

PRELIMINARY CHARACTERIZATION OF THE PHYTOEXTRACTS FROM *ACMELLA OLERACEA* WITH THERAPEUTIC POTENTIAL AND APPLICABILITY IN ACTIVE COSMETICS

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Abstract: The use of plant extracts in dermatocosmetic preparations, instead of chemical synthesis products, is a trend that is becoming more prominent in an effort to meet customer needs and guarantee product quality. In this context, the aim of the work is to present the obtaining and characterization of the extract of *Acmella oleracea*, by classical or combined liquid-solid extraction methods. Their physical-chemical characterization was based on the determination of the content of polyphenols and flavonoids and their antioxidant activity. The emulsion based on *Acmella oleracea* extract was preliminarily characterized in terms of stability and homogeneity.

Keywords: *Acmella oleracea*, cosmetic emulsion, hydro-alcoholic vegetal extract, polyphenol determination, solid-liquid extraction

INTRODUCTION

Among the plants with a rich history in traditional medicine is *Acmella oleracea*. Its recognition as a plant with valuable benefits in internal and external ailments has long been widespread throughout South America and Asia, of which the indigenous flora is a part. Due to the remarkable results in numerous diseases, it attracted the attention of the academic world, therefore *Acmella oleracea* is today a subject of study treated with great seriousness. The results of scientific studies led to its cultivation today in cultures in Eastern Europe, far from the native areas.

In traditional medicine, *Acmella oleracea* is successfully used as: anti-inflammatory, antitumor, antifungal and anthelmintic, analgesic, antipyretic, antioxidant, insecticide, antiseptic, immune stimulator, anti-obesity, antispasmodic, antidiuretic, even as aphrodisiac, sialagogue, wound repair, anti-rheumatic, tonic, antimalarial, hepatic protector with good tolerability [1 – 4].

Studies have shown a high content of bioactive compounds, among which spilanthol is the most abundant and exerts various pharmacological activities [3].

Acmella oleracea can be considered a good natural source for natural bioactive ingredients, with applicability in dermatocosmetics. Its antioxidant, anti-inflammatory, antiseptic and even anesthetic and anti-wrinkle properties are important strengths to be used in dermatocosmetic formulation to obtain preparations designed to protect the skin from the action of free radicals that generate oxidative stress, but also to combat its effects on the epidermis.

An essential step can benefit with the *Acmella oleracea* properties is to obtain the best quality extracts, by using different variants of solid-liquid extraction, such as maceration, reflux in Soxhlet, extraction with supercritical fluids, ultrasounds assisted extraction, microwave-assisted extraction, extraction with eutectic solvents. [5 – 9]. Working parameters (such as: extraction time, ratio between solid/liquid phases, type and concentration of the extraction solvent) can significantly influence both the extraction yield and content in bioactive substances.

The purpose of this paper is to establish the conditions of maceration (M), refluxing (R), ultrasonic (US) assisted extraction (also named sonoextraction) and sonoextraction combined with maceration (USM) for *Acmella oleracea* in order to obtain an hydroalcoholic extract used in the formulation of new preparations with phytotherapeutic action [10 – 14]. In this context, the studies focused mainly on determining the factors that influence the extraction in the case of using the aerial parts of the *Acmella oleracea* plant and on their preliminary characterization and secondly on the preparation and preliminary characterization of a dermatocosmetic formula based on the extract of *Acmella oleracea*.

MATERIALS AND METHODS

Plant

The *Acmella oleracea* plants is originate from one of our own's culture, situated in Popricani village, Iasi County, Romania. After harvesting the entire plants, the aerial parts were separated from the roots and placed in cool spaces away from direct sunlight

to dry. After 6 - 7 weeks, the degree of drying was checked and the dry plant was placed in brown glass containers to be protected from moisture and UV rays until use.

All other reagents (Foling Ciocalteu, AlCl_3 , ethanol, methanol) or reference standards (gallic acid, quercetin and reactive for antioxidant activity determination) that were used were of analytical quality (Sigma Aldrich and Merck Co.).

