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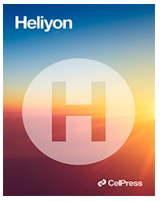

Research article

Phytochemical and colloidal analysis of *Artemisia* hydrolates and their activities against low-density lipoprotein oxidation

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ABSTRACT

Hydrolates, or floral waters, obtained from herbs are using for various purposes in medicinal and cosmetic industries. Biochemical characterization of many *Artemisia* species and their hydrolates, which has not been described before, is important for determining their unique properties for use as the main component in cosmetics. Five *Artemisia* species including *Artemisia absinthium*, *Artemisia serotina*, *Artemisia nitrosa*, *Artemisia sublessingiana*, *Artemisia pauciflora* were studied quantitatively and qualitatively, the content of moisture, ash, extractive substances was determined. Moreover, the mineral composition content, consist of macroelements (K, Na, Mg, Ca) and microelements (Cu, Zn, Fe, Mn) was determined using atomic adsorption spectroscopy. Their antioxidant properties were evaluated using DPPH, ORAC, and TBARS assays. Among them, *A. nitrosa* showed significant inhibition of LDL oxidation (IC_{50} = 3.1 μM) in the TBARS assay, along with prolonged lag time of conjugated diene formation (>180 min) and substantial protection against LDL oxidation in the REM assay. The hydrolates of *Artemisia* species were obtained with the ultrasonic construction using optimal parameters. Moreover, the behavior of *Artemisia* hydrolates has been studied at various phase boundaries – surface tension, wetting and emulsifying properties. It was revealed that the composition of *Artemisia* hydrolates contains surface-active components. Measurements of the wetting properties of *Artemisia* hydrolates showed that they have increased hydrophilizing and, therefore, moisturizing ability, which in turn makes it possible to introduce them into cosmetics. In the result, the cosmetics such as a cream, gel, shampoo based on *A. nitrosa* hydrolates were obtained and their organoleptic properties were studied.

1. Introduction

Currently, the demand for products made from natural raw materials is increasing in the cosmetics market. Hydrolates are one of the important ingredients of cosmetics, due to their beneficial medicinal properties and natural origin. It is a natural aqueous solutions

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obtained by distillation of plant materials with steam [1,2]. Passing through plant materials, water vapor is saturated with valuable water-soluble plant components: essential oils, acids, bioflavonoids and vitamins [3,4]. Hydrolates are of great interest not only for consumers, but also for manufacturers. Firstly, their use is economically advantageous, since such a complex natural product is easily accessible and the method of obtaining is effective. Secondly, compounds obtained from natural sources are hypoallergenic. And finally, products obtained from natural hydrolates have the potential in the world market of skin care cosmetics due to good penetration [5,6]. Thus, the production of cosmetic products using hydrolates obtained from domestic medicinal plants is of practical interest. To develop a scientifically based technology for the production of cosmetics, it is necessary to study their colloidal chemical properties, since all cosmetic products are representatives of dispersed or colloidal systems.

Artemisia genus was selected as a subject of the study due to their wide range of pharmacological properties and diverse content of metabolites, namely terpenoids, flavonoids, lignans, sterols and coumarins, which were founded in more than 200 species [7]. Moreover, the majority of *Artemisia* genus differs with its powerful smell contributing to the presence of essential oils that are useful in natural remedies, dental care, perfume, and other cosmetic industries [8]. For example, one of the representative species of these genus, *Artemisia capillaris* treatment reduced mast cell-induced atopic dermatitis scores in mice by week three and reduced histological signs of the disease, including edema, epidermal keratinization, and cellular infiltration [9]. The genus in question possesses features that promote skin health, including antioxidant, anti-inflammatory, and moisturizing properties. As such, the introducing of this genus into skincare formulations holds promise for fostering skin health and addressing diverse dermatological concerns.

The majority of species growing in Kazakhstan remain understudied or lack comprehensive scientific investigation. Among the rarest species chosen for current study first is a winter wormwood, or *Artemisia serotina*, a plant that grows in Kazakhstan and Uzbekistan in Central Asia. Its major constituents are 1,8-cineol (10.08 %), filifolid A (8.62 %), chrysanthenon (13.00 %), and (*Z*)-jasmone (1.95 %) [10]. The other studies focused on *A. serotina*, which grows in Kazakhstan showed the constituents of essential oils: 1, 8-cineol (6.60 %), camphor (2.00 %), thujone (53.9 %), carvone (5.70 %), isobutyric acid (0.03 %), and phenols (0.05 %) [11,12]. The second species, described in R. Jalmakhanbetova et al. identified organic substances of *Artemisia sublessingiana*: velutin, apigenin, hispidulin, 5,7,3′-trihydroxy-6,4,5-trimethoxyflavone, and eupatilin [13]. The third species, *Artemisia nitrosa* Weber is native to salty desert-steppe environments of Kazakhstan, southern Siberia, and Mongolia [14]. Kazymbetova et al., discovered the presence of guaianolide, germacranolides, sesquiterpene dimer, eudesmanolides from *A. nitrosa* [15]. Another research reports claim on fourth species reports that *Artemisia pauciflora*, is a perennial semi-shrub in the aster family that grows in Altay, East European Russia, Kazakhstan, and Uzbekistan. Its protein content varies from 10 percent (on absolute dry weight) during the winter season to 14 percent during the first part of the vegetative phase and 17 percent in the following crop [16]. According to findings from earlier studies, the leaves of *A. pauciflora* contain a significant number of sugars [17], implying that carbohydrates may play a role in the regulation of intracellular osmotic pressure in *Artemisia* genus.

Antioxidants can be used to prevent and treat oxidative stress-related diseases, including dermatological concerns and skin aging [18–20]. The most harmful aspect of oxidative stress is the generation of reactive oxygen species (ROS), which are regularly produced in living cells as a byproduct of normal oxygen metabolism [21,22]. The ROS can be transformed into more potent radicals by themselves or in the presence of transition metals, causing damage to various biological components such as lipids, low density lipoproteins (LDL), DNA, and proteins [23,24]. Synthetic antioxidants are questionable in use for the medicinal and cosmetic industries because of their potential toxicity and carcinogenic properties [25]. Thus, natural raw materials are becoming increasingly significant means of protecting and improving health [26].

This research aims to examine the phytochemical profile and colloidal chemical properties of hydrolates obtained with *Artemisia* species growing in our country, with the objective of exploring their potential applications in various cosmetic formulations. While existing studies have examined the phytochemical profiles and properties of various plant hydrolates, there is a noticeable lack of research specifically focused on the colloidal chemical properties of *Artemisia* hydrolates in Kazakhstan. This gap underscores the necessity for focused research to assess the distinct biological characteristics of native *Artemisia* species, which may yield insights into their activities, particularly regarding antioxidant properties. The evaluation of antioxidant properties was conducted through various radical scavenging methods, including DPPH, ORAC, TBARS, and oxidative LDL inhibition.

2. Materials and methods

2.1. Plant materials

Five species of *Artemisia* plant were selected as objects of research work based on the previous studies and searched literature review, specifically *A. absinthium*, *A. serotina*, *A. nitrosa*, *A. sublessingiana*, *A. pauciflora*. All the mentioned plants have been collected during its flowering stage in Almaty region and East Kazakhstan region (Tarbagatai and Altai districts) at the end of August 2022.

Collected species were dried in the air, avoiding the direct exposure of sunlight. Air-dried samples were milled to small pieces with laboratory mill and stored in the Research Center for Medicinal Plants, Faculty of Chemistry and Chemical Technology, Al-Farabi Kazakh National University, Almaty, Kazakhstan. The most curious indigenous *Artemisia* species were discovered fully for biochemical composition, antioxidant activity and colloid-chemical properties.

