

Potential of *Artemisia annua* hydroalcoholic extracts in skin care and dermatocosmetic products

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Abstract

The objective of this study was to evaluate the potential of plant extracts derived from the native flora of Iasi region, Romania as valuable natural sources of polyphenols and flavonoids for the development of dermatocosmetic formulations aimed at protecting the skin against oxidative stress. Dried *Artemisia annua* L. samples were used for extracted using three techniques: classical cold maceration (M), heat reflux extraction (R), and Soxhlet heat reflux extraction (Sx). Spectrophotometric analyses were performed to determine the total polyphenol and flavonoid content and to assess the antioxidant capacity using DPPH and ABTS assays. Among the tested methods, heat reflux and Soxhlet extraction proved to be the most efficient, yielding extracts rich in bioactive compounds when using 50% ethanol as solvent and a solid-to-liquid ratio of 1:5. Soxhlet extraction provided the highest total polyphenol content (11.26 mg GAE/mL), while heat reflux extraction resulted in the highest flavonoid concentration (7.97 mg QE/mL). Subsequently, an emulsion containing the most active extract in polyphenols was formulated and subjected to a preliminary evaluation of physicochemical stability. The results demonstrated encouraging stability profiles and antioxidant potential, supporting the use of *A. annua* extracts as promising ingredients in dermatocosmetic applications. Further investigations, including comprehensive cytotoxicity and dermatological testing, are warranted to confirm their efficacy and safety in topical formulations.

Keywords: bioactive compounds; emulsion; liquid - solid extraction; Romania native flora; vegetal extract

Introduction

The growing interest in natural, plant-derived compounds has significantly accelerated the search for new active ingredients with scientifically validated benefits for skin health, driven by their generally lower adverse effects compared with synthetic agents. Despite significant scientific progress, plants remain an invaluable source of biologically active substances and continue to provide valuable compounds that support health and contribute to the prevention and treatment of various disorders.

Among the promising candidates, *Artemisia annua* L. (*A. annua*) (sweet wormwood or black wormwood) has emerged, offering substantial, yet relatively underexplored, dermatological potential. *A. annua* is an annual herbaceous plant native to the temperate regions of Asia, particularly China, where it has been traditionally employed in Chinese medicine. It commonly grows on fallow land, roadsides, fields, and open areas, demonstrating remarkable adaptability to diverse environmental conditions (Ekiert *et al.*, 2021). The species has been introduced and naturalized across various regions of the world, including Europe, Africa, and the Americas (Das, 2012). In recent decades, following the clinical validation of artemisinin's efficacy in malaria control, the global demand for *A. annua* has increased substantially. This rising demand has prompted a shift from reliance on wild populations to large-scale, controlled cultivation on specialized farms.

In Romania, *A. annua* was introduced accidentally or as a medicinal/cultivated plant (for its content of artemisinin, an active antimalarial substance), the first reports in the spontaneous flora of Romania dating back to the second half of the 20th century. Currently, *A. annua* is considered a naturalized species, being established and reproducing spontaneously in the flora of Romania, without direct human intervention, in almost all areas of the country but especially in the south, east and west, where climatic conditions are favourable (hot summers and light soils) (Sarbu and Oprea, 2011). It thrives in sunny habitats and nutrient-poor soils, exhibiting a remarkable tolerance to arid conditions. The plant is commonly found along roadsides, on abandoned agricultural land, degraded pastures, and alluvial soils near riverbanks (Stan *et al.*, 2019; BRGV).

A. annua has a diverse composition influenced by the habitat in which it grows, with different classes of compounds, the most important for the medicinal value of the plant being the sesquiterpene lactones (Nageeb *et al.*, 2013) (Figure 1).

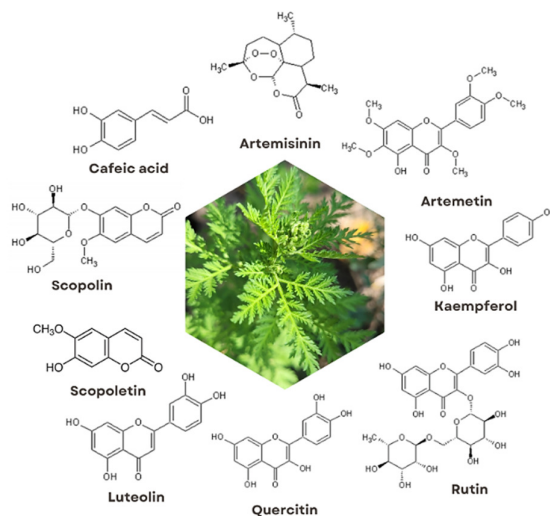


Figure 1. The main biochemical compounds from the composition of the *A. annua* plant

The plant's scientific profile was fundamentally transformed by the pivotal discovery of artemisinin, a sesquiterpene lactone that accumulates primarily in the glandular hairs of its leaves and flowers. Artemisin is

the most important compound in this group and accumulates in the glandular hairs on the leaves and flowers of the plant. The research showed that artemisinin and its derivatives act quickly and effectively on the parasite *Plasmodium falciparum*, the causative agent of malaria, even in cases that are resistant to conventional therapies with chloroquine or other drugs (Tu, 2016). The artemisinin content of the leaves varies considerably, ranging from 0.01% to 1.50% of the dry weight (Laughlin, 1994; Delabays *et al.*, 2001; Mannan *et al.*, 2010; Ivănescu *et al.*, 2011). In wild Romanian *A. annua* plants (collected from the rural environment of Iași, Romania) it varies between 0.17 and 0.21%, in the dry plant (Ivănescu *et al.*, 2011).

The newly revealed *A. Annua's* pharmacological significance (YaNan *et al.*, 2017; Shi *et al.*, 2022) not only revolutionized malaria treatment but also highlighted the broader therapeutic potential of *A. annua*, attracting global attention from researchers and regulatory authorities. Following this milestone, research interest expanded to explore additional applications of the plant, including its potential use against cancer, autoimmune diseases, and bacterial and viral infections, as summarized in Table 1 (Mesa *et al.*, 2015; Wu *et al.*, 2016; Efferth *et al.*, 2015; Feng *et al.*, 2020; Efferth and Oesch, 2021).