Extraction methods

Maceration at room temperature of 20 °C (M) was preliminarily used as a solid-liquid extraction method to determine which part of the *Acmella oleracea* plant, roots or aerial parts, is richer to give a better yields in extractible. The study was continued with three other liquid-solid extraction methods: heat reflux (R), ultrasound-assisted extraction (sonoextraction - US) and a combined method: ultrasound-assisted extraction + maceration (USM) to obtain vegetal extracts from the *Acmella oleracea* plant with the highest content of active compounds, respecting the previously established protocol [15]. The extraction agent was 96 % ethyl alcohol (approved by the cosmetics and dermatocosmetics industry), used as an aqueous solution at three different concentrations: 30 %, 60 % and 75 %. Variables studied for their effects on the extraction process include extraction time, S/L ratio and concentration of extraction agent.

The efficiency of the extraction process carried out under different conditions was assessed by calculating the extraction yield (Eq.1). For this sample of 5 mL from each vegetal extract was evaporated to dryness at constant temperature up to 50 °C using a thermostatic oven.

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

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.

Quantitative characterization of vegetal extracts

The analysis of the plant extracts focused on two types of compounds defining the antioxidant activity, based on a protocol similar to the one used in our previous works [16]:

(1) Total polyphenol content (TPC), as assessed by the Folin-Ciocalteu method. The results, accounting for sample dilution, were represented as μg of gallic acid equivalent (GAE) per mL extract ($\mu\text{g GAE} \cdot \text{mL}^{-1}$). The analyses were carried out twice. Using the same standard methods, the standard calibration curve was generated for various gallic acid concentrations.

(2) The amount of flavonoids (TFC) was ascertained by employing a spectrophotometer-based technique with a 2 % AlCl_3 solution prepared in methanol [15]. The findings, accounting for sample dilution, were reported as mg or mg of quercetin equivalent (QE) per mL extract ($\text{mg QE} \cdot \text{mL}^{-1}$).

Antioxidant assays

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The assay was conducted in accordance with a previously described method [17]. Briefly, 50 μL of each extract was mixed with 150 μL solution of 0.004 % 2,2-diphenyl-1-picrylhydrazyl prepared in methanol. Following a 30 min incubation period at room temperature in dark, the absorbance was measured at 517 nm. DPPH radical scavenging activity was calculated as milligrams of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) equivalents (mg TE/mL extract). Results were reported as the mean \pm standard deviation (SD) of three independent determinations.

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

The assay followed a methodology previously described by Turcov et al. [17]. Initially, $\text{ABTS}^{\bullet+}$ was generated by combining a 7 mM solution of ABTS with 2.45 mM potassium persulfate in a 1:1 (v/v) ratio. This mixture was allowed to incubate in dark at room temperature for 12–16 hours. Prior to the assay, the ABTS solution was diluted with methanol until reaching an absorbance of 0.700 ± 0.02 at 734 nm. Subsequently, 30 μL of the sample was mixed with 200 μL ABTS solution. Following a 30 min incubation period at room temperature, the absorbance was measured at 734 nm. The ABTS radical scavenging activity was calculated as milligrams of Trolox equivalents (mg TE/mL extract). Results were reported as the mean \pm standard deviation (SD) of three independent determinations.

Formulation of an emulsion based on *Acmella oleracea* extract

The extract with the best results in terms of polyphenols content was used to obtain two O/W emulsions formulas which differed in the extract concentration added as active with phytochemical properties to protect the skin from oxidative stress. The composition of the emulsion base is briefly presented in Table 1.

Table 1. The composition of the used emulsion base

Phase/Compounds - Name of INCD	%	Role in the formulation
<i>Lipophilic phase</i>		
<i>Amaranthus Spinosus</i> Seed Oil	5	Emollient
<i>Psoralea corylifolia</i> Seed Oil	5	Emollient
<i>Malus domestica</i> Seed Oil	5	Emollient
<i>Solanum Lycopersicum</i> Seed Oil	5	Emollient
Cetearyl Alcohol (and) Glyceryl Stearate (and) Jojoba Esters (and) <i>Helianthus Annuus</i> (Sunflower) Seed Wax (and) Sodium Stearoyl Glutamate (and) Water (and) Polyglycerin-3	5	Emulsifiers
<i>Hydrophilic phase</i>		
<i>Acmella oleracea</i> Flower Water	71.7	Solvent
Glycerin	2	Conditioning agent
<i>Additives</i>		
Lecithin, Sclerotium Gum, Pullulan, Xanthan Gum	0.3	Viscosity agent
<i>Preservatives</i>		
Benzyl Alcohol, Dehydroacetic Acid	1	Preserved

