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OBTAINING AND CHARACTERIZATION OF PROPANEDIOL PHYTOEXTRACTS FROM *ACMELLA OLERACEA*

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ABSTRACT. Plant extracts are becoming an increasingly important trend in phytocosmetic preparations, replacing chemical synthesis products and ensuring the quality of products in line with consumer requirements. Using propanediol as an extraction solvent, this paper characterizes *Acmella oleracea* extracts obtained by traditional or combined extraction methods. The evaluation of their antioxidant activity and their content of polyphenols and flavonoids served as a basis for their physico-chemical characterization. Based on the best extract of *Acmella oleracea*, an emulsion was prepared and its stability and homogeneity were preliminary evaluated.

Keywords: *Acmella oleracea, antioxidant activity, oxidative stress, propanediol, vegetal extract*

INTRODUCTION

People's shift towards a healthy lifestyle and concern for the environment have led to a change in preferences and choice of personal care products, with phytocosmetics increasingly seen as healthy alternatives to conventional cosmetics.

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Biologically active molecules from plants are increasingly being targeted by scientists as raw materials in various industries, in cosmetics, pharmaceuticals, the food industry or in pest control. Plants are an inexhaustible source of biologically active molecules suitable for cosmetic products *Acmella oleracea* (L.) RK Jansen is a plant with great potential, belonging to the Asteraceae family and cultivated as a medicinal plant in South America, particularly in Mexico, Peru, Bolivia and Brazil, where it is known by various names: jambu, paracress or electric plant [1]. The genus *Acmella* comprises over 30 species, with *Acmella oleracea* being the most commonly cultivated species. It is distinguished from the other species by its cylindrical, disk-shaped flower heads of a golden yellow color with a red tip [2] (Figure 1). In the literature, the names of the genus and species of *Acmella oleracea* are often confused and the genus *Acmella* is often taxonomically confused with the genus *Spilanthes* [3].

Due to numerous revisions, the plant is sometimes referred to as *Acmella oleracea* (L.) R. K Jansen [4], *Spilanthes acmella* L. [5] or *Spilanthes oleracea* L. [6]. It is a medicinal plant, but also a food known above all for its local anesthetic effect, traditionally used in indigenous areas to treat toothache, which is why it has also been called the “toothache plant” or a substitute for pepper in various culinary preparations [7,8]. Although the plant has been known and used for centuries by the tribes of the Peruvian Amazon as an anesthetic for toothache, it was not until 1975 that Dr. Françoise Barbira-Freedman, an anthropologist at the College of Cambridge, brought the anesthetic effect of the plant, which she had discovered during studies on medicinal plants in the Amazon region, to the fore Amazonas, came to the fore [9]. In various systems of traditional medicine, *Acmella oleracea* is used as an analgesic, antibiotic, anticonvulsant, antidiuretic, antifungal, anti-inflammatory, antipyretic, stomach ulcer remedy, antiviral, antifungal, anthelmintic and insecticide [10]. The chemical composition of the plant has been extensively studied in the literature, reporting a high content of biologically active compounds of interest for cosmetic products, including N-alkylamides, vanillic acid, scopoletin, trans-ferulic acid, trans-isoferulic acid, stigmasterol and sitosterone [11].

The biological properties of the plant are mainly due to the content of N-alkylamides, a group of molecules found in the genera Spilanthes and Acmella, represented by the main component spilanthol, the primary metabolite responsible for the anesthetic effect [12].The biomedical interest in N-alkylamides (NAA) has grown enormously, as potential raw materials for various industrial sectors [13]. Spilanthol, the most important secondary metabolite of *Acmella oleracea* L, has aroused particular interest in the cosmetics industry due to its muscle-relaxing effect on the facial muscles and is regarded as a natural alternative to botulinum toxin [14].

In order to develop its effect, spilanthol must overcome the barrier function of the stratum corneum and penetrate deep into the skin. In a study, Veryser et al. [13] analyzed the transdermal pharmacokinetics of spilanthol using human skin in a Franz cell diffusion model. They showed that spilanthol, due to its lipophilicity and low molecular weight, readily penetrates the skin barrier, moves into the dermis and further into muscle tissue, suggesting that the rate of penetration of spilanthol through the epidermis is dependent on the delivery vehicle used. Furthermore, spilanthol is an enhancer of skin penetration of active ingredients from topical compositions [15]. In addition, Demarne and Passaro [14] have demonstrated that spilanthol has a reversible blocking effect on muscle contractions by blocking the activity of calcium and sodium-potassium channels in the cell membrane of neurons, a mechanism that delays the appearance of wrinkles.

