomoc w przygotowywaniu wyciągów, analizie fitochemicznej oraz przeprowadzaniu eksperymentów *ex vivo* dotyczących mikrobioty skóry.
Michał Tomczyk	Methodology of the LC–DAD–ESI–MS/TOF analysis, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF, redakcja manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research, and manuscript editing.
	Koncepcja projektu, metodologia badań nad mikrobiotą oraz redakcja manuskryptu.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript editing, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, redakcja manuskryptu oraz nadzór nad projektem.

KIEROWNIK
Zakład Mikrobiologii Farmaceutycznej i Bioanalizy
Wydział Farmaceutyczny
Warszawski Uniwersytet Medyczny
prof. dr hab. n. med. i n. o zdr. Jakub Piwowarski

21.01.2026 .....

Date / Data

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: **"Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)"** published in *Journal of Ehtnopharmacology* (Volume 318, 26 July 2023, 116968, DOI: 10.1016/j.jep.2023.116968) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **„Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L.)"** opublikowanego w *Journal of Ehtnopharmacology* (tom 318, 26 czerwca 2023, 116968, DOI: 10.1016/j.jep.2023.116968) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Preparation of extract, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktu, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz jego metodologia i analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Dominik Popowski	Assistance in the phytochemical and microbiota analysis, and their visualization.
	Pomoc w analizie fitochemicznej i mikrobiotycznej oraz ich wizualizacji.
Jakub Strawa	Methodology of the LC–DAD–ESI–MS/TOF analysis and assistance in its performance, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF i pomoc w jej przeprowadzeniu, redakcja manuskryptu.
Klaudia Przygodzińska	Assistance with the preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota.
	Pomoc w przygotowywaniu wyciągów, analizie fitochemicznej oraz przeprowadzaniu eksperymentów *ex vivo* dotyczących mikrobioty skóry.
Michał Tomczyk	Methodology of the LC–DAD–ESI–MS/TOF analysis, manuscript editing.
	Metodologia analizy LC–DAD–ESI–MS/TOF, redakcja manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research, and manuscript editing.
	Koncepcja projektu, metodologia badań nad mikrobiotą oraz redakcja manuskryptu.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript editing, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, redakcja manuskryptu oraz nadzór nad projektem.

24.10.2023 ..... Date / Data

Sebastian Granica ..... Legible signature / Czytelny podpis

PROSPECTS
IN PHARMACEUTICAL SCIENCES

Prospects in Pharmaceutical Sciences, -
https://prospects.wum.edu.pl/

Original Article

From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin

Natalia Melnyk[1], Weronika Skowrońska[1], Dominik Popowski[1,2], Jakub Piwowarski[3], Sebastian Granica[1,*]

[1] *Department of Pharmaceutical Biology, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097, Warsaw, Poland*

[2] *Department of Food Safety and Chemical Analysis, Waclaw Dabrowski Institute of Agricultural and Food Biotechnology-State Research Institute, Warsaw, Poland*

[3] *MicrobiotaLab, Department of Pharmaceutical Microbiology and Bioanalysis, Medical University of Warsaw, Banacha 1, Warsaw, Poland*

* Correspondence, e-mail: Sebastian Granica, Department of Pharmaceutical Biology, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland, tel/fax +48 22 5720953, e-mail: sgranica@wum.edu.pl

Received: 27.10.2025 / Revised: 01.12.2025 / Accepted: 01.12.2025 / Published: 11.12.2025

ABSTRACT

The skin represents a complex ecosystem where host cells and microbiota coexist in dynamic equilibrium. Disruption of this balance contributes to inflammation and diseases, while natural compounds may help restore it. For centuries, marigold and chamomile have been among the most valued medicinal plants in traditional herbal medicine, widely used for treating wounds, skin inflammation, and irritations. Their long-standing therapeutic reputation is supported by rich phytochemical profiles – triterpenoids, flavonoids, phenolic acids in marigold, and sesquiterpene lactones, flavonoids, and coumarins in chamomile – known to exert anti-inflammatory, antioxidant, and soothing effects. In this study, *Calendula officinalis* and *Matricaria recutita* flower extracts' effects were investigated on human skin microbiota and dermal cells. Both extracts remained chemically stable under microbial exposure and did not generate new metabolites, highlighting resistance to microbial metabolism. Neither extract disrupted community structure; instead, they selectively modulated microbial taxa, decreasing potentially pro-inflammatory families (*Staphylococcaceae*, *Corynebacteriaceae*, and *Enterococcaceae*) and enriching *the Bacillales and Bacillaceae* families. On the cellular level, at ≤250 µg/mL, both extracts were biocompatible with fibroblasts and keratinocytes. Marigold flower extract showed no significant anti-inflammatory effect in keratinocytes, as IL-6 and IL-8 secretion remained comparable to the stimulated control. In contrast, Chamomile flower extract markedly reduced IL-6 levels in a dose-dependent manner, with moderate effects on IL-8. In fibroblasts, both extracts had strong suppression of IL-6 and IL-8 at higher concentrations. These findings reveal a dual mechanism – direct cellular modulation and indirect microbiota-mediated rebalancing-supporting the traditional therapeutic efficacy of *C. officinalis* and *M. recutita* in skin health.

KEYWORDS: Calendula officinalis, Matricaria recutita, skin microbiota, inflammation

1. Introduction

Skin conditions are a major public health concern, affecting 30–70% of the global population. They are the most common reason for visits to general practitioners. Over 3,000 different skin disorders, both acute and chronic, have been identified, affecting people of all ages and socioeconomic levels [1]. A lot of studies show that skin conditions can significantly alter the microbiota's diversity and composition. For example, in human and animal patients with atopic dermatitis, dysbiosis of the skin microbiota leads to decreased diversity of microbial populations. It is unclear whether these altered microbial populations are the cause or effect of inflammatory skin conditions observed in humans and animals, but there is no doubt that the microbiome plays an important role in skin health. Also, research is growing on how the human microbiome works with exogenous exposures and the body to create a homogenous environment promoting

ultimate health [2].

At the cellular level, keratinocytes and dermal fibroblasts are key players in skin health and disease. Keratinocytes, which constitute the majority of the epidermis, act as the first line of defense against environmental stimuli, releasing cytokines and antimicrobial peptides that orchestrate the immune response [3]. Dermal fibroblasts, embedded in the connective tissue of the dermis, contribute to tissue repair and extracellular matrix remodeling but also secrete mediators that modulate inflammation [4]. Together, these cell types not only respond to exogenous factors but also shape the local microenvironment in which the microbiota resides. Thus, understanding how external interventions – such as natural plant extracts – interplay with cell and microbial communities is crucial for developing innovative therapeutic strategies.

Medicinal plants have a remarkable history of benefiting humanity across nearly every continent. Natural products are essential sources for drug discovery, and many contemporary topically applied medications used in modern pharmacotherapy have their origins in traditional herbal medicine [5]. Medicinal plants still hold great promise for the future, as the phytochemical composition and potential health benefits of numerous species remain unexplored or require further in-depth research [6]. In recent years, there has been growing interest in natural alternatives to synthetic anti-inflammatory agents [7]. Plant extracts, traditionally used in folk medicine for their healing properties, have gained significant attention in dermatological research due to their bioactive compounds that can modulate inflammatory processes [8,9].

Among the most recognized medical herbs used in topical formulations are *Calendula officinalis* L. (marigold) and *Matricaria recutita L.* (chamomile), both officially listed in pharmacopeias and valued for their long-standing use in treating skin inflammation, wounds, and irritations. Marigold is rich in triterpenoids, flavonoids, carotenoids, and phenolic acids, which contribute to its anti-inflammatory, antimicrobial, and wound-healing activities [10]. Chamomile, on the other hand, contains a complex of sesquiterpene lactones (chamazulene, α-bisabolol, matricin), flavonoids (apigenin, luteolin, quercetin glycosides), and coumarins, which collectively exhibit anti-inflammatory, antioxidant, and soothing effects [11]. Chamomile preparations are known to interfere with NF-κB signaling and to downregulate interleukin expression, thus attenuating the inflammatory response associated with various dermatoses [12]. Both species have been incorporated into numerous dermatological and cosmetic preparations designed to restore skin homeostasis, reduce erythema, and support tissue regeneration. Despite their widespread use, the precise mechanisms of action remain insufficiently characterized. Moreover, the interactions between plant materials and the skin are multifaceted. On one hand, plant compounds can influence skin cells, promoting healing, reducing inflammation, and modulating immune responses. On the other hand, these compounds may interact with the skin's microbiota - the diverse community of microorganisms that live on the skin's surface and play a key role in protecting against pathogens, modulating inflammation, and maintaining skin barrier function. Recent studies highlight that this interaction is bidirectional. Phytochemicals can selectively influence microbial growth, inhibit pathogenic bacteria, and promote beneficial commensals, thereby balancing dysbiotic communities [13]. In turn, microorganisms can metabolize plant-derived compounds into bioactive derivatives with altered or enhanced biological activity [14,15].

Taken together, skin health emerges as a dynamic outcome of the interplay between cells, microbiota, and exogenous bioactive compounds, with medicinal plants offering a unique bridge between traditional practices and modern scientific approaches.

The main objective of this work is to evaluate how traditionally used *Calendula officinalis* and *Matricaria recutita* extracts interplay with skin microbiota, as well as their influence on keratinocytes and dermal fibroblasts. Given that the mechanism of action of plant-derived preparations is still not fully elucidated, this approach – combining cellular models with microbiota studies – will contribute to a more comprehensive understanding of how these materials exert their effects. In particular, it will clarify how microbial metabolism may influence the chemical composition of marigold and chamomile extracts, thereby influencing their efficacy and biological relevance.

2. Materials and Methods

The interactions of a 70% (*v*/*v*) ethanolic extracts with human skin microbiota were studied. The impact of a 70% (*v*/*v*) ethanolic extracts on the inflammatory response in skin cells (including fibroblasts and keratinocytes) were examined. The chemical composition of the extracts was characterized using UHPLC-DAD-MS^n.

2.1. Plant sources and extraction

Calendulae officinalis flos and *Matricariae recutita flos* (batch numbers: 2079 and 911.2019, expiration dates: July 2020 and September 2020, respectively) were obtained from Kawon-Hurt (Gostyn, Poland) and Flos (Mokrsko, Poland), respectively. A voucher specimen has been cataloged in the Herbarium of the Department of Pharmaceutical Biology at the Medical University of Warsaw, Poland, under the reference numbers 2020CO07 and 2020CH09. The plant materials were identified as marigold and chamomile flowers through macroscopic and microscopic analysis conducted by Prof. Sebastian Granica, following the methods described by European Pharmacopeia monographs. Additionally, TLC analysis was performed to confirm the plant's identity and compliance with the manufacturer's specifications [16,17].

A total of 50 g of plant material (*C. officinalis flos* or *M. recutita flos*) was subjected to triple extraction with 500 mL of 70% ethanol (*v*/*v*, 7:3) at room temperature under periodic stirring. To improve compound recovery, each extraction step included 15 minutes of ultrasonic bath treatment. The combined extracts were filtered and concentrated under reduced pressure (<45 °C) using a LABORANTA 4000 WB vacuum system (Heidolph, Schwabach, Germany). The concentrate was frozen at −20 °C for 24 h and subsequently lyophilized in a Cryodos freeze-dryer (Telstar, Terrassa, Spain). This process yielded 14,45 g of MFE (marigold flower extract) and 4,04 g of CFE

(chamomile flower extract), which were stored in an airtight container at 4 °C until use.