The final emulsion is an O/W (oil in water) emulsion. After the aqueous phase has been previously gelled with the viscosity agent, both the hydrophilic and lipophilic phases have been heated to a temperature of 75 °C. The lipophilic phase was added over the hydrophilic phase under continuous mixing using a Dynamix® DMX combi 160 homogenizer operating at 13,000 rpm. After 3 shaking cycles of 3 minutes each, the emulsion was cooled on a water bath at a temperature of 40 °C, after which the active ingredients and the preservative were added. Weighed samples (15 g) were placed in brown glass containers for use in future emulsion research. The samples were stored in cold chambers (10 °C) for a maximum of ninety days or until the analysis was completed. The samples were allowed to reach the room temperature before any analysis.

Preliminary evaluation of cosmetic emulsions containing *Acmella oleracea* extracts

The physical analyses listed below were carried out: assessment of conductivity, analysis of microscopic pictures, sensory evaluation (odor, color, texture, and overall aspect), centrifuge and vortex tests, and pH determination [18, 19].

pH Determination

A digital pH meter (Hanna Instrument) was used to measure the pH values of dermato-cosmetic emulsions. The procedure involved dissolving 0.5 g of emulsion in 50 mL of distilled water and letting the mixture sit for two hours. By directly putting the electrode into the sample solution at 24.0 ± 2.0 °C, the pH value was determined. After being diluted with distilled water for a pH test, the emulsions maintained their homogeneity and milky appearance.

Phases Separation

Five grams of sample were used for the centrifugation test, and the centrifuge model XC-Spinplus was operated for thirty minutes at 25 °C and 3000 revolutions per minute. A Multi Speed Vortex MSV-3500 (Grant Instruments Ltd., Cambs, England) was utilized in the vortex test, along with a 5 g sample of emulsion and 30 minutes at 25 °C and 3000 rpm.

Conductivity Measurements

The conductivity tests were carried out on emulsion samples that had been kept at room temperature for 20 days, using a portable Hanna Instruments type conductometer.

Microscopic Images

Using a binocular microscope (Optika B-159, OPTIKA S.r.l., Ponteranica (BG) - Italy), magnification - 1000x, microscopic pictures were captured from samples of the emulsions prepared after seven days of storage under normal temperature circumstances.

RESULTS AND DISCUSSION

To obtain plant extracts from *Acmella oleracea* using solid-liquid extraction, the protocol in Figure 1 was followed. The experimental study followed the influence of certain physical parameters (type and concentration of the extraction solvent, solid/liquid ratio, extraction time), on the extraction yield, but also on the amount of determined bioactive compounds.

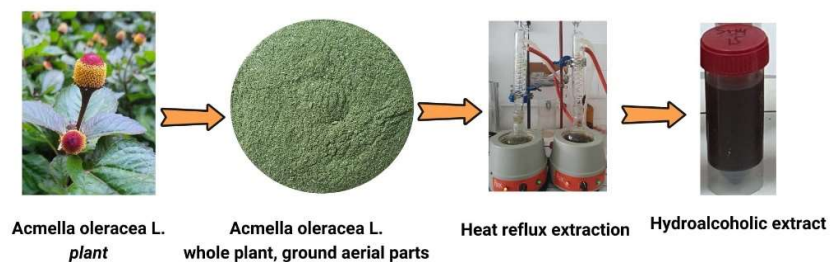


Figure 1. Schematization of the process of obtaining and characterizing *Acmella oleracea* extracts

To determine which part of the plant is richer in the targeted bioactive compounds, maceration was used as a test extraction method, and the roots, leaf, stem and flowers of *Acmella oleracea* were used separately. These parts of plant were crushed separately and sieved using a food mill to obtain particle size between 2 - 5 mm. An aqueous solution with concentration of 60 % ethanol, a solid/liquid (S/L) ratio of 1:5 and temperature of $20^0 \pm 2$ °C were selected as conditions of this extraction process. The efficiency of the method was evaluated based on the yield of the extraction, calculated with Eq.1, after processing the samples of the plant extracts by dry evaporation at a temperature of 50 °C in a thermostatic oven. The obtained results are listed in Table 2.