Acmella oleracea (L.) R. K Jansen is usually native to tropical or subtropical regions as a perennial plant or in Mediterranean areas as an annual plant, but can be successfully acclimatized in temperate areas as an annual plant as it is sensitive to ice [16,17]. Recently, it was also acclimatized in Romania, in a small experimental culture in Iasi County, Popricani locality. The interest in this species lay in the wide range of biological activities on the skin described in the literature, including anti-wrinkle effect, antioxidant effect, antimicrobial effect and reduction of pigmentation spots. However, it is well known that the secondary metabolites of the same plant species vary according to the geographical area in which the plant grows and according to climatic and environmental conditions [18].

The purpose of this article is to investigate the antioxidant capacity of the extracts of *Acmella oleracea* grown in Romania in order to confirm the therapeutic value of the plant and to incorporate it as a biologically active substance in (dermato)cosmetic compositions. In this sense, the aim was to determine the optimal conditions for the application of the extraction methods by maceration (M), extraction by ultrasound (US - sonoextraction) and two combined methods: sonoextraction + maceration (USM) and maceration + sonoextraction (MUS) for obtaining extracts from the plant *Acmella oleracea*, using an aqueous propanediol solution as extraction agent and the aerial parts (stem, leaves, flowers) of the plant as solid phase.

RESULTS AND DISCUSSION

Using solid-liquid extraction under specific experimental conditions (type and concentration of extraction solvent, solid/liquid ratio, extraction time), the protocol shown in figure 1 was followed in order to obtain vegetal extracts from *Acmella oleracea*, a cultivated plant acclimatised in Iasi, Romania.

Figure 1. The steps involved in collecting and obtaining extracts from *Acmella oleracea.*

The efficiency of the extraction methods was evaluated according to the amount of polyphenols and flavonoids obtained, depending on the work methodology and the established extraction conditions.

After filtration and centrifugation, the extracts were stored in cool rooms (refrigerator at 2-5^{0}C) until further processing. The subsequent study aims to calculate the total content of polyphenols and flavonoids and determine the antioxidant activity of the extracts with the most representative content of polyphenols.

Determination of the total content of polyphenols

The total content of polyphenols was determined spectrophotometrically, based on the Foling-Ciocalteu method [19] following a protocol used in a previous work [20] and applied to all plant extracts obtained depending on the extraction conditions. The results obtained were expressed as mg of Gallic acid equivalent (GAE) per mL extract and are presented in Figures 2-5.

In the case of polyphenols, depending on the concentration of the extraction solvent (Figure 2), the best results were obtained through maceration (M)–3.39 mg GAE/mL (60%), also followed by maceration but with solvent of 40% concentration in propanediol with 2.74 mg GAE/mL (40%). Both variants were made using an S/L ratio of 1:5, an extraction time of 7 days.

If the assessment of the amount of extracted polyphenols is done according to the S/L ratio (Figures 3 and 4), it is observed that regardless of the concentration of the extraction reagent, the maceration (performed with a S/L ratio of 1:5 and a time of 7 days) is the method that provides the highest amounts of extracted polyphenols of 2.97 mg GAE/mL (60%), and respectively 2.74 mg GAE/mL (40%). It is followed by sonoextraction under S/L ratio conditions of 1: 5 and 5 minutes extraction time with a value of 1.77 mg GAE/mL (60%), respectively 1.71 mg GAE/mL (40%).