Table 1. Biological activity of *A. annua* and its pharmacological and cosmetological potential

Biological actions	Mechanism of action	Reference
Antimalarial	Infusion extracts of <i>A. annua</i> have been shown to inactivate the calcium pump in protozoa, thereby disrupting cellular homeostasis. Both hydroethanolic and aqueous leaf extracts, primarily through the action of artemisinin, have demonstrated potent antimalarial activity against <i>Plasmodium falciparum</i> and <i>Plasmodium berghei</i> .	(Wright <i>et al.</i> , 2010; Feng <i>et al.</i> , 2020; Ashraf <i>et al.</i> , 2022)
Antibacterial and antifungal	Extracts from <i>A. annua</i> leaves, as well as the plant's essential oil-which is primarily composed of monoterpenes such as 1,8-cineole, camphor, and artemisia ketones-have demonstrated potent antimicrobial activity against several bacterial species, including <i>Escherichia coli</i> , <i>Salmonella enteritidis</i> , <i>Salmonella typhi</i> , <i>Yersinia enterocolitica</i> , and <i>Listeria monocytogenes</i> , as well as against fungal species such as <i>Candida albicans</i> , <i>Candida famata</i> , and <i>Aspergillus fumigatus</i> .	(Donato <i>et al.</i> , 2015; Marinas <i>et al.</i> , 2015; Santomauro <i>et al.</i> , 2016)
Cytotoxic	Compounds derived from <i>A. annua</i> have demonstrated significant anticancer activity through multiple mechanisms. Plant polyphenols inhibit the adhesion of cancer cells to endothelial cells and prevent epithelial–mesenchymal transition, a key process in metastasis. In patients with prostate cancer, combined treatment with <i>A. annua</i> powder capsules and bicalutamide resulted in tumor regression. The extracts have also been shown to inhibit the proliferation of human osteosarcoma cells by inducing apoptosis. Moreover, methanol leaf extracts exhibited notable cytotoxicity against several cancer cell lines, including MCF7 (breast adenocarcinoma), lung cancer cells, and CHO (Chinese hamster ovary) cells. Artemisinin and its derivatives exert anticancer effects through multiple mechanisms, including induction of oxidative stress, DNA damage, apoptosis, and inhibition of angiogenesis. Certain extracts demonstrated selective tumor cell inhibition in vitro and in vivo, even in the absence of detectable artemisinin, with active compounds such as chrysosplenol D, arteannuin B, and casticin identified as promising contributors to their anticancer activity.	(Ferreira <i>et al.</i> , 2010; Efferth, <i>et al.</i> , 2015; Lang <i>et al.</i> , 2019; Stan, 2019; Lang <i>et al.</i> , 2020; Ekiert <i>et al.</i> , 2021; Fu <i>et al.</i> , 2022)
Anti-inflammatory	<i>A. annua</i> has demonstrated significant anti-inflammatory activity through multiple mechanisms, including the reduction of pro-inflammatory cytokine production, such as TNF- α and IL-6, and the inhibition of NF- κ B and MAPK signaling pathways in macrophages.	(Oh <i>et al.</i> , 2014; Wang <i>et al.</i> , 2011; Chen <i>et al.</i> , 2024);

	These anti-inflammatory effects are primarily attributed to sesquiterpene lactones, including artemisinin and arteannuin B.	
Immunosuppressive	Artemisinin derived from <i>A. annua</i> has demonstrated notable immunosuppressive properties, effectively modulating several inflammatory pathways and transcription factors involved in immune responses. Specifically, it inhibits NF- κ B signaling, a central regulator of inflammation and immunity, suppresses pathogenic T cell activation, attenuates B cell function, and promotes regulatory T cell differentiation. These mechanisms highlight its potential therapeutic application in the management of autoimmune diseases.	(Efferth and Oesch, 2021; Long <i>et al.</i> , 2024)
Antiviral	Extracts of <i>A. annua</i> exhibit broad-spectrum antiviral activity against several viruses, including SARS-CoV-2, influenza virus, HIV, HBV, and HSV. The antiviral effects involve both direct interference with viral replication and modulation of host immune responses. Specifically, against SARS-CoV-2, the extracts inhibit viral entry and replication, reduce oxidative stress and inflammation, and mitigate lung tissue damage. Importantly, the crude <i>A. annua</i> extract demonstrated virucidal, antiviral, and antioxidant activities at concentrations that did not compromise cell viability.	(Ahmad <i>et al.</i> , 2022; Allemailen, 2022; Orosco, 2023; Baggieri <i>et al.</i> , 2023)
Antioxidant	Extracts of <i>A. annua</i> exhibit potent antioxidant activity through multiple mechanisms. They have demonstrated significant free radical scavenging capacity against DPPH, ABTS, nitric oxide, and hydroxyl radicals. This antioxidant potential is largely attributed to the plant's high content of polyphenols and flavonoids, including quercetin and chlorogenic acid.	(Yang <i>et al.</i> , 2009; Chukwurah <i>et al.</i> , 2014; Skowrya <i>et al.</i> , 2014; Ejembi <i>et al.</i> , 2021)
Anti-acne	Extracts from the <i>Artemisia</i> genus have demonstrated notable antibacterial activity, effectively inhibiting the growth of acne-causing bacteria such as <i>Propionibacterium acnes</i> , <i>Pseudomonas aeruginosa</i> , and <i>Staphylococcus aureus</i> by disrupting the bacterial cell wall structure. Additionally, <i>Artemisia</i> extracts can attenuate the inflammatory processes associated with acne vulgaris by inhibiting the release of pro-inflammatory cytokines. <i>Artemisia</i> naphta oil, a secondary by-product obtained during artemisinin extraction from <i>A. annua</i> , has been shown to inhibit <i>S. aureus</i> growth, reduce pro-inflammatory cytokine release, and diminish acne lesions and erythema when incorporated at 1% into a gel formulation. Moreover, <i>Artemisia</i> extracts exhibit minimal side effects compared with conventional chemical treatments, highlighting their potential as a promising alternative therapy for acne vulgaris.	(Perez <i>et al.</i> , 2021; Dharsono and Setyowatie, 2022)
Anti-eczema	The aqueous extract of <i>A. annua</i> , which is rich in sesquiterpenes and phenolic acids-the primary bioactive constituents-exerts beneficial effects in the treatment of eczema through its anti-inflammatory and antipruritic properties. It has been shown to increase the itch threshold by inhibiting the expression of inflammatory cytokines, such as thymic stromal lymphopoietin (TSLP), which are involved in eczema-associated inflammation. Additionally, the extract demonstrated therapeutic effects in a mouse model of atopic dermatitis induced by 2,4-dinitrochlorobenzene (DNCB), alleviating dermatitis severity and ear edema by suppressing Th2-type immune responses, which play a central role in disease pathogenesis, and by reducing levels of immunoglobulin E (IgE) and pro-inflammatory cytokines.	(Han <i>et al.</i> , 2022; Zhao <i>et al.</i> , 2024)

The dermatocosmetic potential of *A. annua* has been relatively underexplored, despite its considerable promise for the treatment of various skin disorders (Septembre-Malaterre *et al.*, 2020). Plant extracts have demonstrated wound-healing properties by promoting keratinocyte proliferation and enhancing angiogenesis (Minda *et al.*, 2022). The combination of antioxidant and antimicrobial activities makes *A. annua* a valuable candidate for the development of skin care formulations (Ekiert *et al.*, 2022). In a study involving 25 volunteers with sensitive skin, Yu *et al.* reported that an emulsion containing *A. annua* extract effectively repaired sensitive skin, reduced inflammation, and improved skin barrier function after daily application for 28 days (Yu *et al.* 2020). Furthermore, extracts obtained with polar solvents such as water, hexane, and ethanol exhibited significant tyrosinase inhibitory activity, a key enzyme in melanogenesis and the formation of age spots. These extracts also displayed notable antioxidant effects, protecting the skin from oxidative stress and free radical-induced damage, thereby supporting skin health and delaying signs of aging (Acquaviva *et al.*, 2023). Collectively, these findings suggest that *A. annua* could be effective in addressing premature skin aging, inflammatory skin conditions, and bacterial or fungal infections, highlighting its potential for incorporation into dermatocosmetic products. Thus, our studies align with the current trend of discovering and exploiting new bioactive compounds from easily accessible plant resources. At this time, there are no studies testing preparations based on *A. annua* extract on combating oxidative stress on the skin.