2.2. Reagents and equipment

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), quercetin, dimethyl sulfoxide (DMSO), Folin-Ciocalteu's phenol reagent, gallic acid, sodium carbonate, sodium nitrite and aluminum chloride were

purchased from Sigma Aldrich (St. Louis, MO, USA). All chemicals were analytical grade.

Quantitative analysis of the content of the biologically active components and antioxidant assays of medicinal plants were determined by the methods described above according to previous reported ones with slight modifications to spectrophotometric measuring carried out on a SpectraMaxM3 Multi-Mode Microplate Reader (Molecular device, USA). Colloidal properties of the hydrolates were analyzed using 781 pH/Ion Meter potentiometer, goniometer, tensiometer K6 (KRUSS, Germany).

To obtain the final cosmetic medical product, water bath, heat-resistant jar, thermometer, analytical scales, mini-mixer, chopsticks, cream dishes were used. As reagents almond oil, shea butter, essential oil, carboxymethylcellulose (CMC), glycerin, emulsifier (PEG -100 Stearate (Montanov L), distilled water, polyacrylic acid, ethyl alcohol were applied.

2.3. Authenticity analysis

Using methods described in the monographs [27,28] (full methodology shown in Supplementary material Experimental), the main bioactive ingredients of *Artemisia* species were identified both quantitatively and qualitatively. The plant materials' authenticity, including their moisture, ash contents, mineral compositions, together with the extractive substances with 90 percent ethanol was evaluated based on the State Pharmacopeia of the Republic of Kazakhstan methodology [29,30] (Supplementary material Experimental).

2.4. Mineral composition

About 5 g of raw materials are placed in a pre-calcined finely weighted porcelain, quartz or platinum gel, evenly distributing the material under the lid. Then the crucible is carefully heated, so that the material can be burned or evaporated at a lower temperature. Calcination is carried out at a low heat (about 500 °C) for a constant mass, avoiding melting of the resin, baking of the wall of the stove. After the end of the drying process, the gel is cooled in the exicator and then the resulting ash is burned again at 600 °C until a uniform gray color is obtained.

Ultimately, the precipitate is dissolved in 5 mL of HNO_3 (1:1) under heating conditions. The resulting solution must be heated on a tile to moist salts. The result is dissolved in 10-15 mL of 1N HCl or 1N HNO_3 and transferred to a 25 mL volumetric flask, the volume is adjusted to the mark.

In parallel, an idle experiment is carried out, which consists in preparing a solution of the same concentration from the same acid using the same utensils. The sample prepared according to the above described procedure is examined by atomic adsorption spectroscopy on the ASSIN device of the Carl Zeiss company. The spectra are photographed using DFS-13 (inverse linear dispersion 1A/mm) in the regions of 2100-3600 A. The standard is prepared on a silicon basis. The sensitivity of the analysis is 10^{-2}-10^{-5} [28] (Supplementary material Experimental).

2.5. Extraction processes

Artemisia hydrolates were obtained in the laboratory of Professor Jenis Janar with the application of ultrasonic extraction method that makes it possible to significantly reduce the duration of the process and ensure a more complete extraction of substances. When exposed to ultrasonic waves, the boundary diffusion layer is disrupted, and the penetration of the extractant into the material improves. As a result, the raw material swells much faster, turbulent and eddy flows occur, contributing to mass transfer and dissolution of substances. The aforementioned factors contribute to a marked increase in the rate of transition of active substances from raw materials to extractant.

The raw materials (20g) are placed in a flask, distilled water in the ratio of 1:10 is poured and placed in an ultrasonic construction for 1 h (3 times). Ultrasonic extraction was performed using the KQ5200B ultrasonic unit at a voltage of 40 kHz.

The resulting solution was filtered through a paper filter and concentrated on an EYELA N-1300 rotary evaporator at a temperature of 45 °C. The same procedure was repeated for each *Artemisia* species.

2.6. The total phenolic content (TPC)

The total phenolic content (TPC) was determined according to the Folin-Ciocalteu assay with some modifications [31]. The calibrating curve was made from gallic acid diluted in DMSO (0–1000 μg/mL) as a standard. Samples of *Artemisia* species (40 μL) were mixed with 40 μL of Folin-Ciocalteu's phenol reagent in 360 μL of distilled water using 1.5 mL Eppendorf tube. The mixture was incubated for 5 min at room temperature. Then, 400 μL of sodium carbonate (7 % w/v) solution in 160 μL of water were added to the reaction mixture and incubated for 90 min at room temperature. The resulted reaction mixture was centrifuged at 13,000 rpm for 5 min, 200 μL of supernatant used to the 96 well plates and measured the absorbance at 750 nm against the blank of DMSO. The TPC was indicated as mg of gallic acid equivalents per one g of sample (mg GAE/g).

2.7. The total flavonoid content (TFC)

The total flavonoid content (TFC) was determined by the previously reported method [32] with some modifications. First, the calibration curve was produced with quercetin in different concentrations with DMSO (0–1000 μg/mL). After, samples (50 μL) mixed with 200 μL of water in the 1.5 mL Eppendorf tube, and added 15 μL sodium nitrite (5 % w/v) solution. The mixture was incubated for

5 min at room temperature and then added 15 μL of aluminum chloride (10 % w/v) solution. After incubating for 6 min at room temperature, 100 μL of 1 M sodium hydroxide and 120 μL of distilled water were added to the reaction mixture and then vortexed for 1 min. 200 μL of the resulted mixture putted to the 96 well plates and the absorbance at 415 nm was recorded using a spectrophotometer. The TFC was indicated as mg of quercetin equivalents per one g of sample (mg TE/g).

2.8. Chromatographic conditions for the analysis of flavonoids

Flavonoid profile was quantified through high-performance liquid chromatography (HPLC) on a liquid chromatograph (Shimadzu LC-40), using five standards: gallic acid, catechin, epicatechin, naringin and phloridzin. Sample preparation was conducted by weighing 1 mg of dried sample and dissolving it in 1 mL of methanol. The injection volume was 10 μL, sample entry temperature was 40 °C. The separation of flavonoid constituents was carried out using a chromatographic column of type C18 with a length of 25 cm, an inner diameter of 4.6 mm and a film thickness of 5 μm. The mobile phase consisted of 1 percent acetic acid in water (*v/v*) and acetonitrile at a constant rate of 1 mL/min with the following gradient: starting with 10 percent acetonitrile and ending at 90 percent acetonitrile for 55 min. The detection was carried out at wavelength of 272 nm. Shimadzu LabSolutions software was used to control the liquid chromatography system, record, and process obtained results and data. Data processing included the determination of retention time and peak areas. Compounds were fully identified by comparing their retention times to those of standards under identical chromatographic conditions [33].

2.9. DPPH scavenging activity

The antioxidant activity of plants was evaluated by the ability to scavenge the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH test). The radical scavenging activity (RSA) on the DPPH radical was evaluated according to the method reported previously [34] with modifications adapted to 96-well microplates [35]. Samples (10 μL, in different concentrations in M) were mixed with 190 μL of DPPH solution (0.25 mM) in ethanol inside of 96-well flat bottom micro plates, and incubated in darkness at room temperature for 15 min. The absorbance (A_0 and A) was measured at 517 nm and RSA was expressed as a percentage of inhibition (Equation (1)) [36], relative to a control, containing ethanol in place of the sample and as half-maximal inhibitory concentration (IC_{50}, μM). Trolox (in 3.9–1000 μM) was used as a positive control.

$$\textit{Radical scavenging activity}\ (\%) = \frac{A_0 - A}{A} \times 100 \tag{1}$$