For analysis, the dry extract was dissolved in MeOH to prepare a 10 mg/mL solution for UHPLC-DAD-MS^n analysis, a 12 mg/mL solution in H_2O was prepared for microbiota interaction, and a 180 mg/mL stock solution in DMSO for cell culture experiments.

2.2. Phytochemical screening

The analysis of extracts (MFE and CFE) (10 mg/mL MeOH) and the metabolic experiment samples was conducted using a UHPLC-3000 RS system (Dionex, Leipzig, Germany) coupled with a DAD detector and a splitless connection to an AmaZon SL ion trap mass spectrometer equipped with an ESI interface (Bruker Daltonik GmbH, Bremen, Germany). UV spectra were captured within the wavelength range of 200–450 nm. The mass spectrometer parameters were set as follows: nebulizer pressure at 40 psi, drying gas flow rate at 9.0 L/min, nitrogen gas temperature at 300°C, and capillary voltage at 4.5 kV. Mass spectra were recorded by scanning in the m/z range of 70 to 2200. A Kinetex XB-C18 chromatography column (Phenomenex, Torrance, CA; 150 mm × 2.1 mm; 1.7 µm) was employed. The mobile phase (A) consisted of UPW: HCOOH (99.9:0.1, *v*/*v*), while the mobile phase (B) was MeCN: HCOOH (99.9:0.1, *v*/*v*). The gradient program was set to 1–26% B over 0–60 min (0.416% B/min) and 26–95% B over 60–120 min (1.15% B/min) with a 0.3 mL/min flow rate. An injection volume of 4.0 µL (40 µg/µL) was used for all samples filtered through a 0.45 µm PVDF syringe filter.

2.3. Ex vivo experiments on skin microbiota

Skin microbiota were obtained from the forearms of five healthy volunteers by swabbing each forearm 10 times. Participants were instructed to refrain from showering on the day of sampling and to avoid applying any medications or cosmetic products to the forearm for at least 24h beforehand. The study was conducted in accordance with the Declaration of Helsinki and followed the guidelines of the Ethics Committee of the Medical University of Warsaw (AKBE/151/2021), which approved the collection of skin microbiota for *ex vivo* investigations.

After 24 h of microbiota pre-incubation, 1 mL of the extract was added to 5 mL of each donor sample to reach a final concentration of 2 mg/mL in the culture. Before use, the extract was sterilized by filtration through a 0.22 µm cellulose acetate syringe filter. Two types of controls were prepared: culture medium supplemented with the extract but without microbiota, and a method blank, in which donor microbiota were incubated without the extract. For the control with extract, 1 mL of the extract was added to 5 mL of BHI medium, whereas for the method blank, 1 mL of sterile water was added to 5 mL of the donor sample. All cultures were incubated at 37 °C with shaking at 120-140 rpm to ensure aeration (mini Galaxy A, RS Biotech, Nunc GmbH & Co, Wiesbaden, Germany).

2.3.1. Skin microbiota metabolism of the extract

At three timepoints (24, 48, and 72 h), 0.5 mL aliquots of each culture were collected from the microbiota culture, mixed with 0.5 mL of MeOH containing 0.2% HCOOH (1:1, *v*/*v*), and centrifuged at 10,000 rpm for 3 minutes to separate the microbial pellets. Supernatants were then subjected to UHPLC-DAD-MS^n analysis to evaluate changes in extract composition.

2.3.2. Skin microbiota sequencing

Amplification and sequencing of the 16S rDNA, accompanied by bioinformatic analysis, were conducted on the experimental samples following 24 hours of incubation with the addition of the studied extract. Samples from each donor were individually acquired utilizing the OmniGene-Skin kit. DNA extraction was conducted, followed by amplicon sequencing utilizing a two-stage PCR protocol and an Illumina sequencer. The V3–V4 region of 16S rDNA (341F-785R) was targeted with a universal primer set, producing 2.4 million paired-end sequence reads. The mean length of the aggregated sequences was 409 nucleotides. Chimeric reads were identified and removed using the UCHIME de novo algorithm [18] with the VSEARCH package [19]. The residual high-quality readings were subjected to minimum entropy decomposition [20]. To assign taxonomic information to each OTU, sequences from the clusters were aligned to the Mega BLAST database (https://blast.ncbi.nlm.nih.gov/). The QIIME program (version 1.9.1) was used for OTU processing and taxonomy classification. Abundances were normalized based on lineage-specific copy numbers of the relevant marker genes for more accurate estimates. Alpha diversity indices were calculated following the methods by Chao and Chiu from 2014 [21] and Thukral from 2017 [22]. Box plots were made using the ggplot2 and ggtext programs in R.

2.4. Keratinocytes and fibroblasts cell cultures

HaCaT and NHDF cells were obtained from Lonza Group Ltd. (Basel, Switzerland). DMEM High glucose w/stable glutamine w/sodium pyruvate, Dulbecco's Phosphate Buffered Saline (DPBS) without Ca^{2+} and Mg^{2+}, fetal bovine serum (FBS), penicillin-streptomycin solution, Trypsin-EDTA solution were purchased from Biowest (Nuaille, France). Interferon-γ and TNF-α were obtained from InvivoGen (Toulouse, France), lipoteichoic acid (LTA) from *Staphylococcus aureus*, and dimethylsulfoxide (DMSO) from Sigma Aldrich (United States). IL-6 and IL-8 ELISA kits were from BD Bioscience (San Diego, USA).

Cells were cultured in high-glucose DMEM supplemented with 10% FBS and antibiotics (penicillin and streptomycin). The medium was replaced every 2–3 days using standard aseptic procedures. Cells from flasks that were 70–90% confluent and had over 90% viability were then seeded into 96-well or 24-well culture plates at the specified densities

2.5. Keratinocytes and fibroblasts viability evaluation

HaCaT (7×10^3) and NHDF (5×10^3) cells were seeded into 96-well plates and allowed to grow until reaching 90% confluency. Following this, the cells were treated with previously prepared dilutions of the extracts, 7.8 – 1000 µg/mL, and incubated for 72 hours. Cell fixation was then performed by adding 50 µL/well of 50% trichloroacetic acid (TCA). After a 1-hour incubation at 4°C, the plates were rinsed with water and air-dried. Subsequently, the cell monolayer was stained with 0.04% Sulforhodamine B (SRB) sodium salt. After a 1-hour incubation, the plates were washed with 1% acetic acid

and dried. Finally, 10 mM Tris base was added to solubilize the protein-bound dye, and absorbance was measured at 510 nm.

2.6. Evaluation of IL-6 and IL-8 secretion

To quantify cytokines' release, HaCaT (4×10^4) and NHDF (7×10^4) were seeded into 24-well plates. Once the cultures reached approximately 90% confluence, they were treated with marigold extract at 7.8 – 250 µg/mL concentrations and chamomile extract at 15.6 – 500 µg/mL concentrations. For NHDF, the stimulus (LTA) was added simultaneously with the treatment, whereas for HaCat, the stimuli (mixture of TNF-α and IFN-γ) were added 3h after treatment. After 24h, 200 µL of the supernatant from each well was collected from three independent experiments and stored at − 20 °C until analysis. Interleukin concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) using a commercially available human IL-6 and IL-8 kits (BD Bioscience, USA), following the manufacturer's protocols. Absorbance was measured at 450 nm with correction at 570 nm using a Synergy 4 microplate reader (Biotek).

2.7. Statistical analysis

The results were presented as means ± standard deviation of the means. Statistical significance of differences between means was determined by one-way ANOVA. For comparison of results with the control group, Dunnett's post hoc test was used. Results with *p-value* < 0.05 and *p-value* < 0.001 were considered statistically significant. All analyses were performed using Statistica 10 software.

3. Results and discussion

3.1. Chemical extracts' constituents assessment

The hydroethanolic flower extracts of marigold flowers (MFE) and chamomile flowers (CFE) were subjected to UHPLC-DAD-MS^n analysis, through which a method for assessing their chemical composition was established (**Fig. 1, Table 1**, and **Fig. 2, Table 2** accordingly).

3.1.1. Phytochemical screening of marigold flower extract (MFE)

Peak **1** exhibited a $[M\text{-}H]^-$ ion at 353 *m/z*, identified as chlorogenic acid, with a primary fragment at 191 *m/z* due to the loss of a quinic acid moiety. In the positive mode, a 355 *m/z* ion was observed, further fragmenting into 336, 307, and 163 *m/z* ions [23]. Peak **2** produced a $[M\text{-}H]^+$ ion at 415 *m/z*, corresponding to an undefined compound, with fragmentation resulting in ions at 219 *m/z*. Peak **3** showed a $[M\text{-}H]^-$ ion at 755 *m/z*, representing quercetin-3-O-(2″,6″-di-O-rhamnosyl)-glucopyranoside, with fragments at 737, 609, 591, 573, 547, 489, 465, 409, 343, 300, 271, and 255 *m/z*. It was isolated and identified by Weronika Skowrońska [24]. Peak **4** produced a $[M\text{-}H]^-$ ion at 609 *m/z*, identified as calendoflavobioside, which fragmented into 489, 463, 445, 343, 301, 300, 271, and 179*m/z* ions [23]. The positive mode detected a 611 m/z ion, with further fragmentation into 465, 303, and 315 *m/z* ions. Peak **5** had an $[M\text{-}H]^-$ ion at 769 *m/z*, identified as isorhamnetin-3-*O*-rhamnosylrutinoside, with fragmentation leading to ions at 423, 369, 357, 315, 314, and 300 *m/z* [25]. The positive mode detected at 771 *m/z* revealed additional fragments at 625, 479, 427, and 317 *m/z*. Peak **6** produced a $[M\text{-}H]^-$ ion at 623 *m/z*, corresponding to isorhamnetin-3-*O*-rutinoside, which fragmented into 605, 503, 357, 339, 315, and 299 *m/z* ions [25]. Peak **7** displayed a $[M\text{-}H]^-$ ion at 623 *m/z*, identified as calendoflavoside, with a fragmentation pattern yielding ions at 591, 503, 477, 459, 383, 356, 314b, and 299 *m/z*. In the positive mode, prominent fragments were detected at 479 and 317 *m/z* [23]. Peak **8** showed a $[M\text{-}H]^-$ ion at 609 *m/z*, corresponding to rutin, with fragments at 577, 477, 459, and $[M\text{-}H]^+$ at 611 *m/z*, and further fragmentation into 479 and 317 *m/z* ions in the positive mode [23]. Peak **9** displayed a $[M\text{-}H]^-$ ion at 623 *m/z*, corresponding to narcisin, with fragments at 315, 299, 271, and 255 *m/z*. In the positive mode, a 625 *m/z* ion was observed, which further fragmented into 479 and 317 *m/z* ions [23]. Peak **10**, assigned to isorhamnetin-3-*O*-glucoside, showed a $[M\text{-}H]^-$ ion at 477 *m/z*, yielding fragments at 357, 314, and 285 *m/z*. The positive mode revealed a primary ion at 479 *m/z*, alongside 302 and 165 *m/z* fragments [26]. Peak **11**, linked to isorhamnetin-3-*O*-(6″-acetyl)-glucoside, generated a $[M\text{-}H]^-$ ion at 519 *m/z*, fragmenting into 315 and 300 *m/z* ions, while the $[M\text{-}H]^+$ detected a 565 *m/z* ion [26]. Peak **12**, an unidentified compound, exhibited a $[M\text{-}H]^-$ ion at 589 *m/z*, with fragmentation producing 584 and 485 *m/z* ions. Peak **13**, identified as oleanolic acid glucuronide A, showed a $[M\text{-}H]^-$ ion at 1118 *m/z*, with fragments at 955, 793, 549, and 455 *m/z*, and a $[M\text{-}H]^+$ ion at 439 with fragmentations at 393, 329, 249, and 191 *m/z* [25]. Peak **14**, corresponding to oleanolic acid glucuronide C, displayed a $[M\text{-}H]^-$ ion at 956 *m/z*, with fragmentation resulting in 794, 483 *m/z* ions [25]. Peak **15**, linked to oleanolic acid glucuronide B, produced a $[M\text{-}H]^-$ ion at 793 *m/z*, fragmenting into ions at 673, 631, and 569 *m/z* [25]. Peak **16**, associated with oleanolic acid glucuronide C, showed a $[M\text{-}H]^-$ ion at 955 *m/z*, with fragments at 793, 776, and 713 *m/z*. Peak **17**, representing oleanolic acid glucuronide D, exhibited a $[M\text{-}H]^-$ ion at 793 *m/z*, with fragmentation yielding 731, 613, 595, 569, 551, 537, 483, and 455*m/z* ions [25]. Peak **18**, identified as oleanolic acid glucuronide E, showed a $[M\text{-}H]^-$ ion at 631 *m/z*, fragmenting into 613, 571, 555, 527, 509, and 455 *m/z* ions [25].