Although the safety of *A. annua* in cosmetic products has not yet been evaluated by the Cosmetic Ingredient Review (CIR) expert panel, it is currently authorized for use in cosmetics according to the European CosIng database and is available in 12 different formulations. *A. annua* is incorporated into cosmetic products as a protective and conditioning agent for both skin and hair. Its antibacterial, antioxidant, anti-dandruff, moisturizing, and softening properties further support its use as an active ingredient in skin and hair care formulations (European Commission CosIng, 2020).

Methods for obtaining and characterizing extracts from A. annua specie

Optimizing extraction protocols is essential to ensure that the resulting plant extracts are of high quality, safe, and possess maximum bioactivity. These processes must be rapid, cost-effective, comprehensive, and reproducible. Several established techniques have been explored for *A. annua* (Misra *et al.*, 2013; Ciftici *et al.*, 2018; Prawang *et al.*, 2019; Ilina *et al.*, 2020; Acquaviva *et al.*, 2023), including:

- Conventional solvent-based methods: maceration (with varying solvent concentrations and durations) and Reflux Extraction.
- Assisted Techniques: ultrasound-assisted extraction (using alcoholic solvents or poly (ethylene glycol)) and microwave-assisted extraction.
- Advanced Techniques: supercritical carbon dioxide (SC-CO₂) extraction, sometimes modified with ethanol.

The successful implementation and optimization of these techniques rely heavily on controlling multiple operational parameters, such as the solid-to-liquid ratio, the type and volume of the solvent, its concentration, and the total extraction duration.

A range of chemical and physicochemical methods is employed for the qualitative and quantitative characterization of *A. annua* extracts. Standard methods include the Folin-Ciocalteu and AlCl₃ assays for determining Total Phenolic and Flavonoid contents (Acquaviva *et al.*, 2023). Furthermore, chromatographic techniques are widely utilized to separate and identify specific compounds: thin-layer chromatography (TLC) densitometry (Misra *et al.*, 2013); high-performance liquid chromatography (HPLC) (Ciftici *et al.*, 2018; Stan *et al.*, 2019; Babacan *et al.*, 2022), and gas chromatography–mass spectrometry (GC–MS) analysis (Dobрева *et al.*, 2022).

Although *A. annua* is well recognized for its pharmaceutical importance, its topical applications—particularly in dermatological and cosmetic fields—remain insufficiently investigated in the scientific literature

and underexploited in the dermatocosmetic products market. This gap between the proven biological potential of the plant and its current limited practical application provides a strong rationale for the present study. The research is further motivated by the traditional topical uses of *A. annua* in the treatment of various skin disorders, as documented in folk medicine (Ekiert *et al.*, 2021; Segneanu *et al.*, 2021; Ekiert *et al.*, 2022).

The primary objective of this study was to develop novel, eco-friendly, and effective formulations to mitigate oxidative stress on the skin, thereby supporting the improvement and maintenance of quality of life. To achieve this goal, a series of dermatocosmetic emulsions containing *A. annua* extract as the active ingredient were formulated and characterized. The plant extract was obtained using various liquid-solid extraction techniques, including maceration (M), heat reflux extraction (R), and Soxhlet heat reflux extraction (Sx). The efficiency of each method was rigorously evaluated based on operational parameters, including the solid-to-liquid ratio, extraction time, and solvent concentration. The resulting extracts were further analyzed by quantifying their primary classes of bioactive compounds. Finally, the prepared emulsions were assessed for long-term stability by monitoring critical parameters such as pH, electrical conductivity, phase homogeneity, and microbiological integrity.

Materials and Methods

The plant material used in this study consisted of *Artemisia annua* (*A. annua*), an herbaceous species acclimatized in Romania (Kingdom: Plantae; Phylum: Streptophyta; Class: Equisetopsida; Subclass: Magnoliidae; Order: Asterales; Family: Asteraceae; Genus: *Artemisia*; Species: *Artemisia annua*), collected from spontaneous populations in Iași county, Romania. A total of 10 kg of fresh green plant material (without roots) were harvested prior to flowering, at the stage when flower buds were formed but not yet open. The material was dried in shaded, well-ventilated areas on wooden and mesh shelves to ensure efficient air circulation for 5-7 weeks. After drying, the leaves and inflorescences were separated from the stems, yielding 1 kg of dried plant material for extraction, which was then crushed and stored in brown glass containers to protect it from moisture and sunlight until use (Figure 2).

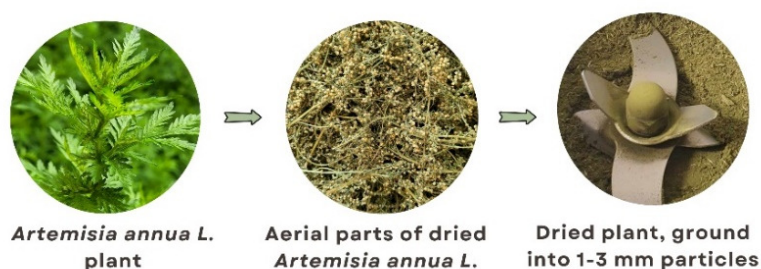


Figure 2. Stages in preparing the *A. annua* plant for the experimental part

The reference standards (gallic acid, quercetin, and reactive for antioxidant activity assessment) and all other reagents (Foling Ciocalteu, AlCl₃, ethanol, and methanol) used were analytical quality (Sigma Aldrich and Merck Co.).

Extraction methods

In this study, three liquid-solid extraction methods were employed to obtain extracts from *Artemisia annua* with the aim of maximizing the content of bioactive compounds, following previously established protocols (Turcov *et al.*, 2022a; Maxim *et al.*, 2024a). The extraction techniques included maceration at room

temperature (22-25 °C) (M), heat reflux extraction (R), and Soxhlet reflux extraction (Sx). The solvent consisted of 96% ethanol-a widely accepted and approved solvent in the cosmetics and dermatocosmetics industry-used as hydroalcoholic solutions at concentrations of 30%, 50%, and 70%. The efficiency of each extraction method, expressed in terms of extraction yield, and polyphenol and flavonoid amounts respectively, was evaluated by varying key operational parameters, including extraction time, solid-to-liquid (S/L) ratio, and solvent concentration.