2.10. ORAC measurement

To evaluate ORAC, initially, 25 μL of an inhibitor was introduced into 150 μL of a sodium fluorescein working solution, followed by an incubation period of 30 min at 37 °C. A stock solution of fluorescein (4 μM) was prepared in 75 mM phosphate buffer at pH 7.4 before being diluted with pH 7.4 phosphate buffer at a 1:200 ratio. The reaction commenced upon the addition of 25 μL of 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) to achieve a final concentration of 29.5 mM. Excitation and emission wavelengths were set at 480 nm and 520 nm, respectively. The ORAC value was determined using the Trolox antioxidant standard curve, which involved plotting the net area under the curve (AUC) on the y-axis. The calculation of AUC and net AUC is presented in Equations (2) and (3) as follows [37]:

$$AUC = 1 + \frac{RFU_1}{RFU_0} + \frac{RFU_2}{RFU_0} + \frac{RFU_3}{RFU_0} + \ldots + \frac{RFU_{59}}{RFU_0} + \frac{RFU_{60}}{RFU_0} \tag{2}$$

$$Net\ AUC = AUC_{antoxidant} - AUC_{blank} \tag{3}$$

2.11. Measurement of TBARS

The absorbance of the reaction product formed between thiobarbituric acid (TBA) and malondialdehyde (MDA) was recorded at 540 nm. In an Eppendorf tube, 10 μL of $CuSO_4$ (final concentration: 10 μM), 10 μL of extracts or probucol, and 220 μL of LDL at a final concentration of 500 μg/mL in a buffer (10 mM PBS, pH 7.4) were mixed. The mixture was then incubated for 4 h at 37 °C. Following the incubation, 100 μL of trichloroacetic acid (TCA) and 100 μL of 0.67 percent TBA/0.05 N NaOH were added. After heating and centrifugation for 15 min at 3000 rpm, the absorbance (A_0 and A) of the supernatant was measured, and the inhibition rate calculated using Equation (4), which was utilized to determine the IC_{50} values.

$$\textit{Inhibition rate}\ (\%) = \frac{A_0 - A}{A} \times 100 \tag{4}$$

2.12. Measurements of formation of conjugated dienes (CD)

To assess the generation of conjugated dienes from oxLDL, we utilized the method outlined previously, employing a clear-bottom 96-well plate. In each well of the plate, 230 μL of LDL at a concentration of 500 μg/mL in a buffer (10 mM PBS, pH 7.4) was combined

with 10 μL of 250 μM $CuSO_4$ (resulting in a final concentration of 10 μM). Additionally, each well contained either extracts or probucol at a final concentration of 10 μM. The plate was then subjected to incubation at 37 °C for 4 h. Following incubation, the response was monitored using a SpectraMax M3 multi-mode microplate reader, with readings taken every 5 min over a duration of 180 min at an absorbance of 234 nm.

2.13. Measurements of relative electrophoretic mobility (REM)

The preparation of the REM for both native LDL and oxidized LDL induced by Cu^{2+} involved minor adjustments to the procedure outlined in a previous publication [35]. Prior to electrophoresis, samples containing 10 μM $CuSO_4$ and 10 μM extracts were incubated with LDL (at a final concentration of 500 μg/mL) for 16 h at 37 °C in the absence of light. The native and pretreated LDL samples were then applied onto a 0.5 percent agarose gel in TAE buffer (40 mM Tris-acetate with 1 mM EDTA, pH 8.0), and electrophoresis was carried out for 50 min at 100 V. Subsequently, the gel was immersed in a fixative solution comprising 60 percent ethanol and 10 percent acetic acid. Following fixation, the gel was stained with 0.15 percent Coomassie Brilliant Blue R250 and visualized using a destaining solution consisting of methanol, acetic acid, and water in a ratio of 1.5:1:7.5.

2.14. Colloid-chemical properties

Solutions of hydrolates AAW, AAP, AAE, ASE, ANE, AUE, APE were used in concentrations of 0.5, 1, 1.5, 2.0 percent (wt.) and the pH of solutions was measured using a 781 pH/Ion Meter potentiometer. The emulsifying and foaming properties of hydrolates were studied using known methods [38].

2.14.1. Wetting ability

Wetting ability was determined by measuring the contact angle θ on a goniometer. Four surfactant solutions of varying concentrations were produced. The surface tension of the prepared solutions and the edge angles at the interface of the polymer plate – surfactant – air solution and polymer plate – water – air are measured using the installation for identifying edge angles.

Measurements of the edge angles and surface tension for each surfactant concentration are carried out 3 times and the values obtained are found as an arithmetic mean. According to Equations (4) and (5), the work of wetting W_{wt} and adhesion W_a for each solution was calculated.

The wetting isotherm and the curve of dependence of the wetting operation on the concentration were constructed, they determine at what concentration of surfactants the surface of the polymer under study becomes hydrophilic, i.e. the wetting inversion point was found.

The phenomena of adhesion and wetting are closely related. The effect of wetting on the adhesive interaction is reflected in equations below. These Equations (5) and (6) show that the better the wetting (less θ), the greater the adhesion work [38].

$$W_a = \sigma_{l-g}(1 + \cos\theta) \tag{5}$$

$$W_{wt} = \sigma_{l-g}\cos\theta \tag{6}$$

2.14.2. Surface tension

Surface tension of surfactant solutions at the gas/liquid interface was measured using the Du Nouy method with the application of tensiometer K6 (KRUSS, Germany). The dependence of the surface tension of surfactant solutions on their concentration (isotherm of surface tension) at room temperature was identified. The adsorption values of surfactants at their various concentrations (1.0, 1.5, 2.0 % (wt.)) at room temperature were determined and adsorption isotherm was constructed.

The Du Nouy ring rinsed with distilled water and heated on a gas burner until it glows red. About 20 mL of the studied hydrolate (0.5 % solution) is poured into a clean cup, it is put on a slide table and raised with a flywheel to the level of the ring (1–2 mm short of the ring). Due to the clockwise rotation of the flywheel, the thread tension increases, and thus the force applied to the ring increases. These alternating operations of increasing the load and lowering the liquid level were repeated until the film breaks, which means that the load applied to the ring has exceeded the surface tension forces [39]. The same procedures were repeated with solutions of 1.0, 1.5, 2.0 percent (wt.) concentration.

2.15. Preparation of the cream

In this work, an attempt was made to obtain a cosmetic product based on wormwood hydrolate in the form of a concentrated emulsion. The oil base consists of carboxymethylcellulose (CMC) and glycerin, the aqueous phase consists of wormwood hydrolate, ethyl alcohol and distilled water. The process of obtaining such a product consists of mixing the components in certain proportions and homogenization. The mass of the finished product should be 100 g. To obtain the gel, the necessary components are weighed. The required amount of cold plant extract is placed in a glass cup with a capacity of 150-200 cm^3, glycerin, alcohol are added and mixed until a homogeneous system is obtained. Then, a gel-forming agent is added to the glass with continuous intensive stirring in small portions [40].

3. Results and discussion

3.1. The obtaining of Artemisia hydrolates

Hydrolates were designated with specific names (as outlined in Table 1) to enhance clarity and conciseness, thereby aiding in identification, and promoting more efficient discourse, ultimately making it more accessible for researchers.

3.2. Authenticity of plant materials

Within the frameworks of study, five *Artemisia* species, which were the base raw material for hydrolates were analyzed for their authenticity. Table 2 illustrates the results of investigation of *A. absinthium*, *A. serotina*, *A. nitrosa*, *A. sublessingiana* and *A. pauciflora.*

According to the obtained results, it can be seen that *A. sublessingiana* has the highest moisture (10.0 %), and *A. nitrosa* has the highest percentage of ash content (19.3 %). Moreover, extractive substances content using 90 percent ethanol as a solvent was identified, corresponding results as next: *A. serotina – 22.68* percent*, A. pauciflora – 20.40* percent*, A. nitrosa – 20.24* percent and *A. absinthium – 12.82* percent.