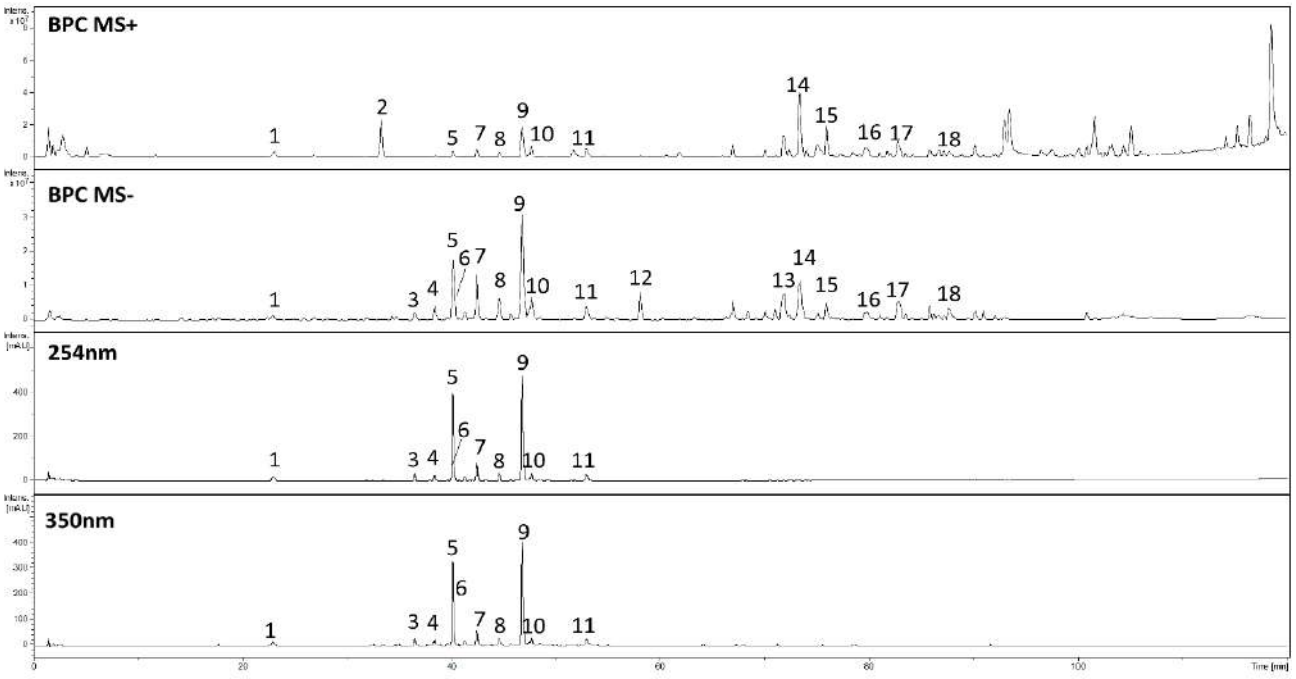


Fig 1. UHPLC-DAD-MS data of the 70% ethanolic extract of marigold flowers (MFE) were recorded in the mode of positive ions (MS+), negative ions (MS-), and UV of 254 and 350 nm.

Table 1. UHPLC-DAD-MS data of detected compounds in the 70% ethanolic extract from the marigold flowers (MFE).

Peak number	Retention time [min]	UV-Vis max [nm]	$[M-H]^-$ *m/z*	MS2 ions (-)	MS3 ions (-)	$[M-H]^+$ *m/z*	MS2 ions (+)	MS3 ions (+)	Compound name	Ref.
1	23.1	248sh, 334	353	191	-	355	336, 307, 163b	-	chlorogenic acid	[23]
2	33.3	-	-	-	-	415	219b	-	unidentified compound	-
3	36.5	254, 265sh, 356	755	737, 609, 591, 573, 547, 489, 465, 409, 343, 300b, 271,255	531, 445, 400, 383, 373, 355, 343b, 313, 301, 297, 271, 267, 254	-	-	-	quercetin-3-*O*-(2″,6″-di-*O*-rhamnosyl)-glucopyranoside	[24]
4	38.4	254, 356	609	489, 463, 445, 343, 301, 300b, 271, 179	373, 343, 301b	611	465, 303b	447, 399, 315, 303b	calendoflavobioside	[23]
5	40.2	253, 265sh, 355	769	737, 624, 605, 503, 423, 369, 357, 315, 314, 300	-	771	625, 479, 427, 317b, 229	-	isorhamnetin-3-*O*-rhamnosylrutinoside	[25]
6	40.2	253, 265sh, 355	623	605, 503, 357, 339, 315b, 299	-	-	-	-	isorhamnetin-3-*O*-rutinoside	[26]
7	42.5	253, 265sh, 354	623	591, 503, 477, 459, 383, 356, 314b, 299	-	625	479, 317b	461, 425, 383, 359, 329, 317b	calendoflavoside	[23]
8	44.7	253, 267sh, 354	609	577, 477, 459, 339, 315b, 271, 179	-	611	479, 317b	-	rutin	[23]
9	46.8	253, 267sh, 354	623	315b, 299, 271, 255	-	625	479, 317b	-	narcissin	[23]
10	47.8	253, 264, 341	477	357, 314b, 285, 271, 151	-	479	302, 165b, 163	-	isorhamnetin-3-*O*-glucoside	[26]
11	53.0	253, 265, 354	519	315b, 300	-	565[c]	317b	-	isorhamnetin-3-*O*-(6″-acetyl)-glucoside	[23]
12	58.1	-	589	584b, 485	-	-	-		unidentified compound	-
13	71.8	-	1118[d+ac]	955b, 793, 549, 455	793b, 569, 549, 455	439	393, 329, 249, 191	-	oleanolic acid glucuronide A	[25]
14	73.5	-	956	794b, 483	614, 595, 551, 537 485, 484 483b, 455	-	-	-	oleanolic acid glucuronide C	[25]
15	75.9	-	793[d+s]	673, 631b, 569	555, 509, 455b	-	-	-	oleanolic acid glucuronide	
16	79.8	-	955	793, 776, 731, 713, 613, 595, 569, 551, 538, 524, 455b	631, 614, 613, 595, 587, 569, 537, 524, 523b, 483, 455, 453	-	-	-	oleanolic acid glucuronide B	[25]
17	82.8	-	793	731, 613, 595, 569, 551, 537, 483, 455	-	-	-	-	oleanolic acid glucuronide D	[25]
18	87.7	-	631	613, 571, 555, 527, 509, 455b	407b	-	-	-	oleanolic acid glucuronide F	[25]

b – base peak (the most abundant ion in the recorded spectrum); sh – shoulder in UV-Vis spectrum; [ac] – acetyl unit $[M+42]^-$; [c] – carboxyl unit $[M+44]^-$; [d] – double mass; [s] – sodium $[M+Na]^-$.

3.1.2. Phytochemical screening of chamomile flower extract (CFE)

Peaks **19** and **20** gave $[M-H]^-$ ions of 355 *m/z*. These were identified as 2-hydroxy-4-methoxyoxycinnamic acid glucoside [27]. They fragmented into a 193 *m/z* peak, during which the hexose was lost, and a 149 *m/z* peak formed by the detachment of an additional CO_2 molecule (-44), indicating that the carboxylic acid in the cinnamic group was free, unesterified. Further loss of the methyl group was observed for peak **19** at 134 *m/z* and was typical of homolytic cleavage of the methyl belonging to the methoxyl group. The differentiation of peak **19** into cis-2-hydroxy-4-methoxy-coccinnamic acid glucoside and peak **20** into trans-2-hydroxy-4-methoxy-coccinnamic acid glucoside was made possible by the occurrence of an additional peak in UV-Vis at 320 nm, being characteristic of the cis isomer [27]. Peak **21** gave a $[M-H]^-$ ion of 479 *m/z*, which indicates the presence of quercetagenin 3-*O*-glucoside. It decayed into a peak with a mass of 317 *m/z*, probably by loss of glucose [28]. Peaks **22** and **23** gave ions $[M-H]^-$ with masses of 463 *m/z* derived from hyperoside and isoquercetin. These compounds have the same masses, but hyperoside has a lower retention time than isoquercetin [29]. Peak **24** was identified as luteolin hexoside with $[M-H]^-$ ion at 447 *m/z*, and $[M-H]^+$ ion at 449 *m/z*, while peak **25** was identified as patuletin 3-*O*-glucoside, as it is derived from the $[M-H]^-$ ion of 493 *m/z*. Peak **26** shows a signal from the $[M-H]^-$ ion with a mass of 493 *m/z*. An absorption maximum was observed at 355 nm, so it is known that the sugar group is in position 3, causing a hypochromic shift relative to the aglycone itself. On this basis, the compound was identified as apigenin-7-*O*-glucoside [27]. In the case of luteolin-7-*O*-glucoside (**27**) and chrysoeriol 7-*O*-glicoside (**28**), the addition of one additional oxygen substitution on C-3′ results in a bathochromic shift of band I at ~ 347 nm, while band II shows a cleavage of its peak, giving rise to a characteristic arm at 267 nm. Compound **29** was recognized as quercetin-7-*O*-glucoside additionally due to the information of its $[M-H]^-$ peak mass of 507 *m/z* [27]. Peak **30** arose from the $[M-H]^-$ ion with a mass of 515 *m/z*, indicating that it is dicaffeoylquinic acid derivative [27]. Peak **31** and **32** with an $[M-H]^-$ mass of 517 *m/z* were identified as isorhamnetin-7-*O*-glucoside and isorhamnetin-3-*O*-glucoside, accordingly [27,29]. Peak **33** showed a signal from the [M-H]+ ion with a mass of 269 *m/z*, and although the chromatogram did not show fragmentation of this ion, due to the signal intensity being too weak, it was identified as apigenin [28]. Peak **34** was recognized as 3-hydroxydecanoic acid, with an $[M-H]^-$ mass of 373 *m/z* and $[M-H]^+$ mass of 375 *m/z* [30]. Peak **35** is from the [M-H]- ion with a mass of 373 *m/z*. It shows absorption maxima at 319 nm and 341 nm. Based on this, the compound was identified as dihydroxytetramethoxyflavone [28]. Peaks **36-40** could not be identified from the available literature.