Table 2. The obtained extraction degree by maceration extraction method (S/L = 1/5)

Maceration time	Alcohol concentration	Extraction yield [%]	
		Part of plant	
		Aerian part	Root
7 days	60 %	22.375	15.875
14 days	60 %	23.625	19.125
30 days	60 %	47.75	23.75
60 days	60 %	21.5	24.5
90 days	60 %	24.5	19.5

The results in Table 2, expressed in the form of yield of maceration, demonstrate that the use of the aerial part of the plant *Acmella oleracea* for extraction is much more efficient than the use of the root. Also, maceration for 30 days is the most effective option for the intended purpose. In this context, the subsequent study will consider only the extraction from the aerial part.

Evaluation of extraction methods depending on the extraction yield

The extraction efficiency by heat reflux (R), sonoextraction (US) and combined method sonoextraction with maceration (USM) was evaluated under the established working methodology and extraction conditions. To calculate the yield of the process, 5 mL samples of each extract were subjected to dry drying in a thermostatic oven (at the constant temperature of 50 °C). The efficiency of the method was evaluated based on the yield of the process, calculated with Eq.1. The results are presented in Table 3.

Table 3. The Realized Extraction Degree by Liquid-Solid Extraction Methods

Extraction Method	Sample	Characteristics			Extraction degree [%]
		Time	Ratio S:L	Solvent concentration [%]	
Heat Reflux (R)	R1	60 min	1:5	30	38.84
	R2			60	32.12
	R3			75	27.87
	R4	60 min	1:15	30	40.08
	R5			60	36.75
	R6			75	32.25
	R7	60 min	1:20	30	40.90
	R8			60	38.00
	R9			75	38.50
	R10	90 min	1:15	30	32.59
	R11	120 min	1:15	30	46.09
Ultrasound Assisted Extraction (US)	US1	5 min	1:5	30	29.75
	US2			60	22.12
	US3			75	16.25
	US4	5 min	1:15	30	31.12
	US5			60	25.50
	US6			75	20.62
	US7	5 min	1:20	30	26.50
	US8			60	22.00
	US9			75	18.00
	US10	10 min	1:15	60	21.37
	US11	15 min	1:15	60	17.25
Ultrasound Assisted Extraction + Maceration (USM)	USM1	5 min+	1:5	30	35.25
	USM2			60	30.00
	USM3			75	26.50
	USM4	5 min+	1:15	30	38.62
	USM5			60	35.62
	USM6			75	30.00
	USM7	5 min + 7days	1:20	30	40.00
	USM8			60	35.50
	USM9			75	32.50
	USM10	10min + 7 days	1:15	60	33.75
	USM11	15min+ 7 days	1:15	60	34.87

The analysis of the data in Table 3 led to the conclusion that the extraction process depends on the type of extraction method and operating conditions (extraction time, concentration of the extraction solvent, solid/liquid ratio). It is also very easy to see that the solvent with the best results, regardless of the extraction technique but also according to the criterion of economic efficiency, turns out to be 30 % ethanol, and the most convenient solid/liquid ratio seems to be 1/15 and 1/20 respectively, depending on the extraction method.

Analyzing the values of the yields obtained in the case of the extraction methods used, it is observed that the highest values were obtained in the case of reflux extraction, the order of efficiency of the extraction techniques used being: $R > USM > US$.