3.3. Mineral composition

Next, mineral composition, namely macro-micro elemental analysis was conducted with the application of atomic adsorption spectroscopy. Among the macro-elements the content of potassium and sodium are mostly presented in *A. serotina,* 24.19 mg/g and 5.27 mg/g, respectively. Additionally, *A. nitrosa* macro-elemental composition includes magnesium – 1.185 mg/g and calcium – 2.353 mg/g. Furthermore, the highest amount of the following microelements was: copper (*A. pauciflora*, 0.019 mg/g), zinc (*A. absinthium*, 0.044 mg/g), iron (*A. serotina*, 0.293 mg/g) and manganese (*A. nitrosa*, 0.062 mg/g) as shown in Table 3.

The role of these elements in specific doses is essential due to their chemical properties in order to offer a variety of advantages for skin health and attractiveness; cosmetic goods frequently contain both macro and microelements. Macro-elements like calcium, magnesium, and potassium may support skin hydration and the structural integrity of skin cells. Microelements that support overall skin health and function include zinc, copper, and manganese. These elements also frequently act as cofactors for enzymes involved in antioxidant reactions, collagen formation, and skin restoration. These ingredients are added to cosmetic compositions in an effort to improve skincare product efficacy and encourage a healthy complexion [41].

Cosmetic products can include supplementary minerals alongside macro and microelements, such as iron oxide for color enhancement or titanium dioxide and zinc oxide for UV protection. The minerals serve various purposes, including imparting color to the product, providing UV protection, and enhancing texture, among other functionalities. The interplay of these components in cosmetic products effectively addresses the functional and aesthetic dimensions of skin health, showcasing a comprehensive approach to skincare [42].

3.4. The amount of total phenolic contents (TPC) and total flavonoid contents (TFC)

Antioxidants play a crucial role in maintaining skin health by neutralizing free radicals, which are unstable molecules that can damage skin cells and accelerate aging. Free radicals generated by factors like UV radiation, pollution, and stress can cause oxidative stress in the skin, leading to collagen degradation, wrinkles, and other signs of aging. Antioxidants help neutralize these free radicals, reducing oxidative stress and preserving skin health.

Phenolic compounds play a leading role in a wide range of degenerative diseases. They are extensively studied for their antioxidant, anticarcinogenic, and antimicrobial attributes. All phenolic or flavonoid compounds exhibit the different level of antioxidant activity. Certain approaches may demonstrate varying levels of effectiveness as a result of structural distinctions. The antioxidant properties of phenolic compounds and flavonoids stem mainly from their capacity to donate electrons, scavenge reactive oxygen species, chelate metal ions, inhibit lipid peroxidation, regulate antioxidant enzymes, and exert anti-inflammatory effects. Therefore, recognizing their quantity in plants provides a clear insight into future antioxidant potential. We evaluated in this study, the comparison of total phenolic (TPC) and total flavonoid (TFC) contents of *A. absinthium, A. serotina, A. nitrosa, A. sublessingiana,* and *A. pauciflora* extracts. The amount TPC in different *Artemisia* ethanol extracts were ranged from 23.55 mg GAE/g to 99.63 mg GAE/g, results are shown in Table 4. As the ethanol extract of *A. absinthium* had the highest extent of TPC (99.63 mg GAE/g) was selected for further evaluation of

Table 1
The species of *Artemisia* used for the preparing of hydrolates, along with the extractants, used extraction method and corresponding abbreviations.

Species	Extractant	Method	Corresponding name
A. absinthium	Water	Ultrasonic extraction	AAW
A. absinthium	Petroleum ether		AAP
A. absinthium	Ethanol		AAE
A. serotina	Ethanol		ASE
A. nitrosa	Ethanol		ANE
A. sublessingiana	Ethanol		AUE
A. pauciflora	Ethanol		APE

Table 2
Authenticity analysis (moisture, ash content and extractive substances in percent) of *Artemisia* species.

Species	Moisture, %	Ash content, %	Extractive substances, %
A. absinthium	7.1	6.4	12.82
A. serotina	7.4	7.3	22.68
A. nitrosa	6.1	19.3	20.24
A. sublessingiana	10.0	12.4	8.60
A. pauciflora	7.9	7.3	20.40

Table 3
The mineral composition given as mg of element per 1 g of plant of *Artemisia* species.

Species/Elements (mg/1g of plant)	Cu	Zn	Fe	Mn	K	Na	Mg	Ca
A. absinthium	0.001	0.044	0.040	0.003	3.483	0.132	0.219	0.967
A. serotina	0.002	0.006	0.225	0.019	2.817	0.229	0.566	2.055
A. nitrosa	0.008	0.009	0.293	0.029	24.19	5.266	0.103	2.110
A. sublessingiana	0.005	0.012	0.075	0.062	7.179	0.898	1.185	2.353
A. pauciflora	0.019	0.005	0.124	0.010	1.844	1.052	0.408	0.462

TPC in different solvents. Water extract (41.15 mg GAE/g) of *A. absinthium* exhibited slightly higher TPC than petroleum ether extract (23.55 mg GAE/g of plant extract) which shown in Table 4.

The highest level of TFC was also recorded in the ethanol extract of *A. absinthium* (76.49 g QE/g of plant extract) followed by other extracts (Table 4). The species *A. absinthium* was selected for further evaluation of TFC using various solvents. Water extract (28.21 mg QE/g of plant extract) of *A. absinthium* exhibited the best trice higher TFC (Table 4) than PE extract (8.06 mg GAE/g of plant extract).

3.5. The high-performance liquid chromatography (HPLC) analysis of flavonoids

For the confirming of the presence of various phenolic compounds HPLC analysis of *Artemisia* extracts was performed. The obtained chromatograms were given below in Figs. 1 and 2, this kind of evaluation commonly present in other studies [43–45]. At Figs. 1 and 2 can be seen that several phenolic and flavonoids constituents can be detected in the wavelength of 272 nm (according to refences given at Table 7), a thus extracts were obviously rich for phenolic and flavonoids content.

The flavonoid profile was measured using high-performance liquid chromatography (HPLC) on a liquid chromatograph (Shimadzu LC-40) with five standards: gallic acid, catechin, epicatechin, naringin, and phloridzin. The chromatogram of standards is shown below at Fig. 1. The retention time in min, standard compounds's concentration in mg/L and compounds' name presented in Table 5.

It can be observed that all species contain gallic acid, catechin, epicatechin, naringin and phloridzin in certain concentrations, as shown in Fig. 2A-E. Table 6 presents the concentration of each compound, expressed in micrograms per gram of dry plant material. Catechin and epicatechin are presented in all the analyzed sample species, but most notably in *A. pauciflora (*384.0 and 83.53 μg/g respectively*)*. Several *in vitro*, *in vivo*, and physical techniques have conclusively demonstrated the antioxidant activity of catechin. The molecular processes behind angiogenesis, the breakdown of extracellular matrix, the control of cell death, and multidrug resistance in cancer and associated diseases are all impacted by catechin [46]. Epicatechin and similar isomers have been shown in Xiong's study to have inhibitory effects on oxidative reactions, including peroxynitrite [47]. Compared to epicatechin alone, the epicatechin tetramer has demonstrated a stronger chemopreventive activity against oxidative damage [48].

The naringin was determined in five *Artemisia* species, the highest concentration was identified in *A. serotina* (108.7 μg/g). Naringin is a member of the flavonoid class known as flavanones. It has been demonstrated that naringin inhibit the growth, spread, and development of cancer [49]. Moreover, naringin belongs to strong antioxidants, which are capable to neutralize free radicals and prevent their development [50]. Additionally, they have a major impact on immunological mechanisms and cells that are crucial to inflammatory processes [50].

Phloridzin's content is mostly found in *A. sublessingiana* with the concentration of 77.70 μg per gram of dried plant material.

Table 4
The antioxidant properties of *Artemisia* extracts expressed by TPC, TFC, DPPH, ORAC and oxidized LDL induced by Cu^{2+}.