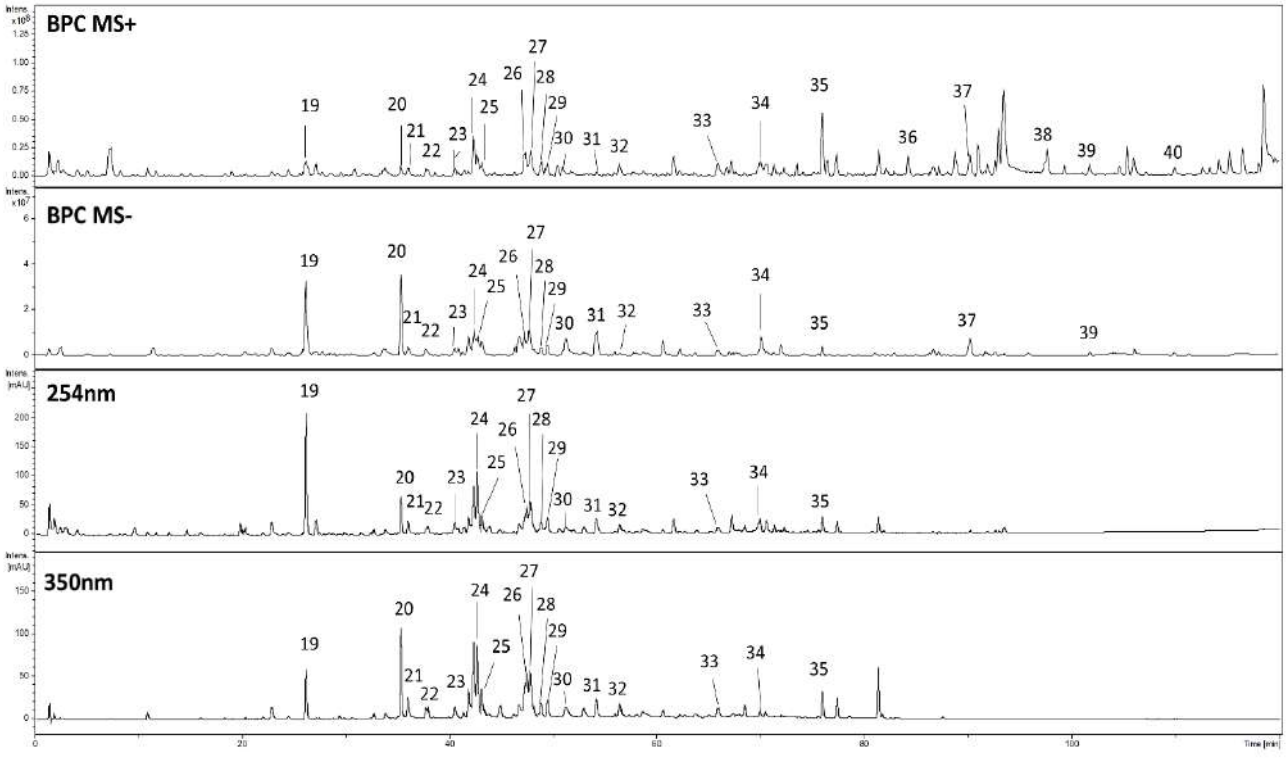


Fig 2. UHPLC-DAD-MS data of the 70% ethanolic extract of chamomile flowers (CFE) were recorded in the mode of positive ions (MS+), negative ions (MS-), and UV of 254 and 350 nm.

Table 2. UHPLC-DAD-MS data of detected compounds in the 70% ethanolic extract from the chamomile flowers (CFE).

Peak number	Retention time [min]	UV-Vis max [nm]	[M-H]- *m/z*	MS2 (-)	[M-H]+ *m/z*	MS2 (+)	Compound name	Ref.
19	26.10	280, 301sh	355	193b, 149, 134	379c	365b, 203, 185, 173	cis-2-hydroxy-4-methoxyoxocinnamic acid glucoside	[27]
20	35.20	287, 320	355	193b, 149	379c	361, 292, 202, 154	trans-2-hydroxy-4-methoxycinnamic acid glucoside	[27]
21	35.90	258, 276, 352	479	317b	481	319b	quercetagenin-3-*O*-glucoside	[27]
22	37.60	278, 377	463	301b	465	421, 303b	quercetin-3-*O*-galactoside (hyperoside)	[29]
23	40.40	253, 368	463	301b, 343	465	303b	quercetin-3-*O*-glucoside (isoquercetin)	[29]
24	42.60	257, 368	447	285b, 286	449	287b, 288	luteolin hexoside	[29]
25	43.00	257, 275, 355	493	331b	495	333b	patuletin 3-*O*-glucoside	[27]
26	47.30	252h, 268, 328	431	269b	433	271b	apigenin 7-*O*-glucoside	[27]
27	47.70	267, 285, 337	447	285b	449	287b	luteolin-7-*O*-glucoside (cynaroside)	[28]
28	48.80	267, 289, 346	461	299b, 446, 284	463	301b, 302	chrysoeriol-7-*O*-glucoside	[27]
29	49.40	257, 272, 364	507[fa]	387, 345b	463	347b	quercetin-7-*O*-glucoside	[27]
30	51.30	300, 330	515	353b, 203	517	353b, 299, 255, 203, 179, 173	dicaffeoylquinic acid derivative	[27]
31	54.10	295sh, 325	517	323b, 281, 179, 251, 355, 193, 2210, 341, 437, 353	541[c]	365, 347b	isorhamnetin-7-*O*-glucoside	[27]
32	56.30	265, 340	517	473, 269b, 268	519	433, 271b	isorhamnetin-3-*O*-glucoside	[27,29]
33	65.80	269, 337	269	-	271	-	apigenin	[28]
34	70.10	350	329	293, 264, 229b, 211, 171	353[c]	-	3-hydroxydecanoic acid	[30]
35	75.90	319, 341sh	373	358b, 359	375	356,342, 311b	dihydroxytetramethoxyflavone	[28]
36	84.30	-	-	-	499	261b	unidentified compound	-
37	90.30	-	295	277b, 233, 170	319[c]	277b, 233, 171,	unidentified compound	-
38	97.70	-	-	-	341	-	unidentified compound	-
39	101.80	-	277	233b	279	261, 243, 223, 205b,	unidentified compound	-
40	110.10	-	-	-	593	533b, 461	unidentified compound	-

b – base peak (the most abundant ion in the recorded spectrum); sh – shoulder in UV-Vis spectrum; [c] - carboxyl unit $[M+44]^-$; [fa] – formic acid $[M+46]^-$.

3.2. *Ex vivo* skin microbiota metabolism of the extract

The hydroethanolic extracts of *Calendulae officinalis* flos and *Matricariae recutita flos* were incubated with skin microbiota for 24 h to assess possible biotransformation processes. Previous studies on microbiota showed that under microbiota influence, secondary metabolites from the extract can be generated, which enhance biological activity [14]. Moreover, our previous study on comfrey root showed that the chemical composition of the extract was biodegraded by skin microbiota [31]. In the case of marigold and chamomile flowers, UHPLC-DAD-MSn analysis revealed that the chemical profile of the extracts remained unchanged after incubation (**Fig. 3-4**).

In both extracts, no new metabolites were detected, and all initially identified constituents of the extracts retained their stability throughout the experiment. This may reflect the relative stability of the main phytochemical constituents, such as flavonoids and terpenoids, which are less prone to microbial transformation compared to glycoside-rich or alkaloid-containing plants. These findings indicate that, under the applied conditions, the skin microbiota did not induce any metabolic conversion of the extracts' components. Importantly, such stability can be considered a favorable outcome, as it suggests that the tested phytochemicals are resistant to microbial transformation within the studied model, which may support their persistence and activity when applied topically.

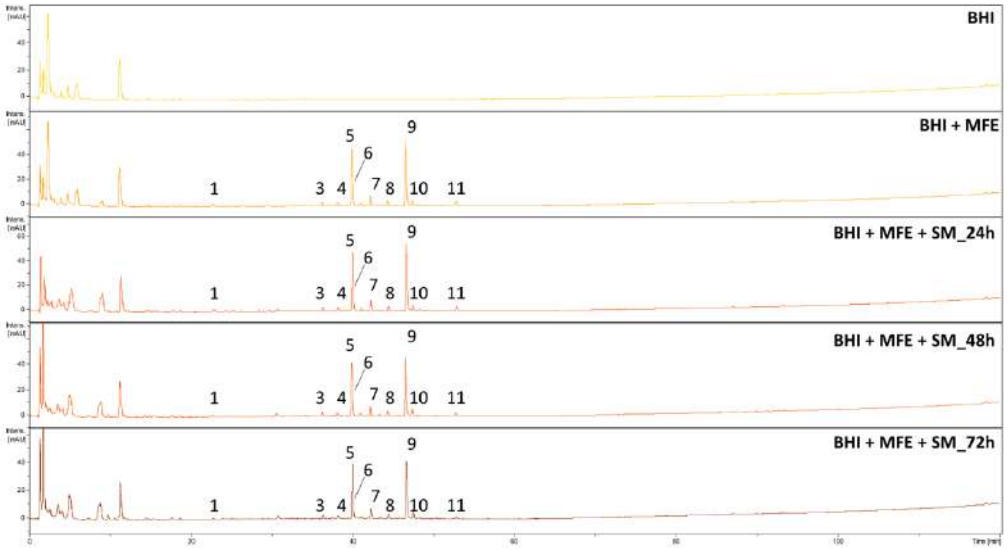


Fig. 3. UHPLC-DAD-MSn chromatograms in the UV of 254 nm of *Calendula officinalis* extract after 24, 48, 72 h incubation with human skin microbiota, showing no detectable metabolite formation, confirming chemical stability of MFE under microbiota influence. BHI, brain heart infusion; MFE, marigold flower extract; SM, skin microbiota.