The extraction yield was calculated to evaluate the effectiveness of the extraction procedure conducted under various conditions (Eq.1).

$$\eta \% = \frac{m_{\text{residue}} \cdot V_{\text{extract}}}{n_{\text{extract}} \cdot m_{\text{solid sample}}} \cdot 100 \quad (1)$$

where, m_{residue} (g) is the mass of the solid residue resulted after the evaporation to dryness of a sample of n_{extract} mL withdrawn from the total obtained liquid extract, V_{extract} (mL); $m_{\text{solid sample}}$ (g) - the mass of plant sample used in the liquid-solid extraction process.

For this sample around 5 mL from each vegetal extract was evaporated to dryness at constant temperature up to 50 °C using a thermostatic oven (Turcov *et al.*, 2022a; Maxim *et al.*, 2024a).

Quantitative characterization of vegetal extracts

Following a methodology similar to that applied in our previous studies (Pavun *et al.*, 2018), the analysis of *A. annua* extracts focused on two classes of compounds primarily responsible for their antioxidant activity:

- *Total polyphenol content (TPC)*: Determined using the Folin–Ciocalteu method, with results expressed as mg of gallic acid equivalent per mL of extract (mg GAE/mL). All analyses were performed in duplicate.
- *Total flavonoid content (TFC)*: Assessed spectrophotometrically using a 2% AlCl_3 solution in methanol (Turcov *et al.*, 2022b), with results expressed as mg of quercetin equivalent per mL of extract (mg QE/mL).

Antioxidant activity assessment

2,2-diphenyl-1-picrylhydrazyl radical scavenging assay

The assay was conducted based on a previously described method (Luca *et al.*, 2022). In summary, 50 μL of sample was mixed with 150 μL of a 0.004% methanol solution 2,2-diphenyl-1-picrylhydrazyl (DPPH). After incubating for 30 minutes at room temperature in the dark, the absorbance was measured at 517 nm. The DPPH radical scavenging activity was expressed in milligrams of Trolox equivalents (mg TE) per mL of sample.

2,2'-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid radical scavenging assay

The assay was performed according to the method previously described (Luca *et al.*, 2022). The $\text{ABTS}^{\bullet+}$ radical was generated by mixing 7 mM of 2,2'-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid (ABTS) solution with a 2.45 mM potassium persulfate solution in a 1:1 (v/v) ratio. The mixture was left to stand in the dark at room temperature for 12–16 hours. Before starting the assay, the ABTS solution was diluted with methanol to achieve an absorbance of 0.700 ± 0.02 at 734 nm. Next, 30 μL of extract was added to 200 μL of the ABTS solution and thoroughly mixed. After incubating for 30 minutes at room temperature, the absorbance was measured at 734 nm. The ABTS radical scavenging activity was expressed as milligrams of Trolox equivalents (mg TE) per mL of sample.

Preliminary error calculation

Each set of experimental determinations was performed in triplicate. For the results obtained, the maximum percentage deviation was calculated in a first step. Error bars represent the maximum relative deviation (%) of the individual measurements from the corresponding mean ($n = 3$). This approach was selected to illustrate the maximum variation within each experimental set.

Formulation of emulsions for dermatocosmetic applications

Dermatocosmetic emulsions containing *A. annua* extract were developed to investigate their potential in skin care applications. A water-in-oil (W/O) emulsion was prepared using 3% of *A. annua* extract (Sx: S/L = 1:15, solvent concentration = 50%, extraction time = 90 min and the TPC content was 11.26 mg GAE/mL). The general composition of the emulsion is presented in Table 2. Ingredients for both the aqueous and lipophilic phases were selected to enhance product performance, ensuring high skin permeability, a pleasant texture, and optimal sensory characteristics for the end user.

Table 2. The main ingredients for the formulation of emulsions

	INCI name raw material	Trade name raw material	%	Ingredient function
Phase A Lipophilic phase	<i>Amaranthus spinosus</i> Seed Oil	Amaranth oil	5	Emollient
	<i>Psoralea corylifolia</i> Seed Oil	Bakuchi oil	5	
	<i>Malus domestica</i> Seed Oil	Apple seed oil	5	
	<i>Solanum lycopersicum</i> Seed Oil	Tomato oil	5	
	Cetearyl alcohol, Glyceryl stearate, Jojoba esters, <i>Helianthus annuus</i> Seed Wax, Sodium stearyl glutamate, Water, Polyglycerin-3	<i>Emulium dolcea</i>	5	Emulsifiers
Phase B Hydrophilic phase	<i>Acmella oleracea</i> flower water	Hydrolate of <i>Acmella oleracea</i>	68.7	Solvent
	Glycerin	Glycerin	2	Conditioning agent
	Lecithin, Sclerotium gum, Pullulan, Xanthan gum	Siligel	0.3	Viscosity agent
Phase C Extract + preservative	Formula 1: Hydro-alcoholic extract of <i>Artemisia annua</i>	Vegetal extract	3	Active
	Benzyl alcohol, Dehydroacetic acid	Salvacosm DB	1	Preserved

The formulation process involved heating both the aqueous and lipophilic phases to 75 °C, followed by the gradual addition of the lipophilic phase to the aqueous phase under continuous mixing using a Dynamix® DMX Combi 160 homogenizer at 13,000 rpm. The mixture was subjected to three shaking cycles of 3 minutes each and subsequently cooled in a water bath to 40 °C. After cooling, active ingredients and preservatives were incorporated. Samples (15 g) were then placed in brown glass containers for storage and further analysis. The prepared emulsions were evaluated for physical, chemical, and microbiological stability over time, including assessments of pH, electrical conductivity, phase homogeneity, and microbial integrity (Maxim *et al.*, 2024b).

Tests for preliminary characterization of emulsions

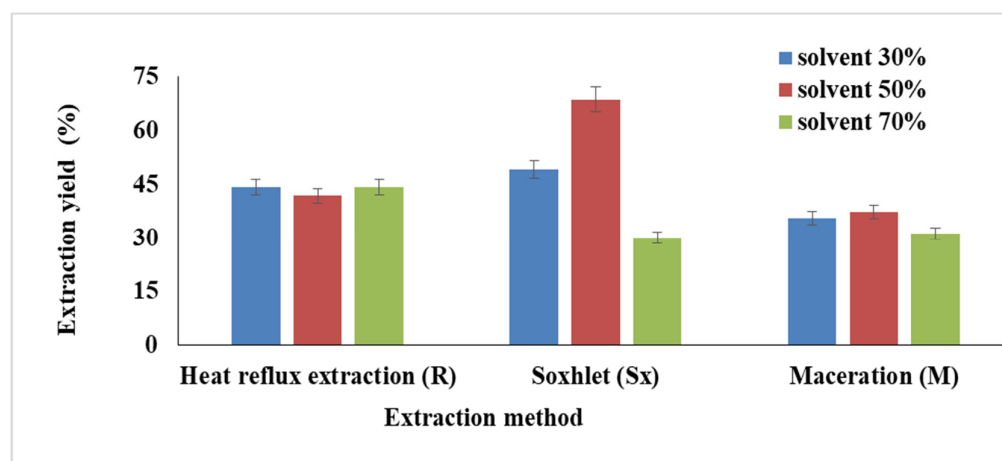
The emulsion was preliminarily evaluated through physicochemical analyses to assess its organoleptic properties and stability under controlled conditions (Mahmood and Akhtar, 2013; Hu *et al.*, 2017; Kim *et al.*, 2020). The characterization included the following parameters:

- assessments of appearance;
- pH: The pH of the dermato-cosmetic emulsions was measured using a digital pH meter (Hanna Instrument). For each analysis, 0.5 g of emulsion was dissolved in 50 mL of distilled water and allowed to equilibrate for two hours. The pH was then recorded by immersing the electrode directly into the solution at 24.0 ± 2.0 °C. After dilution, the emulsions exhibited a milky appearance but remained homogeneous throughout the measurement.
- phase separation under centrifugal and vibrational stress: Stability tests were conducted using 5 g of emulsion. For the centrifugation test, samples were placed in an XC-Spinplus centrifuge and subjected to 3,000 rpm for 30 minutes at 25 °C. For the vortex stability test, a Multi Speed Vortex MSV-3500 (Grant Instruments Ltd., Cambs, England) was used under identical conditions (5 g of sample, 3,000 rpm, 30 minutes, 25 °C).
- electrical conductivity of the emulsions was measured using a portable conductometer (Hanna Instruments) on samples stored at room temperature (25 °C) for 20 days.
- microscopic observations of the emulsions, after 7 days of storage at room temperature, were performed using a binocular microscope (Optika B-159, OPTIKA S.r.l., Ponteranica, Italy) at a magnification of 1000×
- microbiological testing

Prior to each measurement, all samples were equilibrated to room temperature.

Results*Evaluation of extraction methods based on process efficiency*

The extraction efficiency of maceration (M), heat reflux extraction (R), and Soxhlet reflux extraction (Sx) was evaluated according to the established methodology and extraction conditions. The efficiency of each method was determined based on the process yield, calculated using Equation 1. The results are summarized in Figure 3.



(A)

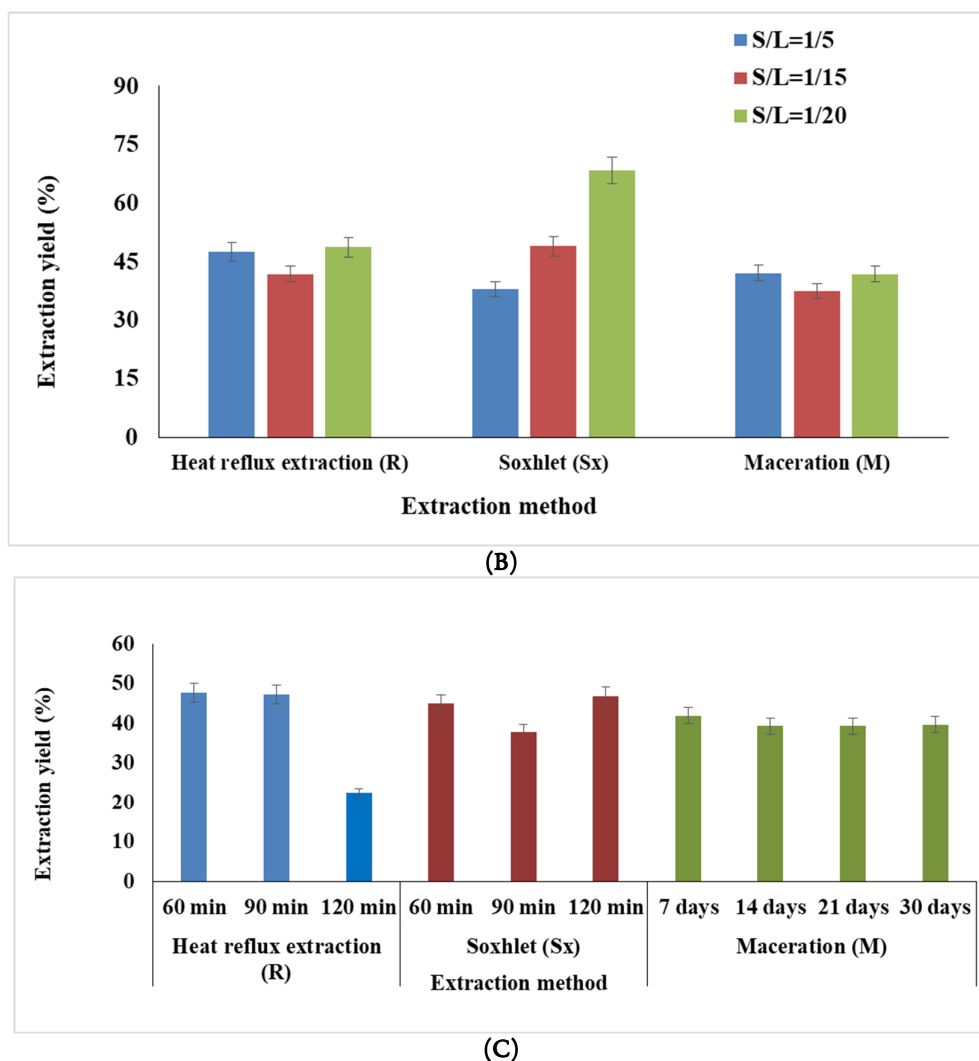


Figure 3. Extraction yield (%) according to the extraction process parameters. Experimental conditions: (a) Influence of solvent concentration (30%, 50%, 70%). S/L ratio: 1:5; (b) Influence of solid/liquid ratio (S/L) (1:5, 1:15, 1:20). Solvent concentration = 50%; (c) Influence of extraction time, Solvent concentration=50%; S/L ratio= 1:15 (maximum relative deviation 5%)

Quantitative characterization of hydro alcoholic vegetal extracts

After filtration and centrifugation, the obtained extracts were subjected to quantitative determination of total polyphenol content (TPC) (Pavun *et al.*, 2018) and total flavonoid content (TFC) (Turcov *et al.*, 2022b).

TPC was expressed as mg of gallic acid equivalent per mL (mg GAE/mL), the analyses were performed in duplicate, and the results, which depended on the extraction technique and operational parameters, are presented in Figures 4A-C.