Samples	TPC, mg GAE/g	TFC, mg QE/g	DPPH, IC_{50} (μg/mL)	ORAC, (μmol TE/g)	TBARS, IC_{50} (μM)	CD lag time, (min)
AAW	41.15	28.21	–	–	–	–
AAP	23.55	8.06	–	–	–	–
AAE	99.63	76.49	79.3 ± 0.2	8.1 ± 0.2	26.1 ± 0.1	105
ASE	22.46	9.48	13.9 ± 0.3	25.6 ± 0.1	4.2 ± 0.3	>180
ANE	39.63	20.98	11.3 ± 0.4	32.4 ± 0.1	3.1 ± 0.2	>180
AUE	62.94	43.43	41.1 ± 0.3	12.1 ± 0.3	13.7 ± 0.2	170
APE	43.60	33.21	49.6 ± 0.2	10.2 ± 0.2	16.5 ± 0.3	125
Trolox	–	–	10.9 ± 0.4	1.0 ± 0.2	–	–
Probucol	–	–	–	–	27.6 ± 0.2	85

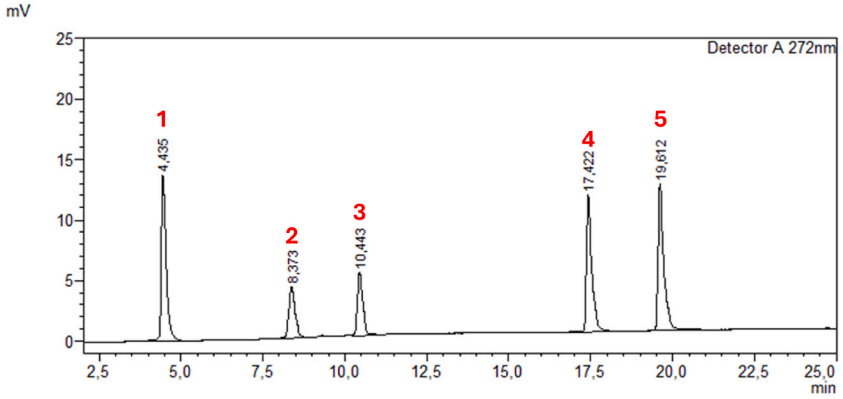


Fig. 1. Chromatogram of flavonoids standards.

Phloridzin differentiates with its strong activity as anti-inflammatory, antioxidant, anti-microbial, anti-aging, anti-cancer and anti-obesity [51].

A. absinthium has the highest content of gallic acid among other species, which is a highly prevalent phenolic acid in the plant kingdom, with powerful antioxidant, anti-inflammatory, and anti-tumor properties [52–54].

3.6. Antioxidant activity

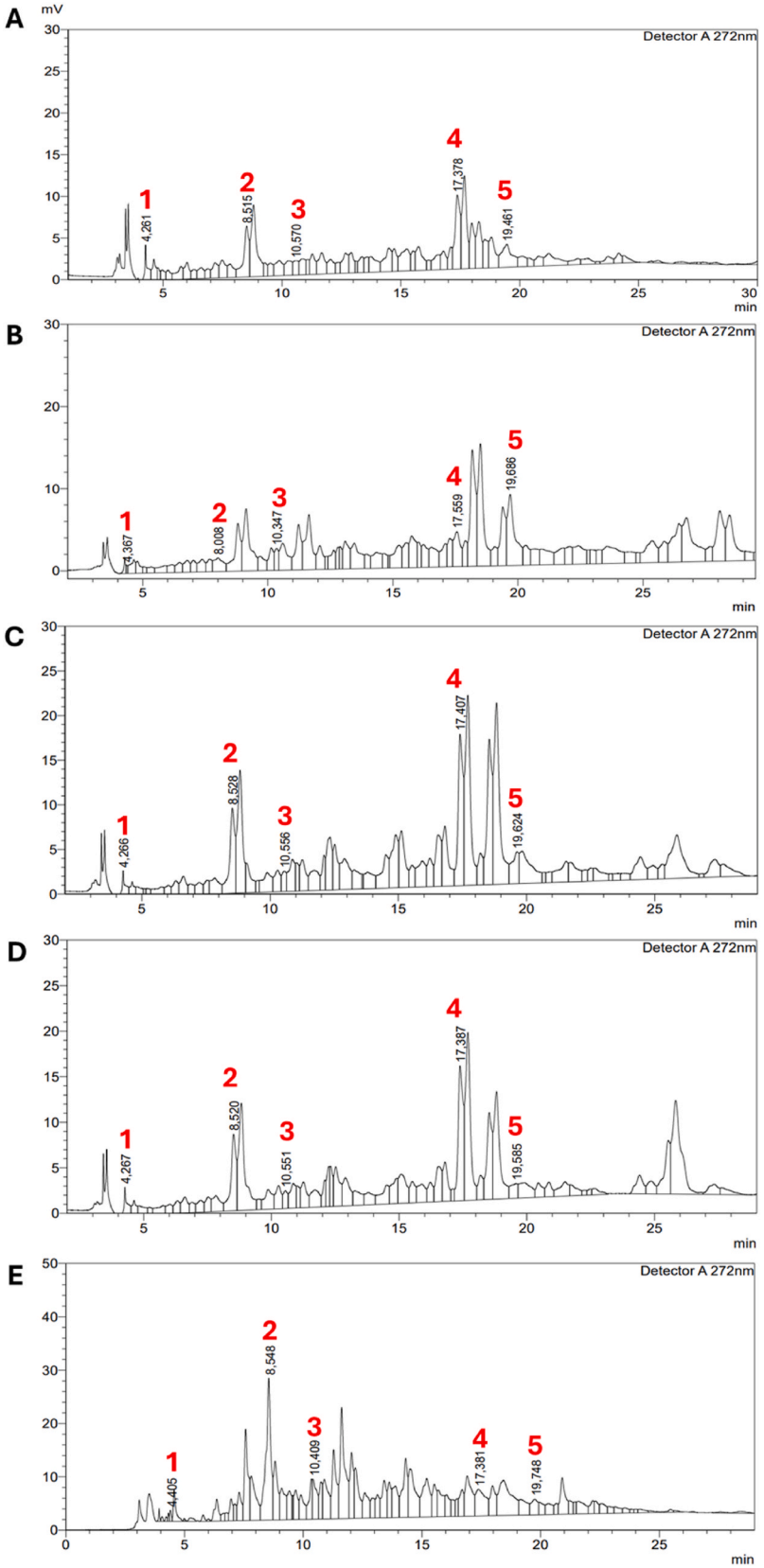
The investigating the different ratios of main constituents in *Artemisia* extracts is essential for understanding their collective influence on antioxidant activity and anti-LDL oxidation potential. The possibility of synergistic interactions between these compounds may increase their efficacy in addressing oxidative stress, while unique mechanisms of action could result in better formulations for therapeutic and cosmetic uses. In our research, we aimed to compare the antioxidant abilities of different species of *Artemisia*, and future studies in this area will be crucial for harnessing the full benefits of these phytochemicals, ultimately advancing their application in treatment of serious diseases associated with oxidative stress. The classical assays named by free radical (DPPH, ORAC, TBARS) scavenging assays were used to assess the antioxidant potential of *Artemisia* extracts. First, the antioxidant activity of extracts was evaluated by the estimation of extract concentration (IC_{50}) required to scavenge 50 percent of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. The DPPH scavenging activity of *Artemisia* extracts were investigated in dose-dependent mode (1.5625–25 μg/mL) and Table 4 shows the IC_{50} values and compared with synthetic antioxidant compounds (Trolox in μM). The DPPH scavenging activity expressed in IC_{50} of *Artemisia* extracts ranged from 11.3 to 79.30 μg/mL (Fig. 3A). The best antioxidant extract is *A. nitrosa* according to DPPH assay.