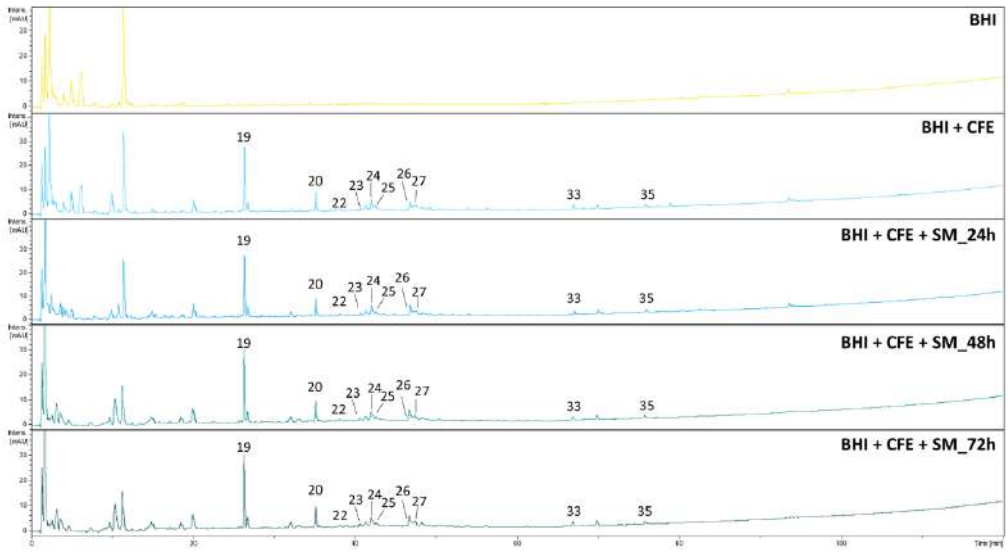


Fig. 4. UHPLC-DAD-MSn chromatograms in the UV of 254 nm of *Matricaria recutita* extract after 24, 48, 72 h incubation with human skin microbiota, showing no detectable metabolite formation, confirming chemical stability of CFE under microbiota influence. BHI, brain heart infusion; CFE, chamomile flower extract; SM, skin microbiota.

3.3. Skin microbiota modulation by MFE and CGE

In a comparative analysis of skin microbiota profiles between untreated controls and samples incubated with plant extracts for 24 h, reproducible shifts in the relative composition of the community were found. Control samples reflect the baseline community structure with the expected dominance of several taxa and the contribution of low-abundant groups.

In the experiment involving *C. officinalis* extract (**Fig. 5**), the microbiota community was dominated by members of the families *Staphylococcaceae*, *Bacillaceae*, *Enterococcaceae*, and unclassified *Bacillales*. After exposure to the extract, a redistribution of the shares of dominant and subdominant taxa is observed. The relative abundance of *Staphylococcaceae, Corynebacteriaceae, Enterococcaceae, Lactobacillaceae, and Leuconostocaceae* decreased, whereas unclassified *Bacillales* relatively increased.

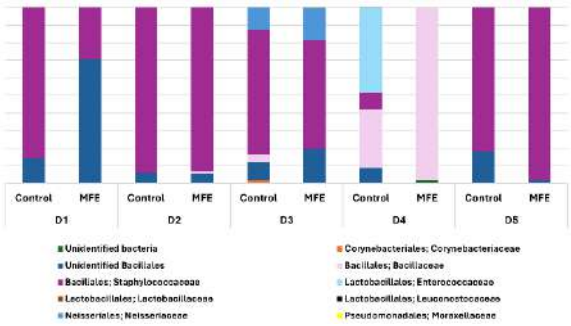


Fig.5. The relative order and family abundance in samples after 24 h incubation. MFE, marigold flower extract; D1-5, donor number.

Notably, several bacterial families (e.g., unclassified *Bacteria* and *Moraxellaceae*) were absent in the control profile, but appeared after 24 h incubation with the extract. This likely reflects taxa that were initially present at very low abundance and became detectable after selective suppression of dominant orders. Such emergence can be interpreted as the result of ecological niche and/or selective stimulation by extract components, rather than true *de novo* colonization. Also, it can be because of the detection effect of sequencing, as taxa could be present in the control in extremely low numbers (below the detection threshold or statistically "masked" by dominant taxa). The presence of extract constituents alters the culture environment, enabling those taxa to increase their abundance above the noise level.

It indicates that the extracts selectively suppressed some taxa while allowing some groups to expand. This suggests a modulatory rather than broad-spectrum antimicrobial effect, leading to a rebalancing of the skin microbial community. Additionally, the maintenance of α-diversity indicates that the extract does not exhibit broad-spectrum antimicrobial activity, although treatment with *C. officinalis* influenced microbial community structure (**Fig. 6**). The Chao-1 index (**Fig. 6, A**) revealed a tendency towards lower richness in the MFE group compared to the control, indicating a reduction in the number of observed taxa. The Shannon (**Fig. 6, B**) and Simpson (**Fig. 6, C**) indices also demonstrated a more uniform distribution in MFE-treated samples, with slightly lower median values relative to the control.

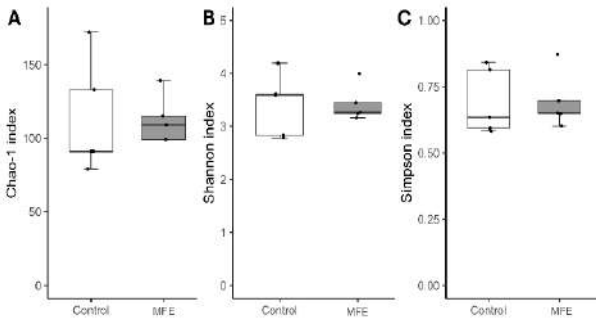


Fig. 6. Boxplots of α-diversity: Chao 1 (A), Shannon (B), Simpson (C) indices. MFE, marigold flower extract.

During the evaluation of *M. recutita* extract, the skin microbiota was primarily composed of the unclassified *Bacillales*, *Staphylococcaceae*, and one donor had *Bacillaceae* (**Fig. 7**). Exposure to the extract resulted in the redistribution of dominant and subdominant taxa as well. A reduction in the relative abundance of unclassified bacteria and *Pseudomonadaceae* was noted, while *Bacillaceae* increased. The response of other bacterial groups varied among donors.

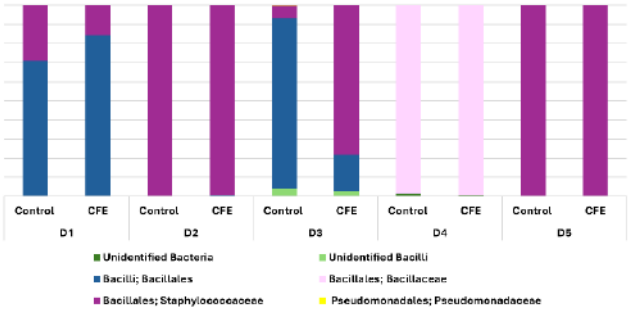


Fig. 7. The relative order and family abundance in samples after 24 h incubation. CFE, Chamomilla flower extract; D1-5, donor number.

Analysis of α-diversity revealed that exposure to chamomile flower extract affected microbial community richness and diversity (**Fig. 8**). The Chao-1 richness (**Fig. 8, A**) showed a broader distribution of values in the CFE group compared with the control, suggesting donor-dependent changes in the number of observed taxa. Similarly, the Shannon (**Fig. 8, B**) and Simpson (**Fig. 8, C**) indices indicated increased variability after CFE treatment, with some samples showing higher diversity than the control group. These results suggest the CFE modulates microbial diversity in a heterogeneous manner, reflecting interindividual differences in microbiota response.

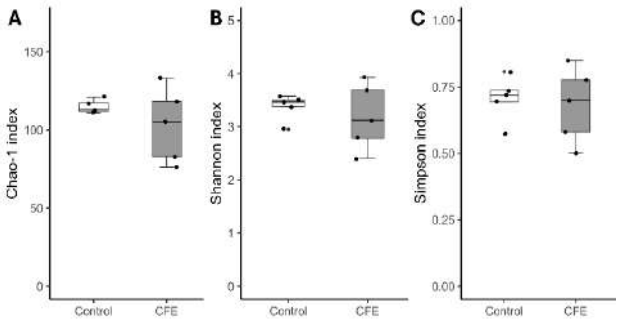


Fig. 8. Boxplots of α-diversity: Chao 1 (A), Shannon (B), Simpson (C) indices. CFE, Chamomilla flower extract

Comparison of the effects of marigold and chamomilla flower extracts on the skin microbiota revealed distinct patterns of modulation. CFE increased inter-donor variability, with some samples showing higher richness and diversity compared with the control. This suggests a more selective and individualized influence on microbial communities, potentially stimulating certain beneficial taxa while suppressing others. In contrast, MFE led to an overall reduction in richness and diversity indices, accompanied by decreased variability between donors. Such an effect indicated a broader and uniform impact on microbial populations, which may reflect a stronger antimicrobial or growth-limiting action. Taken together, these findings highlight that although both extracts modulate microbial communities, chamomile appears to promote donor-dependent diversification, whereas calendula exerts a more homogenizing effect by reducing overall community complexity. These differences may be attributed to their distinct phytochemical compositions, which could differentially influence microbial growth and interactions.

3.4. Keratinocytes and fibroblasts viability evaluation

Keratinocytes and fibroblasts viability was assessed using the colorimetric method, Sulforhodamine B assay, to determine cell viability and growth by quantifying total cellular protein content [32]. The assay is based on the binding of the anionic dye to basic amino acid residues of cellular proteins that remain attached to the culture plate after fixation. The amount of bound dye is directly proportional to the total protein mass and, consequently, to the number of viable cells.

Cell viability is expressed as a percentage of the absorbance of untreated control cells. The SRB assay offers a sensitive and reproducible method for evaluating the impact of plant extracts, drugs, or other agents on cell proliferation and cytotoxicity [33].

3.4.1. Viability of HaCaT keratinocytes

Treatment of keratinocytes with marigold flower extract revealed a pronounced dose-dependent biphasic effect on cell viability (**Fig. 9, A**). At high concentrations (1000 and 500 µg/mL), a significant reduction in metabolic activity was observed ($p < 0.001$), indicating potential cytotoxic or growth-inhibitory effects of the concentrated extracts. Such reduction may be associated with the presence of saponins, flavonoids, or other phenolic constituents known for their membrane-active or pro-oxidant behavior at elevated doses [34]. In contrast, moderate to low concentrations (250-7.8 µg/mL) did not impair HaCaT viability, and in fact, at 125-31.3 µg/mL, the extract markedly stimulated cell proliferation, reaching approximately 107-110% of the control value ($p < 0.05$; $p < 0.001$). This stimulatory response suggests a potential adaptive effect, where sub-cytotoxic doses enhance metabolic and proliferative activity, possibly due to activation of antioxidant defense mechanisms or modulation of growth factor signaling pathways.

Exposure of HaCaT to chamomile flower extract resulted in a mild concentration-dependent response with no marked cytotoxicity across most tested doses (**Fig. 9, B**). Only the highest concentration (1000 µg/mL) significantly reduced cell viability ($p < 0.001$), indicating that excessive amounts of the extract may exert mild cytostatic or membrane-perturbing effects.

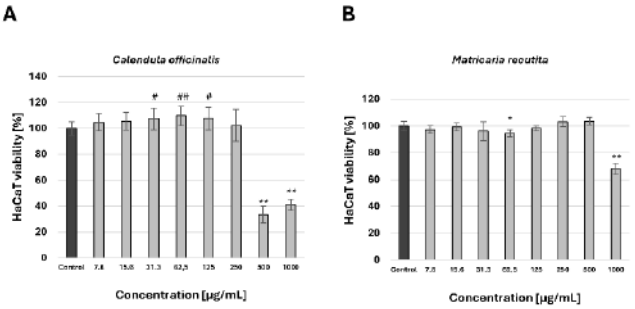


Fig.9. The viability of HaCaT cells assessed after 24 h of treatment with extract of *Calendula officinalis* (A) and *Matricaria recutita* (B) (SRB assay). A statistically significant increase (#$p < 0.05$; ##$p < 0.001$) or decrease (*$p < 0.05$; **$p < 0.001$) was indicated relative to control.