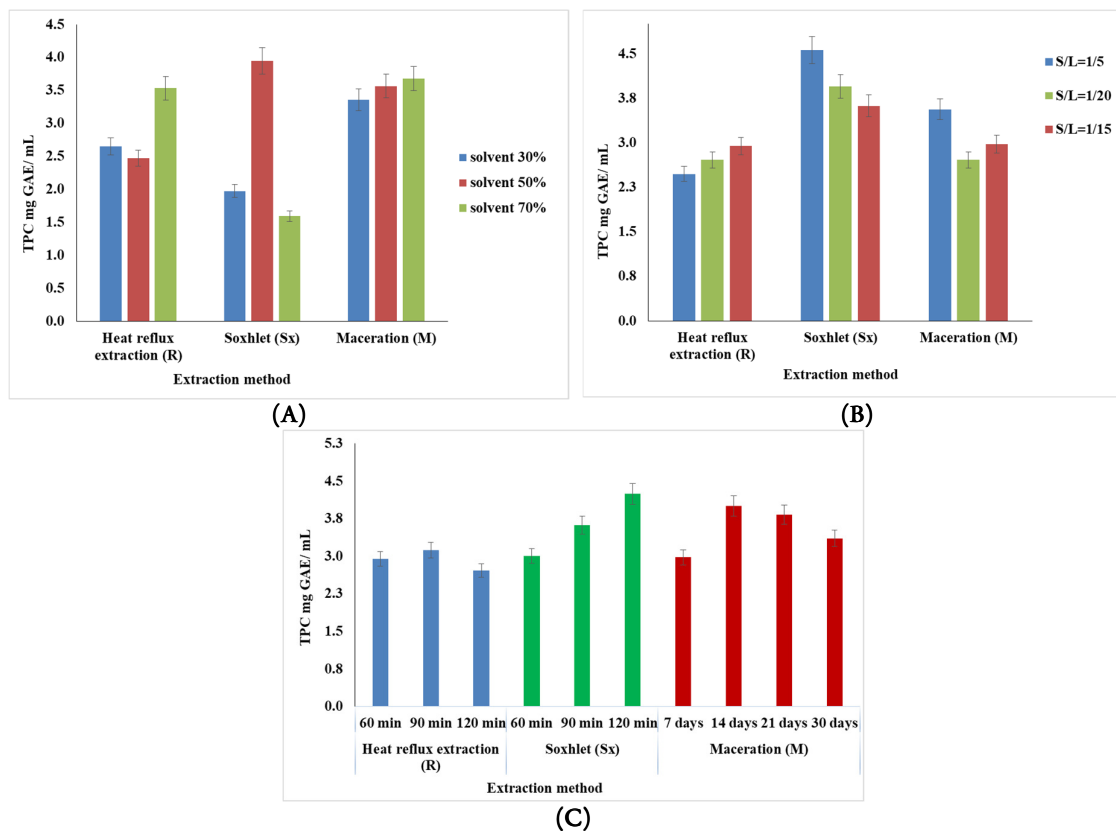
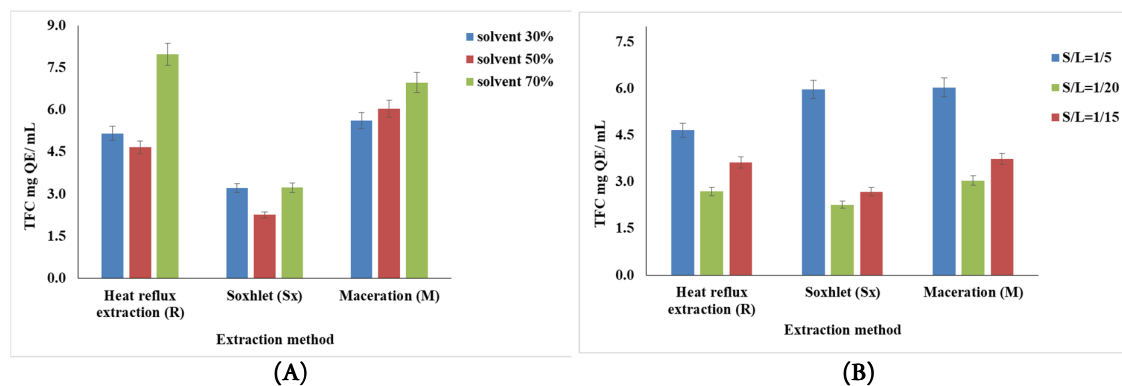


Figure 4. Total polyphenol content (TPC) in mg GAE/mL determined for the extraction methods depending on the parameters considered. Experimental conditions: (A) Influence of solvent concentration (30%, 50%, 70%): S/L ratio = 1:5; (B) Influence of solid/liquid ratio (S/L) (1:5, 1:15, 1:20). Solvent concentration = 50%; (C) Influence of extraction time, solvent concentration = 50%; S/L ratio = 1:15 (maximum relative deviation 5%)

TFC was expressed as mg of quercetin equivalent per mL (mg QE/mL), the analyses were performed in duplicate, and the results, which depended on the extraction technique and operational parameters, are presented in Figures 5A-C.



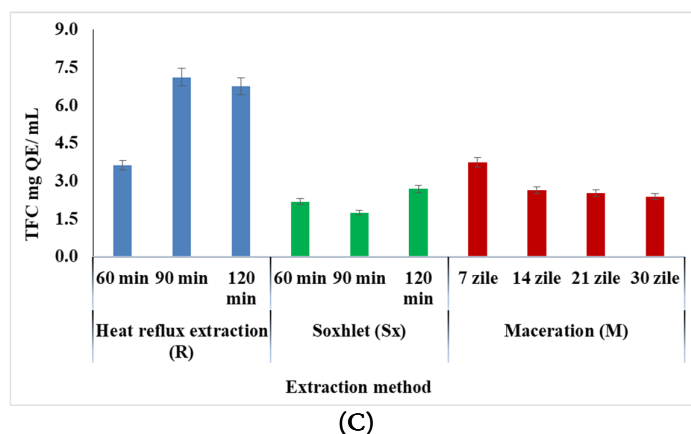
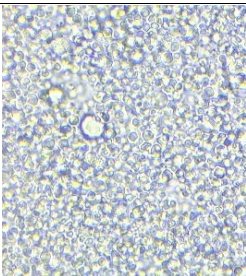
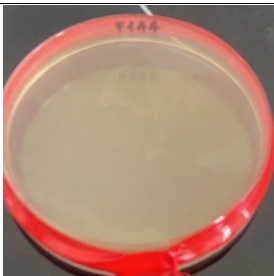
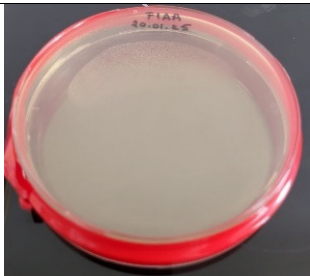


Figure 5. The flavonoids content (TFC) (mg QE/mL) determined for the extraction methods depending on the physical parameters considered. Experimental conditions: (A) Influence of solvent concentration R: S/L= 1:5; 60 min., Sx: S/L= 1:20; 90 min., M: S/L= 1:5; 7 days; (B) Influence of solid/liquid ratio (S/L):solvent concentration = 50 %; R-60 min., Sx- 90 min., M -7 days; (C) Influence of extraction time : solvent concentration = 50 %; S/L= 1:15 (maximum relative deviation 5%)

Preliminary emulsions characterization

The results of the preliminary characterization of the prepared emulsion, compared with the base formulation, are presented in Table 3.