The subsequent ORAC assay conducted in current study presents several advantages compared to the DPPH assay. By employing the peroxyl radical as a representative model for antioxidant reactions with ROS commonly present in food, it establishes a more pertinent system for assessing antioxidant activity. The results presented in Table 4 were ranged from 8.1 to 32.4 μmol TE/g, and the highest ORAC value was at *A. nitrosa* (Fig. 3A). In summary, with comparison of previous works (Table 8), the outcomes obtained by antioxidant evaluations showed promising results.

Low-Density Lipoprotein (LDL) is primarily known for its role in cardiovascular health as it transports cholesterol through the bloodstream, contributing to plaque buildup in arteries. However, emerging research suggests that LDL may also have implications for skin health. The link between the anti-LDL oxidative activity of *Artemisia* and their ability to protect skin cells from oxidative stress lies in the common mechanisms of oxidative damage. Plants that exhibit strong anti-LDL oxidative activity likely have potent antioxidant properties that can neutralize ROS. These same antioxidants can protect skin cells by reducing oxidative stress, which contributes to premature aging, inflammation, and skin barrier damage. The dual advantage reinforces the argument for their incorporation in skin care formulations. Some studies have suggested a potential link between elevated LDL levels and certain skin conditions such as acne, psoriasis, and accelerated skin aging. High levels of LDL may contribute to inflammation and oxidative stress in the skin, which can exacerbate these conditions and accelerate the aging process. Furthermore, LDL cholesterol is believed to play a role in the formation of advanced glycation end products (AGEs), which are compounds that contribute to skin aging by damaging collagen and elastin fibers.

Firstly, the extracts underwent testing using the thiobarbituric acid reactive substances (TBARS) assay, which quantifies the formation of lipid peroxidation products. The IC_{50} values ranged from 3.1 to 26.1 μM, as indicated in Table 4. Notably, *A. nitrosa* demonstrated significant inhibition of LDL oxidation, with an IC_{50} value of 3.1 μM. Secondly, the extracts were assessed for their capacity to prolong the lag time, reflecting resistance to LDL oxidation, by monitoring the formation of conjugated dienes at 234 nm over a 180- min period. The control LDL incubated with 10 μM $CuSO_4$ exhibited a lag time of 80 min (Fig. 3B). The lag times reported in Table 4 corresponded with the findings from the TBARS assay. For instance, *A. nitrosa* displayed a lag time of 185 min ($IC_{50} = 3.1$ μM), *A. pauciflora* had a lag time of 125 min ($IC_{50} = 16.5$ μM), *A. absinthium* demonstrated a lag time of 105 min ($IC_{50} = 26.1$ μM), and probucol exhibited a lag time of 85 min ($IC_{50} = 27.6$ μM).

The oxidation of LDL can be evaluated using the relative electrophoretic mobility (REM) assay, where oxidized LDL (oxLDL)



(caption on next page)

Fig. 2. The HPLC spectra of *Artemisia* species: **(A)** *A. absinthium*, **(B)** *A. sublessingiana*, **(C)** *A. serotina*, **(D)** *A. nitrosa*, **(E)** *A. pauciflora*.

presents higher REM compared to native LDL due to alterations in its surface charge and size resulting from the oxidation process. However, the introduction of antioxidants can impede or delay this oxidation process and consequently reduce the REM of oxLDL, which can be quantified using agarose gel electrophoresis. The REM assay depicted in Fig. 3C was conducted as follows: lane 1 represents native LDL, lane 2 shows $CuSO_4$ (10 μM) oxidized LDL, and lanes 3–6 illustrate LDL oxidized with $CuSO_4$ (10 μM) and subsequently incubated for 16 h with 10 μM of extracts. The extracts exhibiting higher IC50 values in the TBARS assay notably decreased the mobility of LDL. *A. nitrosa* and *A. serotina* demonstrated the most potent anti-LDL oxidation activities, indicating a strong correlation between the REM and TBARS results.

As summary, the phenolic compounds provide significant protective benefits for skin health by interacting with multiple cellular pathways to combat oxidative stress. Their ability to directly neutralize ROS prevents damage to cellular lipids, proteins, and DNA, protecting the structural integrity and function of skin cells. By enhancing the activity of the body's natural antioxidant enzymes and inhibiting the process of lipid peroxidation, phenolic compounds help maintain the skin's barrier function, hydration, and elasticity. In addition, their anti-inflammatory properties and ability to chelate metal ions that trigger oxidative reactions further reduce the impact of environmental stressors such as UV radiation and pollution, which accelerate skin aging. These extensive protective benefits underscore the significance of phenolic compounds in skin care formulations aimed at preventing premature ageing, inflammation, and

Table 5
The results of the HPLC analysis of the standard compounds content in *Artemisia* species.

Peak No	t_R, min	Concentration, mg/L	Compound
1	4.43	65.03	Gallic acid
2	8.37	64.83	Catechin
3	10.44	64.27	Epicatechin
4	17.42	64.31	Naringin
5	19.61	64.18	Phloridzin

Table 6
The standard flavonoids content of *Artemisia* species analyzed by HPLC.

Plant	Concentration (μg/g of dry weight plant)				
	Gallic acid	Catechin	Epicatechin	Naringin	Phloridzin
A. absinthium	9.69	97.32	23.45	65.02	37.33
A. sublessingiana	1.23	45.37	25.86	38.36	77.70
A. serotina	8.18	137.1	24.83	108.7	29.07
A. nitrosa	8.42	119.6	21.49	97.10	13.85
A. pauciflora	3.35	384.0	83.53	69.86	25.15

Table 7
UV absorbance properties and reasons of absorbance of class of compounds (with their main representatives) present in plants: phenolics, flavonoids, terpenoids, and tannins.

Class of Compound	Examples and Characteristic Peaks (nm)	Typical Absorbance Range (nm)	Reason for Absorbance
Phenolic Compounds	Gallic acid (272), Caffeic acid, Ferulic acid	270–280	Presence of hydroxyl groups attached to aromatic rings
Flavonoids	Quercetin (255, 370), Kaempferol, Rutin, Luteolin	250–400	Conjugated double bonds and benzopyran structure (A-ring, B-ring absorption)
Cinnamic Acid Derivatives	Caffeic acid (320), Chlorogenic acid, Ferulic acid	310–320	Extended conjugation of cinnamic acid structure
Coumarins	Umbelliferone (325–330), Scopoletin	320–340	Benzopyrone structure with conjugated systems
Monoterpenes	Limonene (205), Pinene, Myrcene	190–210	Some absorb due to unsaturation in the structure (C=C bonds)
Sesquiterpenes	β-Caryophyllene (200), Humulene	190–220	Similar to monoterpenes, with UV absorbance related to unsaturation
Diterpenes	Retinol (Vitamin A) (260–280), Taxol	210–300	Contain multiple rings and unsaturated bonds
Triterpenes	Squalene (210, 280), Betulin	210–300	Absorbance related to conjugated double bonds and ring structures
Tetraterpenes	Carotenoids (e.g., β-Carotene) (450)	400–500	Strong absorbance due to extensive conjugation in the structure
Hydrolyzable Tannins	Gallotannins (250, 270), Ellagitannins	240–300	Presence of multiple hydroxyl groups and phenolic structures
Condensed Tannins	Proanthocyanidins (e.g., catechins) (278)	220–280	Aromatic rings and hydroxyl groups contribute to UV absorbance
Mixed Tannins	Both hydrolyzable and condensed	240–300	Different functional groups lead to variable UV absorbance