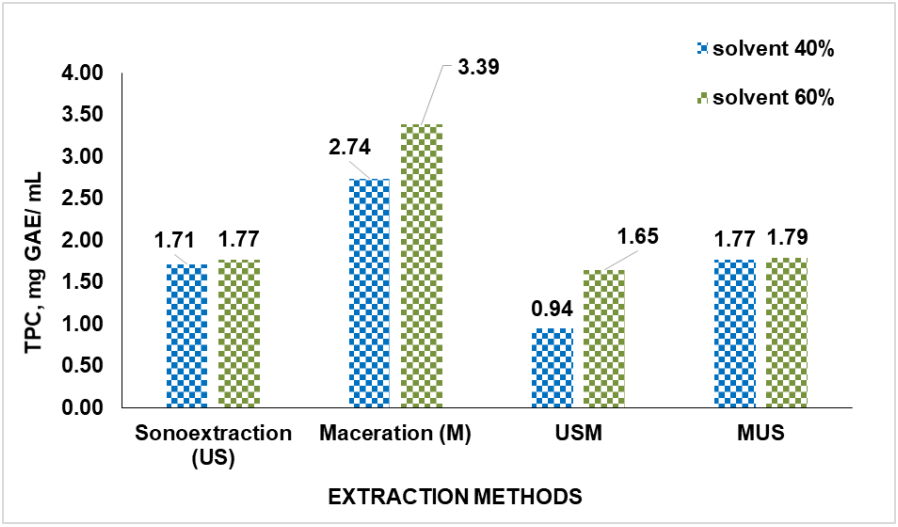


Figure 2. The total polyphenol content (TPC) in mg GAE/mL determined depending on the influence of solvent concentration. *Conditions*: (a) S/L= 1:5; M-7 days, US- 5 min., USM – 5 min+7 days; MUS : S/L= 1:20, 30 days +10 min.

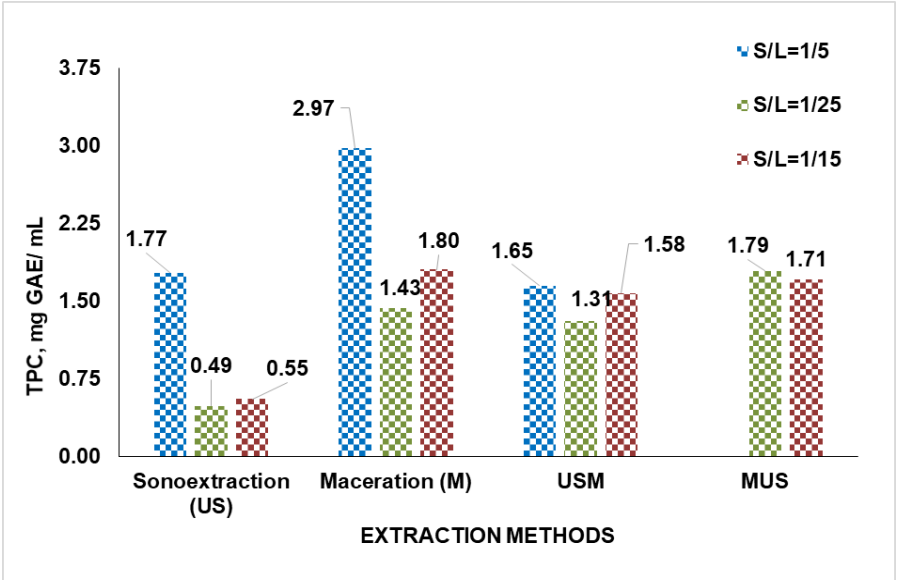


Figure 3. The total polyphenol content (TPC) in mg GAE/mL determined depending on the influence of ration solid (S)/liquid (L) when 60% propanediol was used as extraction agent . *Conditions*: M-7 days, US- 5 min., USM – 5 min+7 days; MUS- 30 days +10 min.