Table 3. Initial physicochemical and microbiological determinations of emulsions with *Artemisia annua* extract (3%)

		
Appearance after centrifugation test	Appearance after vortex test	Organoleptic analysis
		
Microscopic images after 24 h storage at 25 °C	Microbiological test at 24 h after preparation	Microbiological test at 30 h after preparation

Discussion

Evaluation of extraction methods based on extraction yield

The comparative analysis of the extraction methods, considering the concentration of the hydroalcoholic solution, revealed significant differences among the three techniques applied (Figure 3a). Soxhlet extraction with 50% ethanol exhibited the highest efficiency (68.50%), followed by Soxhlet with 30% ethanol (49.00%). Heat reflux extraction showed relatively consistent yields for 30% and 70% ethanol (44.25%), whereas maceration produced the lowest yields. These results indicate that, for *Artemisia annua* (*A. annua*), a 50% ethanol solution is optimal, particularly in the Soxhlet method, which yielded nearly double that of 70% ethanol. The solid-to-liquid (S/L) ratio (Figure 3b) influenced extraction efficiency differently depending on the method. Soxhlet extraction reached a maximum yield of 68.50% at an S/L ratio of 1:20, demonstrating efficient extraction under high dilution conditions. Heat reflux extraction provided similar results across the tested ratios, with a slight advantage at 1:20 (48.75%). Maceration also achieved its highest yield at 1:20 (41.87%), suggesting that a larger solvent volume enhances solubilization of active compounds for this species. Extraction time (Figure 3c) affected the methods in distinct ways. Heat reflux extraction yields remained nearly constant at 60 and 90 min but decreased markedly at 120 min (22.50%), likely due to thermal degradation of sensitive compounds. In contrast, Soxhlet extraction efficiency increased progressively, peaking at 120 min (46.88%), indicating good thermal tolerance. Maceration provided stable yields over 14-30 days (39.38-42%), with a slight increase during the first 7 days (42%), highlighting that, although slow, this method enables continuous transfer of bioactive compounds without significant degradation over time.

Overall, Soxhlet extraction using 50% ethanol and an S/L ratio of 1:20 was the most efficient strategy for *A. annua*, achieving yields close to 70%. While maceration is a milder technique suitable for temperature-sensitive applications, it produces lower yields. Higher ethanol concentrations (70%) did not improve extraction efficiency, and excessively prolonged extraction at high temperatures can negatively affect yields, as observed with refluxing for 120 min.

Quantitative characterization of hydroalcoholic vegetal extracts

The total polyphenol content (TPC) in hydroalcoholic extracts of *A. annua* was evaluated to assess the influence of experimental parameters-solvent concentration, solid-to-liquid (S/L) ratio, and extraction time-across three extraction methods: Soxhlet reflux (Sx), heat reflux extraction (R), and maceration (M) (Figure 4).

Effect of solvent concentration (Figure 4a)

Soxhlet extraction (Sx) yielded the highest TPC (11.26 mg GAE/mL) using 50% ethanol, whereas Heat reflux extraction (R) provided an optimal TPC of 10.48 mg GAE/mL with the same solvent concentration. For maceration (M), the maximum TPC (9.03 mg GAE/mL) was obtained with a more dilute 30% ethanol solution. These results indicate that optimal solvent concentration is method-dependent. Thermal methods (Soxhlet and heat reflux) benefit from energy input that enhances cell wall permeability, facilitating release of less accessible compounds; a medium-polarity solvent (50% ethanol) balances solubilization of both polar and partially non-polar compounds. In contrast, maceration relies more on intrinsic solubility and concentration gradient, favouring a more dilute solvent (30% ethanol) to efficiently extract predominantly polar phenolics.

Effect of solid-to-liquid ratio (Figure 4b)

Heat reflux extraction was highly sensitive to S/L variations, achieving maximum TPC at the most concentrated ratio, 1:5 (10.48 mg GAE/mL), likely due to enhanced mass transfer at high temperature and low solvent volume. Soxhlet extraction showed similar yields for ratios 1:5 and 1:15 (10.12 and 9.39 mg GAE/mL), while maceration reached optimal TPC values at higher solvent volumes (1:15 and 1:20, 9.19 and 9.34 mg GAE/mL), indicating that extended solvent contact promotes efficient extraction under mild conditions.

Effect of extraction time (Figure 4c)

Soxhlet extraction demonstrated stable TPC values with a slight increase at 120 min (9.44 mg GAE/mL), reflecting good thermal tolerance. Heat reflux extraction showed no significant increase with time, and a slight decrease after 90 min (8.72 mg GAE/mL at 120 min) suggested thermal degradation of sensitive phenolics. Maceration maintained stable polyphenol concentrations over extended periods (up to 30 days), peaking at intermediate times (14-21 days, 9.76-9.91 mg GAE/mL), confirming its suitability for preserving heat-sensitive compounds.

Overall, careful selection of extraction conditions is critical. Soxhlet extraction under moderate solvent concentration and short duration provided the highest TPC. Heat reflux extraction was most effective at concentrated S/L ratios for rapid extraction, whereas maceration, though slower, allowed gradual and stable extraction under mild conditions.

The behavior of flavonoids under the same extraction conditions used for polyphenols was evaluated, considering solvent concentration, solid-to-liquid (S/L) ratio, and extraction time across three methods: Soxhlet reflux (Sx), heat reflux extraction (R), and maceration (M) (Figure 5).

Effect of solvent concentration (Figure 5a)

Flavonoid extraction efficiency generally increased with ethanol concentration from 30% to 70%. Heat reflux extraction (R) showed the highest TFC, increasing from 5.16 mg QE/mL (30% ethanol) to 7.97 mg QE/mL (70% ethanol), indicating thermal stability of flavonoids under these conditions. Maceration followed a similar trend, achieving 6.97 mg QE/mL at 70% ethanol, reflecting compatibility of flavonoids with concentrated ethanol in static conditions. In contrast, Soxhlet extraction (Sx) yielded significantly lower TFC, with a maximum of 3.23 mg QE/mL at 70% ethanol, suggesting potential thermal degradation or volatilization during prolonged high-temperature extraction.

Effect of solid-to-liquid ratio (Figure 5b)

The S/L ratio significantly influenced flavonoid extraction. For all three methods, a low ratio (1:5) produced the highest TFC, indicating optimal solute-solvent interaction at minimal solvent volumes. Maceration achieved the highest TFC (6.04 mg QE/mL), followed by Soxhlet (5.98 mg QE/mL) and heat reflux extraction (4.66 mg QE/mL). Increasing the solvent volume (ratios 1:15 and 1:20) led to consistent decreases in TFC across all methods, reflecting reduced extraction efficiency in more dilute systems.

Effect of extraction time (Figure 5c)

Extraction time influenced TFC differently depending on the method. In heat reflux extraction, the optimal time was 90 min, yielding 7.12 mg QE/mL; extending to 120 min slightly decreased TFC (6.77 mg QE/mL), likely due to thermal degradation. Soxhlet extraction showed minimal variation, with a modest maximum at 120 min (2.68 mg QE/mL), indicating the limitations of prolonged high-temperature exposure. For maceration, TFC peaked at 7 days (3.74 mg QE/mL) and gradually decreased to 2.38 mg QE/mL at 30 days, likely due to slow oxidation or partial adsorption of flavonoids onto residual plant solids.