In the case of the same extraction technique, the dependence of the extraction yield on the concentration of the solvent used for the extraction, the extraction time, and the solid/liquid ratio used is obvious, thus:

- in the case of reflux (R), maximum yields are recorded between 46.09 % (S/L conditions = 1:15; 120 min; solvent 30 %) and 40.09 % (S/L conditions = 1:20; 60 min; solvent 30 %);
- using ultrasound-assisted extraction (US), the following maximum yields were obtained: 31.12 % (S/L conditions = 1:15; 5 min; solvent 30 %) and 29.75 % (S/L conditions = 1:5; 5 minutes; solvent 30 %);
- when using the combined method ultrasound-assisted extraction + maceration (USM), it is observed that the yields obtained are higher than those achieved in the case of the use of ultrasound-assisted extraction, which proves that maceration contributed to an increase in extraction yields. Maximum yields obtained were between 40.00 % (S/L conditions = 1:20; 5 min+7 days; solvent 30 %) and 38.62 % (S/L conditions = 1:15; 5 min+7 days; solvent 30 %).

From the point of view of the extraction yield obtained, the conclusion is that the best results were obtained by extraction with reflux in conditions of S/L = 1:15; for 120 min; using a solvent of 30 % concentration (46.09 %), followed also by refluxing (40.09 %) under conditions of S/L = 1:20; for 60 min; using a solvent of 30 % concentration and the combined method: sonoextraction + maceration (40.00 %) under conditions of S/L = 1:20; extraction time of 5 min+7 days; using a 30 % concentration solvent.

Quantitative characterization of hydroalcoholic vegetal extracts

After filtration and centrifugation, the obtained extracts it was proceeded to the quantitative determination of the total content of polyphenols [20, 21] and flavonoids [16], represented as μg of gallic acid equivalent (GAE) per mL ($\mu\text{g GAE}\cdot\text{mL}^{-1}$) and mg of quercetin equivalent (QE) per mL ($\text{mg QE}\cdot\text{mL}^{-1}$), respectively. The results of these research, carried out in duplicate, were dependent on the extraction technique and the working parameters and are presented in Figures 2(a-c) and Figures 3(a-c).