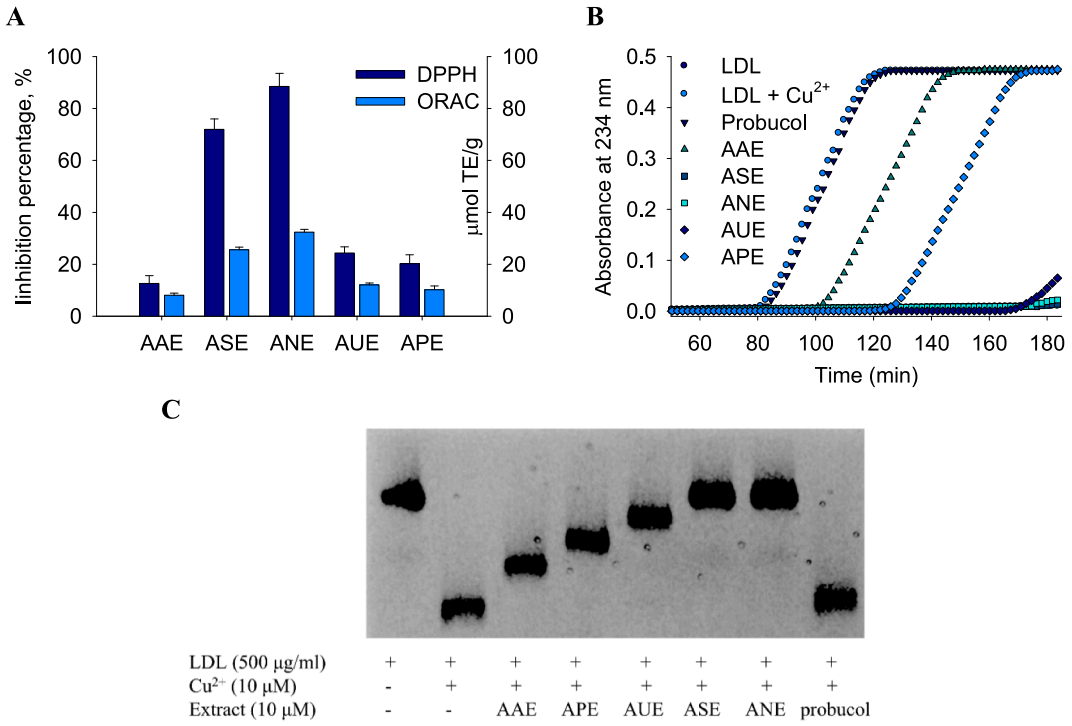


Fig. 3. Antioxidant capacity of *Artemisia* ethanol extracts (**A**) DPPH radical scavenging percentage of extracts at 20 µg/mL and ORAC values of extracts. (**B**) Effects of extracts on the generation of conjugated diene on Cu^{2+} induced LDL oxidation. (**C**) Effect of extracts and probucol on the Cu^{2+} mediated LDL oxidation by relative electrophoretic mobility (REM), full image is shown at Supplementary materials Fig. S1.

Table 8
Antioxidant activity, IC_{50} values, and extraction details of various *Artemisia* species in relation to radical scavenging assays.

Artemisia Species	IC_{50} (µg/mL)	Radical/Assay Used	Plant Part	Extraction Solvent	Authors
Artemisia annua	17.8	DPPH	Aerial parts	Ethanol	Ferreira et al. (2010) [55]
Artemisia absinthium	28.4	DPPH	Leaves	Methanol	Wojdyło et al. (2007) [56]
Artemisia herba-alba	24.7	TBARS	Whole plant	Ethanol	Mohamed et al. (2014) [57]
Artemisia dracunculus	35.1	DPPH	Leaves	Methanol	[58]
Artemisia vulgaris	30.6	DPPH	Leaves	Methanol	Bajpai et al. (2007) [59]
Artemisia sieberi	15.5	ORAC	Aerial parts	Ethanol	Souri et al. (2008) [60]
Artemisia scoparia	32.2	ORAC	Leaves	Ethanol	[61]
Artemisia campestris	12.5	TBARS	Aerial parts	Methanol	[62]
Artemisia afra	22.4	ORAC	Leaves	Methanol	Wintola & Afolayan (2011) [63]

oxidative damage.

3.7. Colloid-chemical properties

Hydrolates are wonderful moisturizers. The components of hydrolates can exhibit properties such as penetration into the deep layers of the skin; involvement of water molecules in the skin; regeneration of new cells and improved blood circulation. As a result, immunity and elasticity of the skin are improved, and the functioning of the sebaceous glands is normalized [64,65]. The evaluation of colloidal chemical properties in the development of hydrolates increases their productivity in the extraction of bioeffective substances, promoting both activity and the yield of valuable resources [66,67]. Understanding the effects of particle size and surface charge, particularly negative zeta potential values, is critical to optimizing bioavailability and skin penetration. Smaller particle sizes can enhance absorption and therapeutic benefits, while surface charge affects interaction with skin cells, influencing formulation stability and performance. Negative zeta potential provides the necessary electrostatic repulsion that enhances the stability of colloidal suspensions, minimizing the risks of aggregation and sedimentation. This stability ensures consistent delivery of active ingredients, enhancing product efficacy and consumer satisfaction.

For the scientifically based use of plant hydrolates in the composition of cosmetics for various purposes, it is necessary to study their colloidal chemical properties. First of all, it is necessary to measure the surface tension of aqueous solutions, since a decrease in the

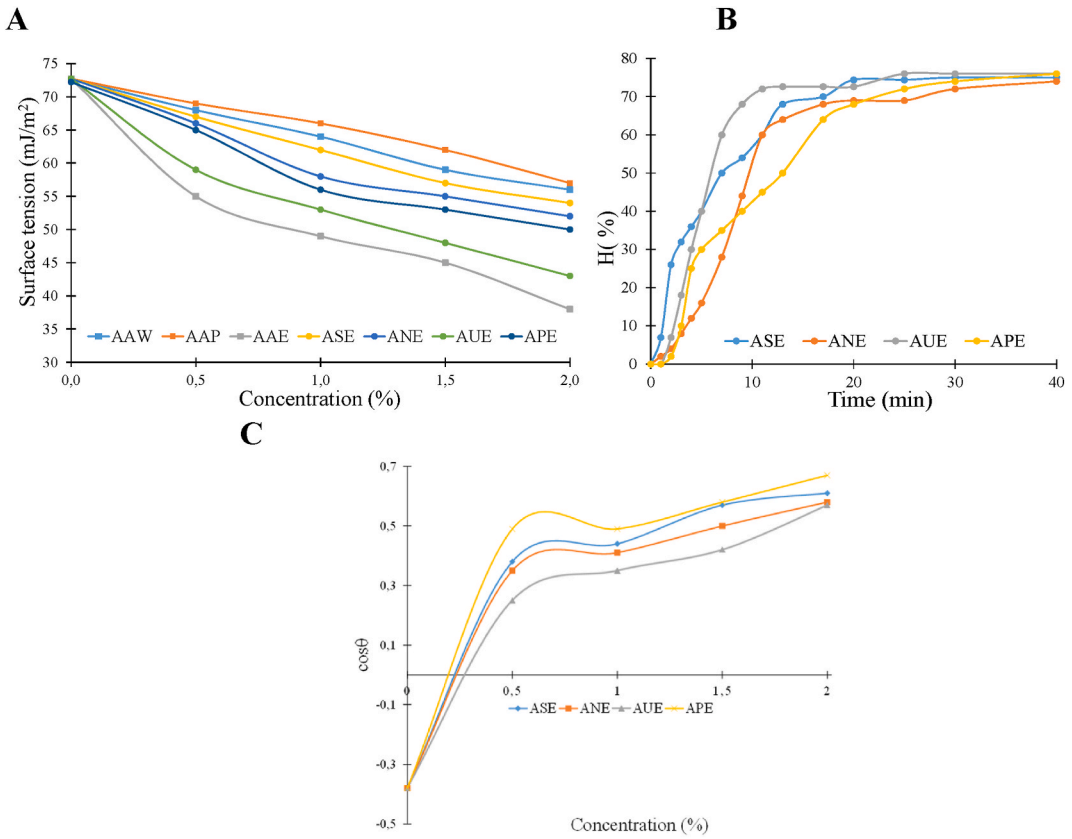


Fig. 4. (**A**) Surface tension isotherms of aqueous solutions of *Artemisia* hydrolates. (**B**) Kinetic curves of emulsion separation. (**C**) Wetting isotherms of aqueous solutions of *Artemisia* hydrolates.