Such outcomes at high doses are consistent with the presence of sesquiterpene lactones and phenolic acids, which may induce oxidative stress or alter mitochondrial function when accumulated intracellularly [35]. At lowest concentrations, 250 – 7.8 µg/mL, no notable proliferative effect was detected, indicating that CFE primarily stabilizes cellular metabolism rather than directly stimulating proliferation. This neutral or protective action might reflect a predominance of phenolic acids and flavonoids with anti-inflammatory and antioxidant activity, which help preserve keratinocytes' homeostasis without overactivation.

Both extracts exhibited dose-dependent cytotoxicity at the highest concentrations, while maintaining good biocompatibility at ≤ 250 and 500 µg/mL, respectively. These findings confirm that both extracts are safe and non-cytotoxic for epidermal application at moderate concentrations.

3.4.2. Viability of NHDF fibroblasts

The viability of NHDF after exposure to extracts of *Calendula officinalis* and *Matricaria recutita* demonstrated distinct but concentration-dependent trends (**Fig. 10**).

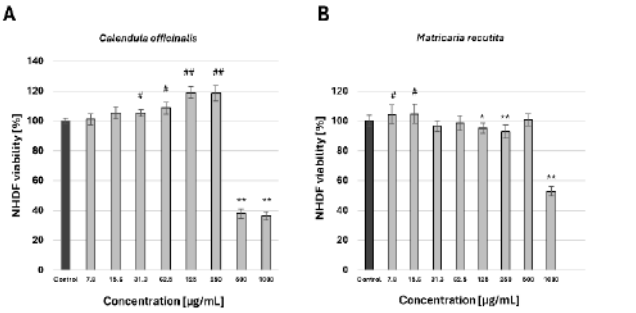


Fig.10. The viability of NHDF cells assessed after 72 h of treatment with extract of *Calendula officinalis* (A) and *Matricaria recutita* (B) (SRB assay). A statistically significant increase (#$p < 0.05$; ##$p < 0.001$) or decrease (*$p < 0.05$; **$p < 0.001$) was indicated relative to the control.

In the case of marigold (**Fig. 10, A**), fibroblasts responded in a biphasic manner. At low and moderate concentrations (7.8 - 250 µg/mL), a slight but consistent increase in viability was observed, with a maximum of

approximately 120% compared to the control at 125-250 µg/mL. This suggests that MFE at sub-cytotoxic levels may stimulate fibroblasts' metabolic activity and proliferation. Such effects are typical for phytochemicals with antioxidant and trophic properties, which can promote dermal repair and collagen synthesis. However, at 500-1000 µg/mL, cell viability dropped below 40%, indicating cytotoxicity likely associated with excessive accumulation of bioactive compounds.

CFE (**Fig. 10, B**) maintained fibroblast viability near the control level across most concentrations, demonstrating good cytocompatibility and absence of toxic effects. A moderate decrease was observed only at the higher concentrations (1000 µg/mL), similar to MFE, suggesting a threshold-dependent response. Unlike MFE, no notable proliferative effect was detected, indicating that CFE primarily stabilizes cellular metabolism rather than directly stimulating proliferation. Just at low concentrations, 15.6 – 7.8 µg/mL, the extract maintained slightly increased cell viability compared to the control, with values remaining 105% of baseline. This suggests that *Matricaria recutita* is well tolerated by NHDF cells and can support cell survival even under prolonged exposure. The observed biocompatibility aligns with its traditional dermatological applications and reported antioxidant, anti-inflammatory, and soothing effects.

Overall, both extracts were well tolerated by NHDF cells at concentrations ≤ 250 and 500 µg/mL, respectively. The biphasic pattern observed for MFE supports the hypothesis that plant-derived antioxidants may exhibit dose-dependent duality – protective at low doses and cytotoxic when overloaded. These findings highlight the potential of both extracts as safe and biologically active components for dermal applications.

3.5. Anti-inflammatory potential of extracts

Skin inflammation represents a complex, multi-stage biological response that involves both epidermal and dermal cell populations. It is initiated primarily by keratinocytes, which, upon exposure to pro-inflammatory cytokines such as tumor necrosis factor and interferons, release a wide range of mediators, including interleukin-6 and interleukin-8 [36]. These cytokines act as central regulators of the cutaneous immune response – IL-6 contributes to leukocyte activation, acute-phase protein synthesis, and modulation of keratinocyte proliferation, while IL-8 serves as a potent chemoattractant for neutrophils and macrophages, amplifying the inflammatory cascade [37,38]. Sustained overproduction of these mediators is associated with chronic inflammatory skin disorders such as atopic dermatitis, psoriasis, and delayed wound healing [38].

In the dermis, fibroblasts further sustain and propagate inflammation through cytokine secretion and paracrine crosstalk with immune cells and keratinocytes. Thus, simultaneous assessment of inflammatory markers in both HaCaT and NHDF provides an integrated model for evaluating the dual epidermal and dermal anti-inflammatory actions of bioactive compounds.

Plant extracts rich in polyphenols, triterpenoids, and glycosides have been shown to modulate inflammatory signaling pathways, including NF-κB, MAPK, and STAT1/3, thereby reducing cytokine production and oxidative stress [39–41]. In this study, the anti-inflammatory potential of the tested extracts from *Calendulae officinalis flos* and *Matricariae recutita flos* was investigated by measuring their ability to regulate IL-6 and IL-8 secretion in both types of skin cells.

3.5.1. Modulation of IL-6 and IL-8 secretion in HaCaT keratinocytes

HaCaT cells were stimulated with TNF-α/IFN-γ to induce cytokine production and to evaluate the potential of the tested extracts to modulate this response. Stimulation markedly increased the secretion of IL-6 and IL-8 compared with the non-stimulated control. Urolithin A was used as a positive control, reducing both IL-6 and IL-8 secretion in a dose-dependent manner.

Treatment with *Calendula officinalis* extract did not result in significant suppression of IL-6 secretion at any of the tested concentrations (7.8 – 250 µg/mL) (**Fig. 11**). Similarly, IL-8 release remained unchanged, with values close to those of the stimulated control (**Fig. 12**). These results suggest that, within the tested concentration range, *Calendula officinalis* exhibits a lack of pronounced anti-inflammatory efficacy in keratinocytes under TNF-α/IFN-γ challenge.

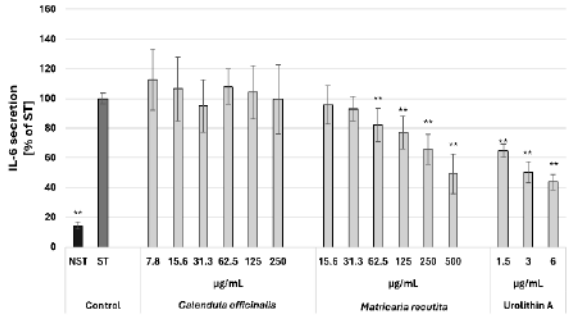


Fig.11. Effect on IL-6 release in HaCaT cells stimulated with a TNF-α/IFN-γ mixture (10 ng/mL each). A statistically significant increase (#p < 0.05; ##p <0.001) or decrease (*p < 0.05; **p < 0.001) was observed compared with stimulated control.

In contrast, *Matricaria recutita* extract demonstrated a distinct modulatory effect. A concentration-dependent decrease in IL-6 secretion was observed, with reductions of approximately 50-70% at concentrations of 250 and 500 µg/mL (*p < 0.05; **p < 0.001, respectively) **(Fig. 11).** The suppression of IL-8 secretion was less pronounced and did not consistently reach statistical significance, indicating a more selective action towards IL-6 modulation **(Fig. 12).**

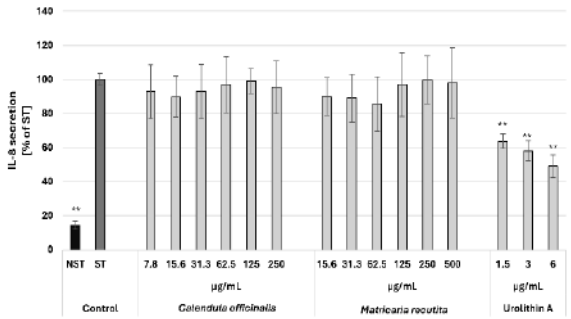


Fig.12. Effect on IL-8 release in HaCaT cells stimulated with a TNF-α/IFN-γ mixture (10 ng/mL each).

A statistically significant decrease (*p < 0.05; **p < 0.001) was observed compared with the stimulated control.

3.5.2. Modulation of IL-6 and IL-8 secretion in NHDF fibroblasts

To assess the anti-inflammatory effects of the tested extracts in dermal fibroblasts, NHDF cells were stimulated with lipoteichoic acid (LTA), a Toll-like receptor 2 agonist derived from Gram-positive bacteria, known to induce IL-6 and IL-8 secretion.

Both extracts demonstrated a clear dose-dependent inhibitory effect on IL-6 and IL-8 secretion **(Fig. 13-14).** At high concentrations (125 – 250 µg/mL) of *Calendula officinalis* extract, the levels of both cytokines decreased to 20-30% of stimulated control. *Matricaria recutita* extract also suppressed cytokine secretion, with high doses (250-500 µg/mL) causing profound suppression of IL-6 and IL-8 to 15-30% of stimulated control. However, for IL-6 at 15.6 µg/mL, a biphasic response was observed: the value exceeded stimulated control by almost 20%, whereas for IL-8 at the same dose, the effect was close to control.

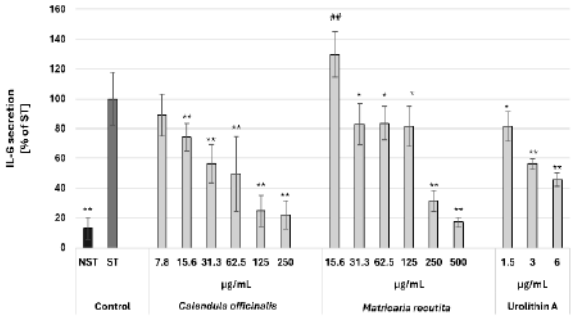


Fig. 13. Effect on IL-6 release in NHDF cells stimulated with LTA (10 ng/mL). A statistically significant increase (#p < 0.05; ##p <0.001) or decrease (*p < 0.05; **p < 0.001) was observed compared with stimulated control.

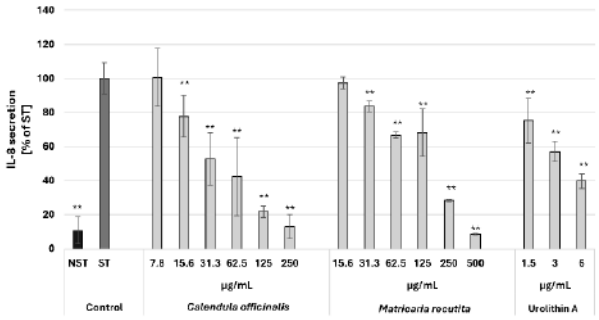


Fig. 14. Effect on IL-8 release in NHDF cells stimulated with LTA (10 ng/mL). A statistically significant decrease (*p < 0.05; **p < 0.001) was observed compared with the stimulated control.