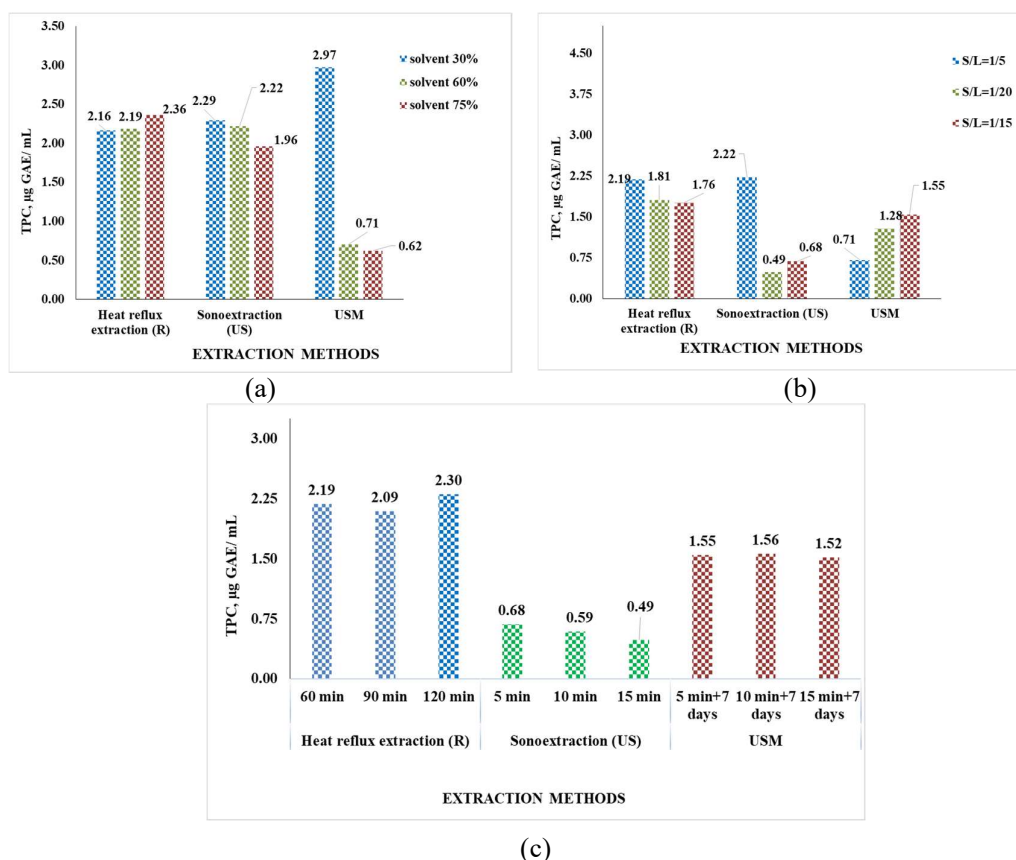


Figure 2. The total polyphenol content (TPC) in $\mu\text{g GAE}\cdot\text{g}^{-1}$ determined comparatively for the extraction methods used depending on the physical parameters considered.

Conditions: (a) S/L= 1:5; R-60 min., US- 5 min., USM - 5 min+7 days;

(b) solvent concentration = 60 %; R-60 min., US- 5 min., USM - 5 min+7 days;

(c) solvent concentration = 60 %; S/L(R)- 1:5., S/L(US)- 1:15, S/L(USM)- 1:15

In the case of polyphenols (Figure 2), depending on the concentration of the extraction solvent (Figure 2a), the best results were obtained following the USM method - $2.97 \mu\text{g GAE}\cdot\text{g}^{-1}$ (30 %), followed by US with $2.29 \mu\text{g GAE}\cdot\text{g}^{-1}$ (30 %), $2.22 \mu\text{g GAE}\cdot\text{g}^{-1}$ (60 %), and reflux extraction (R): $2.36 \mu\text{g GAE}\cdot\text{g}^{-1}$ (75 %), $2.19 \mu\text{g GAE}\cdot\text{g}^{-1}$ (60 %) and $2.16 \mu\text{g GAE}\cdot\text{g}^{-1}$ (30 %).

If the evaluation is done according to the S/L ratio (Figure 2b), the best results were recorded in the case of (R) in the case of all three concentrations, respectively $2.19 \mu\text{g GAE}\cdot\text{g}^{-1}$ (1/5), $1.81 \mu\text{g GAE}\cdot\text{g}^{-1}$ (1/20) and $1.76 \mu\text{g GAE}\cdot\text{g}^{-1}$ (1/15) followed by US - $2.22 \mu\text{g GAE}\cdot\text{g}^{-1}$ (1/5).

Considering the influence of extraction time (Figure 2c), under the selected conditions, the best values were obtained by extraction by reflux (R).

In conclusion, when we consider the content of polyphenols the best results were recorded in the case of the USM method applied under the following conditions: S/L ratio of 1:5, solvent concentration 30 % and time 5min + 7 days ($2.97 \mu\text{g GAE}\cdot\text{g}^{-1}$). This method is followed by reflux (R) carried out under conditions of S/L ratio of 1:5, solvent concentration 75 % and time of 60 min. ($2.36 \mu\text{g GAE}\cdot\text{g}^{-1}$).

In the case of flavonoids (Figure 3), depending on the concentration of the extraction solvent (Figure 3a), the best results were obtained following the combined method (USM) - $13.77 \text{ mg QE} \cdot \text{g}^{-1}$ (60 %; S/L = 1:5; 5 min + 7 days), followed by reflux (R) with $12.35 \text{ mg QE} \cdot \text{g}^{-1}$ (30 %; S/L = 1:5, 60 min.).