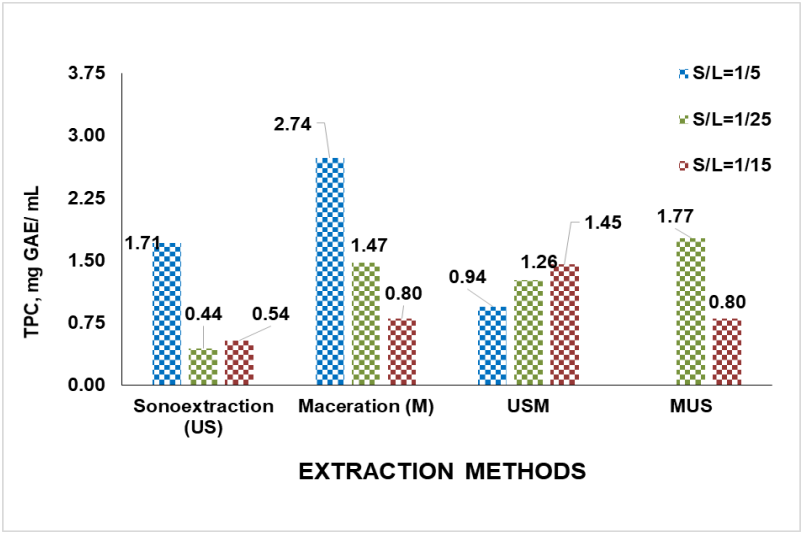


Figure 4. The total polyphenol content (TPC) in mg GAE/mL determined depending on the influence of ration solid (S)/liquid (L) when 40% propanediol was used as extraction agent . *Conditions*: M-7 days, US- 5 min., USM – 5 min+7 days; MUS- 30 days +10 min.

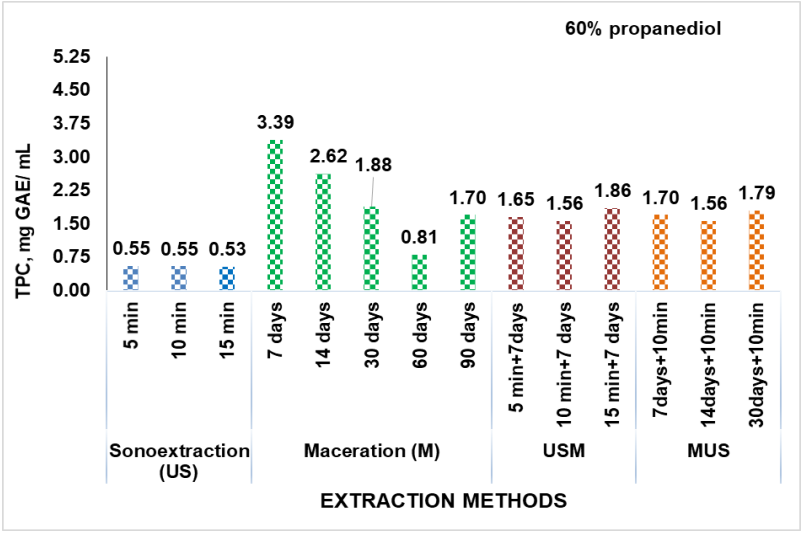


Figure 5. The total polyphenol content (TPC) in mg GAE/mL determined depending on the influence of extraction time, when 60% propanediol was used as extraction agent. *Conditions*: M, US, USM- S/L=1:5; MUS – S/L=1:20.

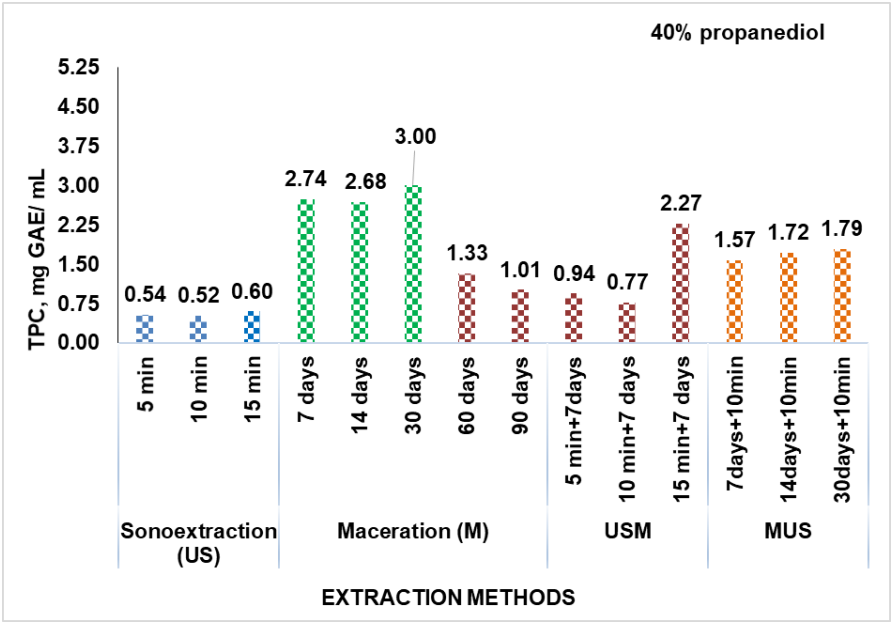


Figure 6. The total polyphenol content (TPC) in mg GAE/mL determined depending on the influence of extraction time, when 40% propanediol was used as extraction agent. *Conditions*: M, US, USM- S/L=1:5; MUS – S/L=1:20.