The data obtained in the present study align well with those previously reported in the scientific literature (Table 4). Table 4 summarizes a selection of values describing the polyphenol and flavonoid content in extracts obtained from different parts of *A. annua*, using extraction methods similar or comparable to those applied in this work. In the case of polyphenols, the results are generally expressed relative to gallic acid equivalents (GAE), maintaining consistency across most studies. However, for flavonoids, the mode of expression varies considerably, depending on the reference standards employed by each research group (e.g., catechin (CE), rutin (RE), or quercetin (QE) equivalents). This variability highlights the importance of standard selection when comparing quantitative data from different studies.

Table 4. Comparative evaluation of polyphenol and flavonoid content with literature data

Plant part used	Extraction method	TPC	TFC	References
Aerial parts	Maceration with solvent based on 70 % (v/v) ethanol	27.06 ± 0.19 mg GAE/g extract	5.18 ± 0.32 mg RE/g extract	(Acquaviva <i>et al.</i> , 2023)
Leaves	Maceration with solvent based on 50 % (v/v) ethanol	23.36 ± 0.92 mg GAE/g	2.68 ± 0.07 mg CE/g	(Skowrya <i>et al.</i> , 2014)
Flowers (inflorescences)	Maceration + ultrasound assisted extraction based on solvent: 80% Ethanol (v/v)	37.41 ± 0.67 mg GAE / g of dried extract	36.98 ± 0.81 TFC mg QE / 100 g d.w.	(Țiclea <i>et al.</i> , 2025)
Aerial parts	Maceration with solvent based on 70 % (v/v) ethanol	66.74 mg GAE/ g of dried extract	12.57 ± 0.21 mg QE mg/g of Dried extract	(Gavarić <i>et al.</i> , 2025)
Leaves	Maceration + ultrasound assisted extraction based on solvent 80% ethanol (v/v)	21.57 ± 0.51 mg GAE/g	19.57 ± 0.61 TFC mg QE / 100 g d.w.	(Țiclea <i>et al.</i> , 2025)
Aerial parts	Maceration + ultrasound assisted extraction based on solvent 80% ethanol (v/v)	129.28 ± 2.09 mg GAE / g of dried extract	-	(Minda <i>et al.</i> , 2022)
Leaves and flowers	Maceration based on solvent ethanol 50% and 70% respectively (v/v)	3.27 mg GAE/mL extract	6.97 mg QE/mL extract	the present study

Initial Assessment of Emulsion Properties

The centrifugation and vortex stability tests (Table 3) demonstrated that emulsions containing *A. annua* extracts remained physically stable, maintaining a homogeneous appearance and consistent texture throughout the evaluation period, with no observable signs of phase separation or other physical instability phenomena.

The initial pH measurements (Table 3) indicated values within the neutral to slightly acidic range. The base emulsion exhibited a pH of 6.01, while the incorporation of *A. annua* extracts led to a slight increase in pH. The hydroalcoholic extract raised the value to 6.13, an effect likely attributable to the presence of polyphenolic compounds and organic acids extracted from the plant matrix. All formulations slightly exceeded the optimal physiological skin pH range (5.2-5.8), suggesting that minor pH adjustment may be required to ensure dermal compatibility and maintain the integrity of the skin's acid mantle.

Conductivity measurements (Table 3) revealed relatively low values, particularly for the *A. annua* formulation (0.39 mS/cm), which is characteristic of weakly ionic systems. This finding suggests a moderate contribution of ionizable constituents extracted in the hydroalcoholic phase. Even so, the low conductivity values are indicative of good colloidal stability within the emulsified systems.

The organoleptic assessment of the *A. annua* emulsion revealed a uniform and smooth appearance with a glossy surface and a light yellowish-white coloration, characteristic of the botanical raw material employed. The formulation displayed a homogeneous, semisolid consistency with an unctuous texture and an overall pleasant sensory profile.

Microscopic analysis (Table 3) confirmed a homogeneous microstructure, characterized by a stable and uniform dispersion of internal phase droplets within the continuous phase. As indicated in Table 3, the emulsion exhibited consistent droplet distribution and no evidence of coalescence, confirming its microstructural stability.

Microbiological evaluation (Table 3) demonstrated that, 24 hours post-preparation, the formulation containing the hydroalcoholic extract exhibited a single isolated colony (1 CFU/g), a value well within the acceptable limits defined by current quality standards. Upon retesting after 30 days of storage at room temperature, no colonies were detected, confirming the isolated nature of the initial contamination event and

the effectiveness of the preservative system employed. All microbiological parameters complied with ISO17516:2014 requirements, confirming the formulations' safety and microbiological stability.

The results regarding the stability and characterization of the emulsion obtained with *A. annua* extract confirm the obtaining of a new product whose properties comply with current regulations and meet consumer acceptability standards. From a sensory, physical, and physicochemical standpoint, the emulsion is comparable to other dermatocosmetic formulations previously developed by our team using the same base and *Acmella oleracea* extract as the active ingredient (Maxim *et al.*, 2024a; Maxim *et al.*, 2024b). Similarities are also observed when compared to emulsions formulated with different bases and other plant extracts, such as *Crocus sativus* L. (Turcov *et al.*, 2022a) or *Galium verum* (Turcov *et al.*, 2022b).

Conclusions

The study of *Artemisia annua* extracts demonstrates that this plant is a valuable source of bioactive compounds suitable for the preparation of alcoholic extracts intended for phytopharmaceutical and dermatocosmetic applications. Based on the experimental results, two main conclusions can be drawn, proving a rationale for further research focused on developing dermatocosmetic formulations aimed at mitigating oxidative stress in the skin: (1) content of polyphenol in extract: Optimal conditions for each method were as follows: *Soxhlet extraction*: 50% ethanol, S/L = 1:15, 90 min, TPC = 11.26 mg GAE/mL; *Heat reflux extraction*: 50% ethanol, S/L = 1:5, 60 min, TPC = 10.48 mg GAE/mL and Maceration: 50% ethanol, S/L = 1:15, 21 days, TPC = 9.91 mg GAE/mL; (2) content of flavonoid in extract: The most efficient conditions were achieved by *Heat reflux extraction* using 70% ethanol, an S/L ratio of 1:5, and an extraction time of 90 min, resulting in a maximum TFC of 7.97 mg QE/mL.

The *Artemisia annua* extract-based emulsions exhibited satisfactory physicochemical and structural stability, as demonstrated by the results of mechanical stress testing, pH and conductivity analyses, organoleptic evaluations, and microscopic observations. The formulations maintained consistent homogeneity and integrity under the applied stress conditions. Furthermore, all emulsions displayed a uniform microstructure and a pleasant sensory profile, with no observable signs of physical separation, coalescence, or other instability phenomena, confirming their overall formulation robustness.

Authors' Contributions

Conceptualization: DS and CM; Data curation: MB; Formal analysis: CM; Funding acquisition: DS; Investigation: CM and MB; Methodology: CM and DS; Project administration: DS; Resources: DS; Software: CM and DS; Supervision: DS; Validation: DS and DT; Visualization: DS and MB; Writing - original draft: DS, DT and MB; Writing - review and editing: DS and DT.

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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