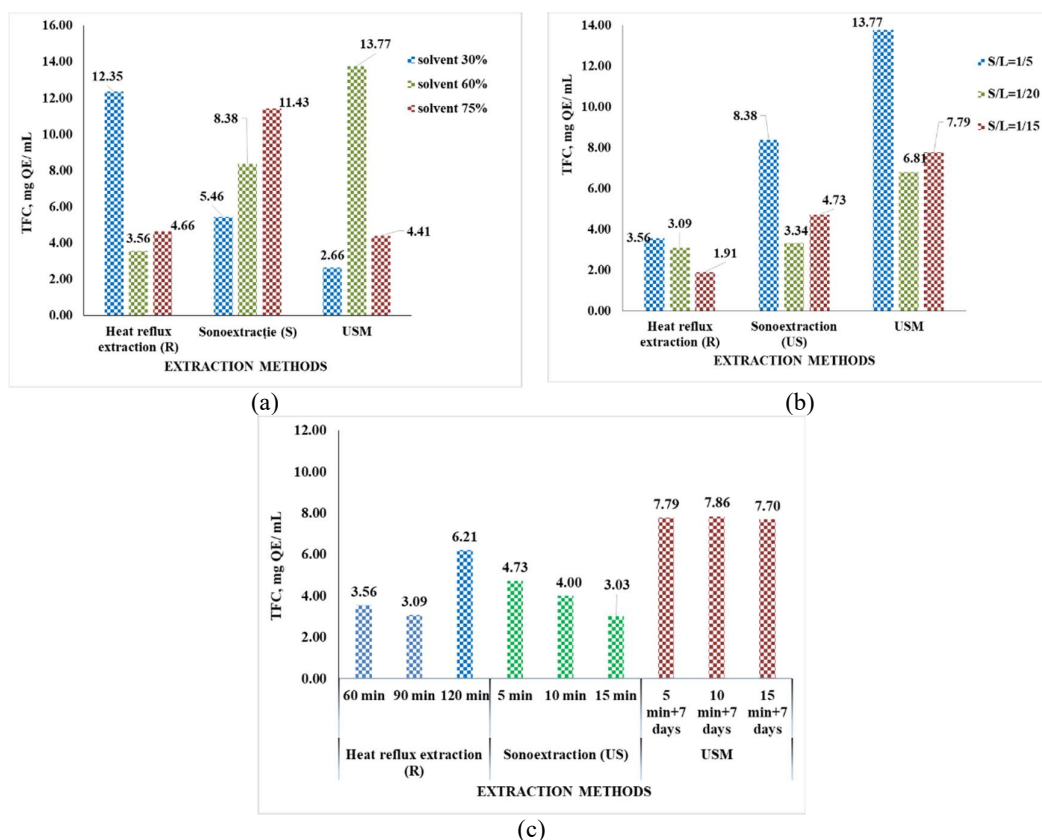


Figure 3. The flavonoids content (TPC) in $\text{mg QE} \cdot \text{g}^{-1}$ determined comparatively for the extraction methods used depending on the physical parameters considered. Conditions: (a) S/L = 1:5; R-60 min., US- 5 min., USM - 5 min+7 days; (b) solvent concentration = 60 %; R-60 min., US- 5 min., USM - 5 min+7 days; (c) solvent concentration = 60 %; S/L(R)- 1:5., S/L(US)- 1:15, S/L(USM)- 1:15

If the evaluation is done according to the S/L ratio (Figure 3b), the best results were recorded in the case of the S/L=1:15 ratio in the case of USM method - $13.77 \text{ mg QE} \cdot \text{g}^{-1}$ (60 %) followed by the US method - $8.38 \text{ mg QE} \cdot \text{g}^{-1}$ (60 %). The positive contribution of maceration on the increase in the amount of flavonoids extracted after performing the extraction by sonoextraction is observed.

Depending on the extraction time (Figure 3c), in the selected conditions, the best values were obtained by the extraction by USM.

In conclusion, the best results in terms of flavonoid content were recorded in the case of the extracts obtained by USM applied under conditions of S/L ratio of 1:15, solvent concentration 60 % and time of 5 min +7 days ($13.77 \text{ mg QE} \cdot \text{g}^{-1}$). This method is followed by the R method carried out under conditions of S/L ratio of 1:5, solvent concentration 30 % and time of 60 min. ($12.35 \text{ mg QE} \cdot \text{g}^{-1}$).

Antioxidant assays

The antioxidant activity was determined for a series of extracts, selected according to the yield obtained and the content of polyphenols and flavonoids obtained. Results were reported in Table 4 as the mean \pm standard deviation (SD) of three independent determinations.