Analyzing the amount of polyphenols obtained according to the extraction time (Figures 5 and 6), the best values were obtained by maceration method (M) when working with an S/L ratio of 1:5, a time of extraction of 7 days and using as extraction solvent popanediol with 60% concentration.

The content of flavonoids (TFC) in all the plant extracts obtained was determined using the method involving treatment with a methanolic solution of 2% $AlCl_3$, following a protocol used in previous work [20]. The results obtained were expressed in mg quercitin equivalent (QE) per ml of extract and are shown in Figures 7-11.

The analysis of the amount of extracted flavonoids was similar to that of the polyphenols, as the factors influencing the extraction were the same. Thus, depending on the concentration of the extractant (Figure 37), the best results were obtained with the combined method: USM (11.91 mg QE/mL), under the working conditions: S/L=1:5, an extraction time of 5 min+7 days and 60% concentration of the extraction solvent propanediol, followed by the combined MUS method (10.73 mg QE/mL), performed under the working conditions: S/L=1:20, an extraction time of 30 days+10 min and an extraction solvent concentration of 40%.

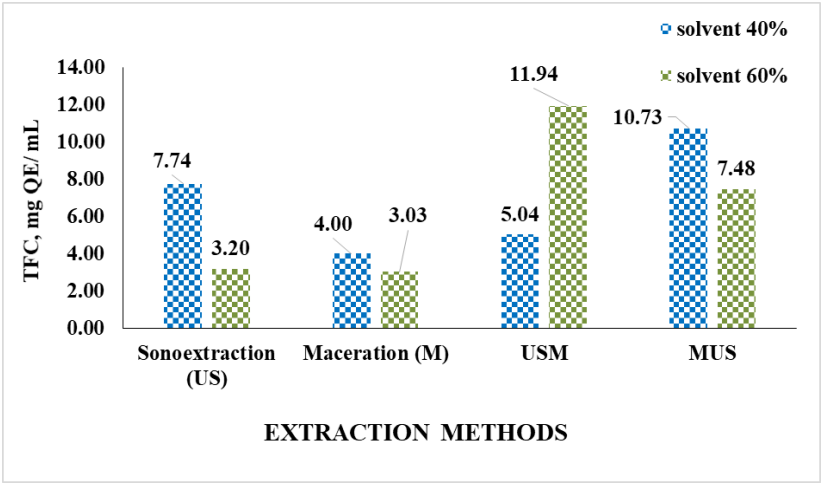


Figure 7. The flavonoids content (TFC) in mg QE/mL determined determined depending on the influence of solvent concentration.*Conditions*: S/L= 1:5; M-7 days, US- 5 min., USM – 5 min+7 days; MUS : S/L= 1:20, 30 days +10 min.