4. Conclusions

Calendula officinalis and *Matricaria recutita* extracts, characterized by distinct phytochemical profiles, demonstrated a complex yet balanced interaction with both skin microbiota and dermal cells. The marigold flower extract (MFE), rich in phenolics, flavonoids, and saponins, and the chamomile flower extract (CFE), containing mainly flavonoids and caffeic acid derivatives, both remained chemically stable under microbiota exposure, as no new metabolites were identified after incubation with human skin microbial communities. This stability indicates a resistance of the major constituents to microbial degradation, suggesting that their biological effects arise primarily from native phytochemicals rather than microbiota-derived metabolites.

Chamomile extract induced donor-dependent changes, with certain samples showing increased richness and diversity compared with the control, reflecting a more variable and individualized modulatory potential. In contrast, marigold extract consistently reduced richness and diversity, resulting in a more uniform microbial structure. Importantly, neither extract caused collapse of the microbial ecosystem; instead, they selectively modulated the relative abundance of dominant and subdominant taxa while preserving overall community complexity. Such selective remodeling may represent a beneficial ecological mechanism, supporting skin homeostasis through microbiota rebalancing rather than broad-spectrum antimicrobial activity.

Moreover, the observed decrease in the relative abundance of *Staphylococcaceae, Corynebacteriaceae,* and *Enterococcaceae* suggests a potential anti-inflammatory rebalancing of the skin microbiota, as these taxa are often associated with dysbiosis and inflammatory skin conditions. Concurrently, the increase in unclassified *Bacillales* and *Bacillaceae* may indicate the enrichment of species with antimicrobial or immunomodulatory properties, contributing to a more resilient and less pro-inflammatory microbial ecosystem. The reduction in unclassified bacteria and *Pseudomonadaceae*, which include opportunistic and pro-inflammatory species such as *Pseudomonas aeruginosa,* further supports the hypothesis of a decreased pathogenic and inflammatory potential.

At the cellular level, both extracts showed favorable biocompatibility with human skin cells at a concentration of ≤ 250 µg/mL. Fibroblasts appeared more resilient and responsive to low-dose stimulation, while keratinocytes displayed higher sensitivity to concentrated treatments. *C. officinalis* showed a clear dose-dependent response in keratinocytes: higher concentrations were cytotoxic, intermediate levels promoted cell proliferation, and the lowest doses produced no measurable traditional application in wound healing and tissue repair. In contrast, *M. recutita* showed a stabilizing and cytoprotective profile, helping to maintain cellular homeostasis without inducing excessive proliferation or stress responses.

Summarizing, these findings emphasize a dual mechanism of action: direct cellular modulation and indirect microbiota-mediated balance. The extracts act as gentle regulators – stabilizing the skin microbial ecosystem while simultaneously protecting and modulating inflammatory responses in skin cells. Notably, the relative abundance of potentially pro-inflammatory and opportunistic taxa decreased, whereas beneficial and immunomodulatory groups increased. This selective microbial modulation reflects a shift toward a more balanced, resilient, and anti-inflammatory skin environment. This dual interaction between phytochemicals, microbiota, and host cells provides a mechanistic basis for the traditional therapeutic efficacy

of *Calendula officinalis* and *Matricaria recutita* and highlights their potential as multifunctional components in formulations aimed at restoring skin health and homeostasis.

Appendix

List of abbreviations:

CFE chamomile flower extract

BLAST basic local alignment search tool

DMEM Dulbecco's modified Eagle medium

DPBS Dulbecco's phosphate-buffered saline

FBS fetal bovine serum

HaCaT human epidermal keratinocyte line

MFE marigold flower extract

NHDF normal human dermal fibroblasts

NST non-stimulated control

IL interleukin

ST stimulated control

SRB sulforhodamine B

ELISA enzyme-linked immunosorbent assay

IFN interferon

TNF tumor necrosis factor

LTA Lipoteichoic acid

UHPLC-DAD-MS ultra-high performance liquid chromatography (UHPLC) coupled to diode array detection (DAD) and multi-stage mass spectrometry (MS)

OTU operational taxonomic unit

PCR polymerase chain reaction

QIME quantitative insights into microbial ecology

UCHIME algorithm for detecting chimeric sequences

VSEARCH versatile open-source tool for metagenomics

Author Contributions: Conceptualization, **N.M.**; methodology, **W.S., J.P.P., S.G.**;investigation, **N.M.;** resources, **S.G.;** data curation, **N.M.;** writing—original draft preparation, **N.M.;** writing—review and editing, **W.S., D.P., S.G.**; visualization, **N.M., D.P.;** supervision, **J.P.P., S.G.**;project administration, **N.M., S.G.**; funding acquisition, **S.G.** All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by by the National Science Centre Poland grant Preludium Bis 2 No. 2020/39/O/NZ7/01109.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Acknowledgments: The authors express their sincere gratitude to **Kamila Konstancja Lis**, **Aleksandra Anna Nyczka**, and **Anastasiya Kharyna**, the students of the Department of Pharmaceutical Biology, Medical University of Warsaw, for their valuable assistance in conducting the experiments and data collection

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Richard MA, Paul C, Nijsten T, et al. Prevalence of most common skin diseases in Europe : a population-based study. *J Eur Acad Dermatology Venerelogy* **2022**; *1088–1096*. DOI: 10.1111/jdv.18050

2. Hoffmann AR. The cutaneous ecosystem : the roles of the skin microbiome in health and its association with inflammatory skin conditions in humans and animals. V*et Dermatol* **2017***; 28: 60-e15*. DOI: 10.1111/vde.12408

3. Simmons J, Gallo RL. The Central Roles of Keratinocytes in Coordinating Skin Immunity. *J Invest Dermatol* **2024**; *144: 2377-2398*. DOI: 10.1016/j.jid.2024.06.1280

4. Atit R, Thulabandu V, Chen D. Dermal fibroblast in cutaneus development and healing. *Wiley Interdiscip Rev Dev Biol* **2018***; 7: 1-19*. DOI: 10.1002/wdev.307.Dermal

5. Khan H. Medicinal Plants in Light of History : Recognized Therapeutic Modality. *J Evidence-Based Compementary Altern Med* **2014**; *19: 216-219*. DOI: 10.1177/2156587214533346

6. Marelli M. Medicinal Plants. Plants 2021; 10. DOI: 10.3390/plants10071355

7. Proestos C. The Benefits of Plant Extracts for Human Health. *Foods* **2020**; *10-12*. DOI: 10.3390/plants10071355

8. Khumaidi A, Murwanti R, Damayanti E, et al. Empirical use, phytochemical, and pharmacological effects in wound healing activities of compounds in Diospyros leaves: A review of traditional medicine for potential new plant-derived drugs Akhmad Khumaidi. *J Ethnopharmacol* **2025**; *337*. DOI: 10.1016/j.jep.2024.118966

9. Fernandes A, Rodrigues PM, Pintado M, et al. Phytomedicine A systematic review of natural products for skin applications : Targeting inflammation , wound healing , and photo-aging. *Phytomedicine* **2023**; *115*. DOI: 10.1016/j.phymed.2023.154824

10. Nicolaus C, Junghanns S, Hartmann A, et al. In vitro studies to evaluate the wound healing properties of *Calendula officinalis* extracts. *J Ethnopharmacol* **2017**; *196*. DOI: 10.1016/j.jep.2016.12.006

11. Santos DS dos, Barreto R de SS, Serafini MR, et al. Phytomedicines containing *Matricaria species* for the treatment of skin diseases: A biotechnological approach. *Fitoterapia* **2019**; *138*. DOI : 10.1016/j.fitote.2019.104267

12. Flemming M, Kraus B, Rascle A, et al. Revisited anti-inflammatory activity of matricine in vitro: Comparison with chamazulene. *Fitoterapia* **2015**; *106*: *122–128*. DOI: 10.1016/j.fitote.2015.08.010

13. Krizkovska B, Hoang L, Brdova D, et al. Modulation of the bacterial virulence and resistance by well-known European medicinal herbs. *J Ethnopharmacol* **2023**; *312*. DOI: 10.1016/j.jep.2023.116484

14. Skowrońska W, Pawłowska KA, Obrębski M, et al. Chemical composition, skin microbiota metabolism, antimicrobial potential and anti-inflammatory properties of witch hazel bark (*Hamamelis virginiana* L.). *J Ethnopharmacol* **2025**; *353*: *120433*. DOI: 10.1016/j.jep.2025.120433

15. Piwowarski JP, Granica S, Zwierzyńska M, et al. Role of human gut microbiota metabolism in the anti-inflammatory effect of traditionally used ellagitannin-rich

plant materials Jakub P. Piwowarski. *J Ethnopharmacol* **2014**; *155*: *801–809*. DOI: 10.1016/j.jep.2014.06.032

16. Polish Pharmacopoeia, 12th Edition. Monograph 04/2020:1297 *Calendulae flos* , Warsaw: Office for Registration of Medicinal Products, Medical Devices and Biocidal Products; 2020.

17. Polish Pharmacopoeia, 12th Edition. Monograph 07/2019:0404 *Matricariae flos* , Warsaw: Office for Registration of Medicinal Products, Medical Devices and Biocidal Products; 2020.

18. Edgar RC, Haas BJ, Clemente JC, et al. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **2011**; *27: 2194–2200*. DOI : 10.1093/bioinformatics/btr381

19. Rognes T, Flouri T, Nichols B, et al. VSEARCH: A versatile open source tool for metagenomics. *PeerJ* **2016**; *1–22*. DOI: 10.7717/peerj.2584

20. Eren AM, Esen C, Quince C, et al. Anvi'o : an advanced analysis and visualization platform for 'omics data. *PeerJ* **2015**; *1–29*. DOI: 10.7717/peerj.1319

21. Chao A, Chiu C. Species Richness : Estimation and Comparison. *Wiley StatsRef Stat Ref Online* **2014**; DOI: 10.1002/9781118445112.stat03432.pub2

22. Thukral AK. A review on measurement of Alpha diversity in biology. *Agric Res J* **2017**; *54: 1–10*. DOI: 10.5958/2395-146X.2017.00001.1

23. Olennikov DN, Kashchenko NI, Chirikova NK. Isorhamnetin and Quercetin Derivatives as Anti-Acetylcholinesterase Principles of Marigold (*Calendula officinalis*) Flowers and Preparations. *Int J Mol Sci* **2017**; *18: 1–17*. DOI: 10.3390/ijms18081685

24. Skowrońska W, Granica S, Piwowarski JP, et al. Wound healing potential of extract from *Sambucus nigra* L. leaves and its fractions. *J Ethnopharmacol* **2024**; *320*. DOI: 10.1016/j.jep.2023.117423

25. Budan A, Bellenot D, Freuze I, et al. Potential of extracts from *Saponaria officinalis* and *Calendula officinalis* to modulate in vitro rumen fermentation with respect to their content in saponins. *Biosci Biotechnol Biochem* **2014**; *78: 288–295*. DOI : 10.1080/09168451.2014.882742

26. Liu J, Guo Y, Zhang J, et al. Phytomedicine Systematic chemical analysis of flavonoids in the Nelumbinis stamen. *Eur J Integr Med* **2014**; *21: 1753–1758*. DOI : 10.1016/j.phymed.2014.09.003

27. Tsivelika N, Irakli M, Mavromatis A, et al. Phenolic Profile by HPLC-PDA-MS of Greek Chamomile Populations and Commercial Varieties and Their Antioxidant Activity. *Foods* **2021**; DOI: :10.3390/ foods10102345

28. Lin L, Harnly JM. LC-PDA-ESI/MS Identification of the Phenolic Components of Three *Compositae Spice*s: Chamomile, Tarragon, and Mexican Arnica. *Nat Prod Commun* **2012**; *7: 749–752*