Table 9
Colloidal chemical properties of *Artemisia* hydrolates, including critical surface tension, work of adhesion, pH and emulsion "lifetime".

Hydrolate	Critical surface tension σ_{cr}, mJ/m^2	Work of adhesion W_{max}, mJ/m^2	pH	Emulsion "lifetime", min
AAW	38.0	82.75	6.12	5
AAP	37.5	85.48	5.21	10
AAE	32.0	74.55	5.33	7
ASE	42.4	107.69	6.57	3
ANE	42.5	134.65	6.04	25
AUE	38.2	124.81	7.08	10
APE	37.3	264.07	6.45	8

surface tension of water provides a thermodynamic factor for the stability of dispersed systems, which include cosmetics [5]. Therefore, the surface tension of aqueous solutions of hydrolates AAW, AAP, AAE, ASE, ANE, AUE, APE at the liquid-gas interface was measured at concentrations of 0.5, 1.0, 1.5, 2.0 percent (wt.) and obtained surface tension isotherms $\sigma = f(c)$, presented in Fig. 4A.

The obtained isotherms have a classic appearance; with increasing concentration of hydrolates, the surface tension decreases, which indicates the presence of surface-active components in the hydrolates. Based on surface tension isotherms, it is clear that hydrolates AAE and AUE have greater surface activity, which reduce the σ of water to 40–50 mJ/m^2. The remaining hydrolates slightly reduce the surface tension of water. To increase surface activity in the future, it is necessary to introduce surfactants and study the effect of surfactants on the surface properties of hydrolates.

For the practical use of hydrolates in the compositions of cosmetic detergents and cleansers, it is important to study their wetting ability. In this regard, at the next stage, the contact angles of wetting of aqueous solutions of hydrolates on a Teflon (polytetrafluoroethylene), which was used as hydrophobic surface, were measured. The wetting isotherms presented in Fig. 4C show that all hydrolates, even in small concentrations, have good hydrophilizing ability.

Also, from the dependences $\cos\theta = f(\sigma)$, the values of the critical surface tension (Table 9) at which complete hydrophilization of the Teflon surface is achieved are determined. The results of calculating the work of adhesion, presented in Table 9, show that APE

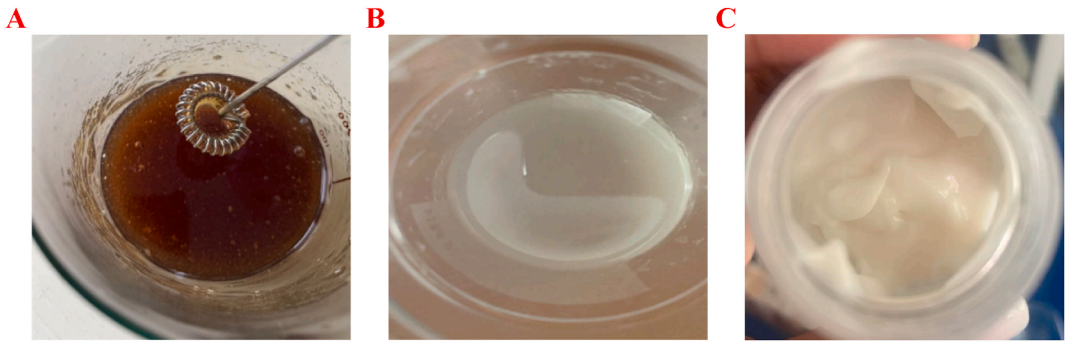


Fig. 5. **(A)** Process of obtaining cosmetic hydrogel on the base of *A. nitrosa* hydrolate. **(B)** Prepared cosmetic hydrogel. **(C)** Final cosmetic moisturizing cream based on ANE hydrolate.

solutions have the greatest adhesion, and, therefore, moisturizing properties. This circumstance indicates that they can be used in the formulation of moisturizers.

Various cosmetics, for example, creams, ointments, hydrophilic oils, etc., are developed on the basis of disperse systems called emulsions [68,69]. In this regard, in this work an attempt was made to obtain emulsions based on *Artemisia* hydrolates. In order to carry out this task, emulsions were created using various ratios of sunflower oil and water. It was found that the most stable emulsions were achieved with a volume phase ratio of 6:4. Kinetic separation curves of sunflower oil/hydrolate emulsions (6:4) for ASE, ANE, AUE, APE are presented in Fig. 4B. Similar dependencies were obtained for other studied hydrolates.

As the results obtained show, all the studied hydrolates have approximately the same emulsifying ability. From the kinetic separation curves of the resulting emulsions, the values of their "lifetime" were determined, which are presented in Table 9. From these values it can be seen that the most stable emulsions were obtained based on ANE hydrolate, the "lifetime" of which was 25 min. These results allow us to recommend the use of this hydrolate in creams and ointments.

One of the important parameters for the practical use of plant hydrolates is their pH. The measured pH values presented in the table show that hydrolates are slightly acidic (AAP and AAE) and neutral (ASE, ANE, AUE, APE and AAW), this makes it possible use of *Artemisia* hydrolates in cosmetics for various purposes.

In this work, an attempt was made to obtain a cosmetic product in the form of a concentrated emulsion based on *Artemisia* hydrolates. Fig. 5A shows a process of obtaining hydrogel, Fig. 5B represents photo of a prepared hydrogel, and Fig. 5C is a picture of the final moisturizing cream based on ANE *Artemisia* hydrolate. The oil base consists of carboxymethylcellulose (CMC) and glycerin, the aqueous phase is made of ANE *Artemisia* hydrolate, ethyl alcohol and distilled water. The process of obtaining such a product consists of mixing the components in certain proportions and homogenization.

4. Conclusion

Considering obtained results, five species of *Artemisia* plant (*Artemisia absinthium*, *Artemisia serotina*, *Artemisia nitrosa*, *Artemisia sublessingiana*, *Artemisia pauciflora*) were explored phytochemically for the authenticity and the presence of biologically active substances together with the mineral composition (macro-, microelements). In the conclusion of colloid-chemical studies of *Artemisia* hydrolates, determined that the *Artemisia* hydrolates consist of surface-active components with average surface activity (σ_{cr} were 32.0–42.5 mJ/m^2), have good wetting and adhesive properties, and therefore, moisturizing properties (W_{max} were 74.55–264.07 mJ/m^2). They have approximately the same emulsifying ability, slightly acidic and neutral medium (pH were 5.21–7.08). Following assessment *via* three distinct radical scavenging assays (DPPH, ORAC, and TBARS), determined that the *Artemisia* extracts exhibited notable antioxidant characteristics. Notably, *A. nitrosa* demonstrated effective inhibition of LDL oxidation in the TBARS assay (IC_{50} = 3.1 μM), prolonged the lag time of conjugated diene formation (>180 min), and significantly protected against LDL oxidation in the REM assay. The results obtained allow us to recommend these *Artemisia* species hydrolates in use as part of various cosmetic products – creams, gels, hydrophilic oils, etc.

CRediT authorship contribution statement

Ayaulym Minkayeva: Writing – review & editing, Methodology, Investigation. **Saltanat Kumargaliyeva:** Methodology, Investigation. **Orynkul Yessimova:** Writing – original draft, Supervision, Project administration, Methodology, Investigation. **Yasmina Ulfanova:** Methodology, Investigation. **Shang Xiaofei:** Methodology, Investigation. **Aizhamal Baiseitova:** Writing – review & editing, Visualization, Validation, Methodology, Investigation. **Janar Jenis:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Data availability statement

Supplementary material containing full gel image is available online. Data will be made available on request. For requesting data, please write to the corresponding author.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e42677.

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