Table 4. DPPH and ABTS radicals scavenging activity of investigated extracts

Sample	DPPH [mg TE·mL ⁻¹]	ABTS [mg TE·mL ⁻¹]
R8	0.81 \pm 0.06	2.36 \pm 0.06
US10	0.53 \pm 0.02	2.22 \pm 0.05
USM7	0.45 \pm 0.03	2.43 \pm 0.03

Results are expressed as mean \pm standard deviation (SD) of three determinations. Legend: ABTS - 2,2'-azino-bis (3-ethylbenzothiazoline) 6-sulfonic acid; DPPH - 1,1-diphenyl-2-picrylhydrazyl; TE - Trolox equivalents.

It can be seen from Table 4 that the antioxidant activity is correlated with the content of polyphenols determined previously, respecting the order of the extraction methods established then.















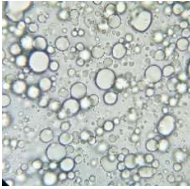
Preliminary analyzes for emulsions based on *Acmella oleracea* extract




Using the extract of *Acmella oleracea* obtained after extraction by maceration 7 days in following conditions: S/L = 1:5, time of extraction = 7 days, solvent: 30 % hydroalcoholic solution of ethanol, characterized by a total polyphenol content of 3.798 $\mu\text{g GAE}\cdot\text{g}^{-1}$ (not characterized in this work), we proceeded to obtain two types of emulsions using the same base. Their composition is presented in Table 1.

Placing a product on the market requires a series of analysis regarding product stability and storage and preservation conditions. Depending on the results obtained, the studies will be continued with *in vitro* and *in vivo* analyses.

Stability under various settings is a factor for evaluating dermatocosmetic preparation quality. In this regard, three physical evaluations were conducted to assess the stability of the resulting dermatocosmetic emulsions: once at room temperature, 24 hours after formulation, and once in the emulsion container. The results of the preliminary analysis carried out (pH and conductivity determinations, sensory analysis, the action of centrifugal and vibrational forces, obtaining microscopic images) are presented concisely in Table 6.

Table 6. Preliminary Characterization of Emulsions with *Acmella oleracea*

Parameters	Emulsions		
	Base emulsion	Formula 1 with 3 % active	Formula 2 whis 5 % active
Appearance of the emulsion after centrifugation			
	The sample is stable, homogeneous, compact		The sample is stable, a thin layer of foam is observed due to the air incorporated when mixing the actives
The appearance of the emulsion after the vortex test			
	Homogeneous compact texture and appearance		
Measuring pH			
	Initial emulsion pH value: 6.01	The pH value 5.86 is a compliant value, it does not require adjustment	The pH value 5.75 is a compliant value, it does not require adjustment
Organoleptic analysis			
	Compact, homogeneous texture. Characteristic, pleasant smell. Yellowish, glossy color, derived from the raw materials used		
Microscopic images after 24 hours of emulsion preparation storage 25 °C			
	Uniform particles of different sizes, without cremation, flocculation or sedimentation phenomena		

Conductivity measurement (mS) after 24 hours from emulsion preparation, storage at 25 °C			
	0.25, without phase separation phenomena	0.48 after adding the percentage of 3 % actives. The emulsion is stable	0.62 after adding 5 % actives. The emulsion is stable

The results presented in Table 6 show two stable products, corresponding to the continuation of the analyzes regarding the structure, rheology, microbiological behavior, diffusion through membranes similar to the structure of the skin, in vivo and in vitro analyses.

CONCLUSIONS

The combined method: ultrasound-assisted extraction + maceration (US+M) proved to be the most efficient of the solid-liquid extraction methods studied to obtain plant extracts from *Acmella oleracea*. Choosing the working parameters carefully leads to extracts rich in biologically active compounds with antioxidant action. Based on the working synergy of the compounds from the extracts, they were used in the preparation of phytocosmetic emulsions to combat / treat oxidative stress on the skin. These two emulsions based on *Acmella oleracea* extract were preliminarily characterized from the point of view of stability during preparation and storage, the results being positive and encouraging for further in-depth analysis *in vivo* and *in vitro*.

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