29. Caleja C, Barros L, Amilcar LA, et al. Development of a functional dairy food : Exploring bioactive and preservation effects of chamomile (*Matricaria recutita* L .). *J Funct Foods* **2015**; *16: 114–124*. DOI: 10.1016/j.jff.2015.04.033

30. Luan Y, Cui J, Zhai J. High-throughput sequencing reveals differential expression of miRNAs in tomato inoculated with *Phytophthora infestans*. *Planta* **2015**; *1405–1416*. DOI: 10.1007/s00425-015-2267-7

31. Melnyk N, Popowski D, Strawa JW, et al. Skin microbiota metabolism of natural products from comfrey root (*Symphytum officinale* L .). *J Ethnopharmacol* **2024**; *318*. DOI: 10.1016/j.jep.2023.116968

32. Orellana EA, Kasinski AL. Sulforhodamine B (SRB) Assay in Cell Culture to Investigate Cell Prolifiration. *Bio Protoc* **2016**; *6*. DOI: 10.21769/BioProtoc.1984.Sulforhodamine

33. Vichai V, Kirtikara K. Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nat Protoc* **2006**; *1: 1112–1116*. DOI: 10.1038/nprot.2006.179

34. Verma PK, Raina R, Agarwal S, et al. Phytochemical ingredients and pharmacological potential of *Calendula officinalis* Linn. *Pharm Biomed Res* **2018**; *4: 1–17*. DOI: 10.1007/s11101-005-4053-9

35. Mihyaoui A El, Esteves JCG, Charfi S, et al. Chamomile (*Matricaria chamomilla* L .): A Review of Ethnomedicinal Use , Phytochemistry and Pharmacological Uses. *Life* **2022**; *12: 1–41*. DOI: 10.3390/life12040479

36. Dong L, Lee H, Liu Z, et al. Anti-Inflammatory Activity of Compounds Isolated from *Digitalis purpurea* L . in TNF- α / IFN- γ -Induced HaCaT Keratinocytes and a Three-Dimensionally Reconstructed Human Skin Model. *Molucular Sci* **2022**; *26: 1088–1069*. DOI: 10.3390/ijms26167747

37. Grebenciucova E, Vanhaerents S. Interleukin 6 : at the interface of human health and disease. *Front Immunol* **2023**; *6: 1–10*. DOI: 10.3389/fimmu.2023.1255533

38. Prokop A, Magiera A, Olszewska MA. Proanthocyanidins as Therapeutic Agents in Inflammation - Related Skin Disorders. *Int J Mol Sci* **2025**; DOI: 10.3390/ijms262010116

39. Clausen ML, Kezic S, Olesen CM, et al. Cytokine concentration across the stratum corneum in atopic dermatitis and healthy controls. *Sci Rep* **2020**; *2–9*. DOI: 10.1038/s41598-020-78943-6

40. Zheng Z. Cellular and Molecular Mechanisms of Phytochemicals Against Inflammation - Associated Diseases and Viral Infection. *Cell Biol Int* **2025**; *606–633*. DOI: 10.1002/cbin.70011

41. Kopalli SR, Annamneedi VP, Koppula S. Potential Natural Biomolecules Targeting JAK / STAT / SOCS Signaling in the Management of Atopic Dermatitis. *Molecules* **2022**; *27: 1–23*. DOI: 10.3390/molecules27144660

42. Yin Q, Wang L, Yu H, et al. Pharmacological Effects of Polyphenol Phytochemicals on the JAK-STAT Signaling Pathw. *Front Pharmacol* **2021**; *12*. DOI: 10.3389/fphar.2021.716672

Manuscript co-author statement

As a one of co-authors of the manuscript: "**From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin**" published in *Prospects in Pharmaceutical Sciences* (Volume 24, 11 Dec 2025, DOI: 10.56782/pps.792) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: "**From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin**"opublikowanego w *Prospects in Pharmaceutical Sciences* (tom 24, 11 grudnia 2025, DOI: 10.56782/pps.792) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name **Imię i nazwisko współautora**	**Scope of contribution** **Zakres wkładu**
Natalia Melnyk	Preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, performance of *in vitro* experiments on skin cells and their analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktów, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz ich metodologia i analiza, przeprowadzenie eksperymentów *in vitro* na komórkach skóry i ich analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Weronika Skowrońska	Methodology of the cell experiments; contribution through consultations and conceptual suggestions; manuscript revision.
	Metodologia doświadczeń komórkowych; konsultacje i sugestie koncepcyjne; korekta manuskryptu.
Dominik Popowski	Assistance in the microbiota analysis and its visualization; manuscript revision.
	Pomoc w analizie mikrobioty i jej wizualizacji; korekta manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research.
	Koncepcja projektu, metodologia badań nad mikrobiotą.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript revision, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, korekta manuskryptu oraz nadzór nad projektem.

26.01.2026

Date / Data

Natalia Melnyk

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: **"From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin"** published in *Prospects in Pharmaceutical Sciences* (Volume 24, 11 Dec 2025, DOI: 10.56782/pps.792) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **"From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin"**opublikowanego w *Prospects in Pharmaceutical Sciences* (tom 24, 11 grudnia 2025, DOI: 10.56782/pps.792) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, performance of *in vitro* experiments on skin cells and their analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktów, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz ich metodologia i analiza, przeprowadzenie eksperymentów *in vitro* na komórkach skóry i ich analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Weronika Skowrońska	Methodology of the cell experiments; contribution through consultations and conceptual suggestions; manuscript revision.
	Metodologia doświadczeń komórkowych; konsultacje i sugestie koncepcyjne; korekta manuskryptu.
Dominik Popowski	Assistance in the microbiota analysis and its visualization; manuscript revision.
	Pomoc w analizie mikrobioty i jej wizualizacji; korekta manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research.
	Koncepcja projektu, metodologia badań nad mikrobiotą.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript revision, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, korekta manuskryptu oraz nadzór nad projektem.

21.01.2026 — Date / Data

Weronika Skowrońska — Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: **"From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin"** published in *Prospects in Pharmaceutical Sciences* (Volume 24, 11 Dec 2025, DOI: 10.56782/pps.792) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **"From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin"**opublikowanego w *Prospects in Pharmaceutical Sciences* (tom 24, 11 grudnia 2025, DOI: 10.56782/pps.792) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name **Imię i nazwisko współautora**	**Scope of contribution** **Zakres wkładu**
Natalia Melnyk	Preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, performance of *in vitro* experiments on skin cells and their analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktów, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz ich metodologia i analiza, przeprowadzenie eksperymentów *in vitro* na komórkach skóry i ich analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Weronika Skowrońska	Methodology of the cell experiments; contribution through consultations and conceptual suggestions; manuscript revision.
	Metodologia doświadczeń komórkowych; konsultacje i sugestie koncepcyjne; korekta manuskryptu.
Dominik Popowski	Assistance in the microbiota analysis and its visualization; manuscript revision.
	Pomoc w analizie mikrobioty i jej wizualizacji; korekta manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research.
	Koncepcja projektu, metodologia badań nad mikrobiotą.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript revision, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, korekta manuskryptu oraz nadzór nad projektem.

22/12/2025

Date / Data

Dominik Popowski

Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: **"From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin"** published in *Prospects in Pharmaceutical Sciences* (Volume 24, 11 Dec 2025, DOI: 10.56782/pps.792) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **"From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin"**opublikowanego w *Prospects in Pharmaceutical Sciences* (tom 24, 11 grudnia 2025, DOI: 10.56782/pps.792) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name Imię i nazwisko współautora	Scope of contribution Zakres wkładu
Natalia Melnyk	Preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, performance of *in vitro* experiments on skin cells and their analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktów, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz ich metodologia i analiza, przeprowadzenie eksperymentów *in vitro* na komórkach skóry i ich analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Weronika Skowrońska	Methodology of the cell experiments; contribution through consultations and conceptual suggestions; manuscript revision.
	Metodologia doświadczeń komórkowych; konsultacje i sugestie koncepcyjne; korekta manuskryptu.
Dominik Popowski	Assistance in the microbiota analysis and its visualization; manuscript revision.
	Pomoc w analizie mikrobioty i jej wizualizacji; korekta manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research.
	Koncepcja projektu, metodologia badań nad mikrobiotą.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript revision, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, korekta manuskryptu oraz nadzór nad projektem.

KIEROWNIK
Zakład Mikrobiologii Farmaceutycznej i Bioanalizy
Wydział Farmaceutyczny
Warszawski Uniwersytet Medyczny

21.01.2026
.....
Date / Data

prof. dr hab. n. med. i n. o zdr. Jakub Piwowarski
.....
Legible signature / Czytelny podpis

Manuscript co-author statement

As a one of co-authors of the manuscript: **"From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin"** published in *Prospects in Pharmaceutical Sciences* (Volume 24, 11 Dec 2025, DOI: 10.56782/pps.792) I agree to include this publication into a collection of thematically related articles constituting a doctoral dissertation prepared by mgr farm. Natalia Melnyk.

Additionally, I confirm that the scope presented below corresponds to my contribution to the project.

Oświadczenie współautora manuskryptu

Jako jeden ze współautorów manuskryptu: **"From Tradition to Mechanism: Anti-inflammatory and Microbiota-Modulating Effects of *Calendula officinalis* and *Matricaria recutita* Extracts on the Skin"**opublikowanego w *Prospects in Pharmaceutical Sciences* (tom 24, 11 grudnia 2025, DOI: 10.56782/pps.792) wyrażam zgodę na włączenie tej publikacji do zbioru powiązanych tematycznie artykułów naukowych, stanowiących część pracy doktorskiej przygotowanej przez mgr farm. Natalię Melnyk.

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

Co-author's name **Imię i nazwisko współautora**	**Scope of contribution** **Zakres wkładu**
Natalia Melnyk	Preparation of extracts, phytochemical analysis, and performance of *ex vivo* experiments on skin microbiota and its methodology and analysis, performance of *in vitro* experiments on skin cells and their analysis, visualization of the obtained results, and writing of the original text of the manuscript.
	Przygotowanie ekstraktów, analiza fitochemiczna i przeprowadzenie eksperymentów *ex vivo* dotyczących mikrobioty skóry oraz ich metodologia i analiza, przeprowadzenie eksperymentów *in vitro* na komórkach skóry i ich analiza, wizualizacja uzyskanych wyników i napisanie oryginalnego tekstu manuskryptu.
Weronika Skowrońska	Methodology of the cell experiments; contribution through consultations and conceptual suggestions; manuscript revision.
	Metodologia doświadczeń komórkowych; konsultacje i sugestie koncepcyjne; korekta manuskryptu.
Dominik Popowski	Assistance in the microbiota analysis and its visualization; manuscript revision.
	Pomoc w analizie mikrobioty i jej wizualizacji; korekta manuskryptu.
Jakub P. Piwowarski	Conceptualization of the project, methodology of the microbiota research.
	Koncepcja projektu, metodologia badań nad mikrobiotą.
Sebastian Granica	Conceptualization of the project, methodology of the phytochemical and microbiota analysis, manuscript revision, and project supervision.
	Koncepcja projektu, metodologia analizy fitochemicznej i mikrobioty, korekta manuskryptu oraz nadzór nad projektem.

.....

Date / Data

.....

Legible signature / Czytelny podpis