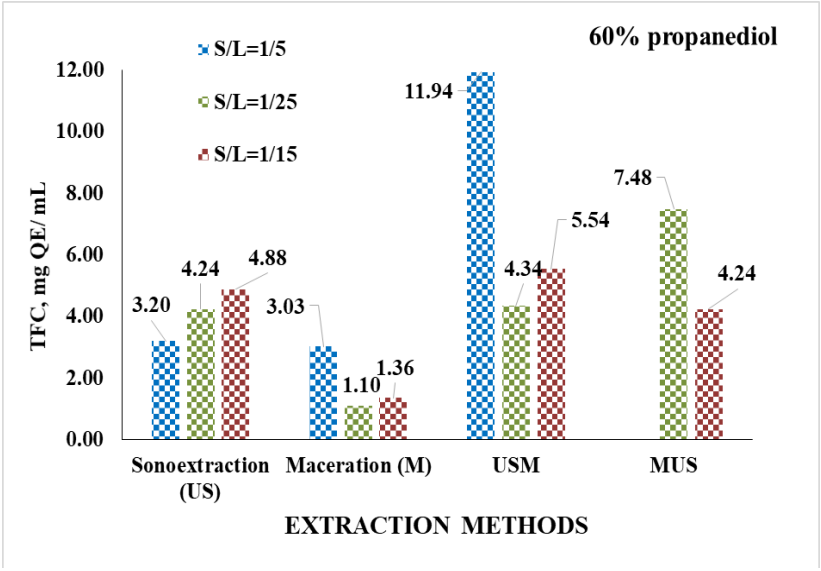


Figure 8. The flavonoids content (TFC) in mg QE/mL determined depending on the influence of ration solid (S)/liquid (L) when 60% propanediol was used as extraction agent. *Conditions*: M-7 days, US- 5 min., USM – 5 min.+7 days; MUS- 30 days +10 min.

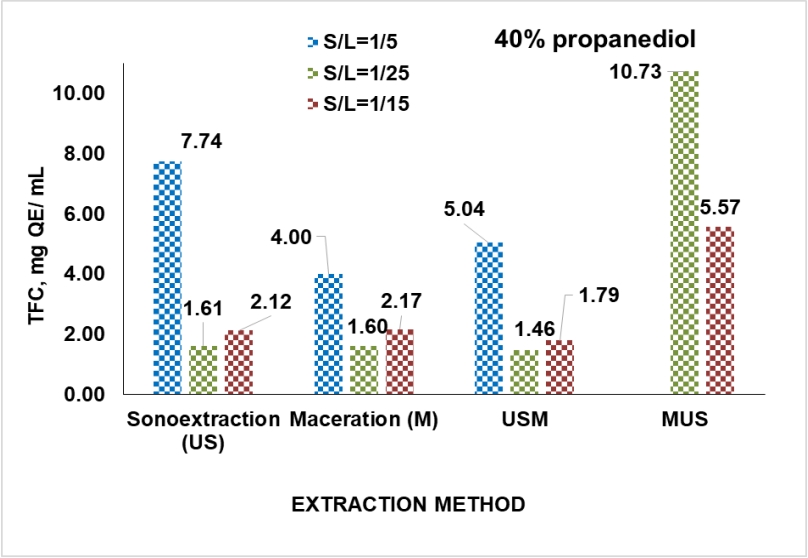


Figure 9. The flavonoids content (TFC) in mg QE/mL determined depending on the influence of ration solid (S)/liquid (L) when 40% propanediol was used as extraction agent. *Conditions*: M-7 days, US- 5 min., USM – 5 min+7 days; MUS- 30 days +10 min.

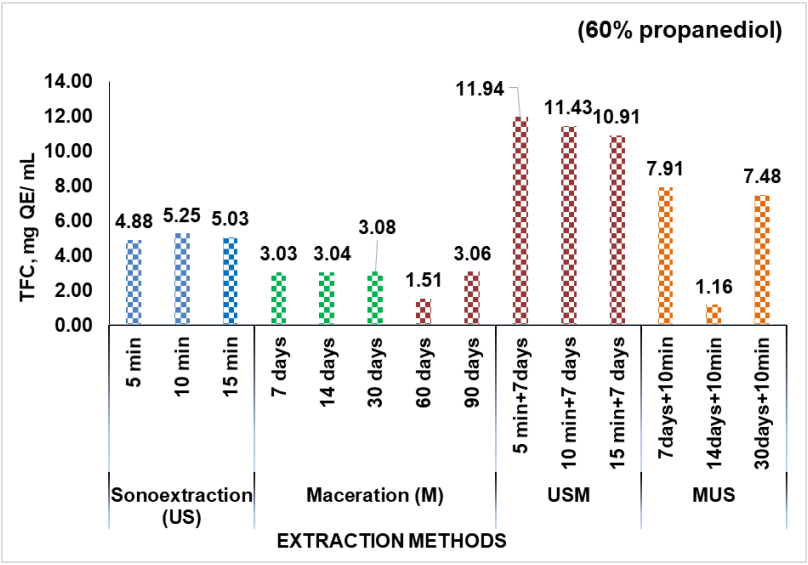


Figure 10. The flavonoids content (TFC) in mg QE/mL determined depending on the influence of extraction time, when 60% propanediol was used as extraction agent. *Conditions*: M, US, USM- S/L=1:5; MUS – S/L=1:20.

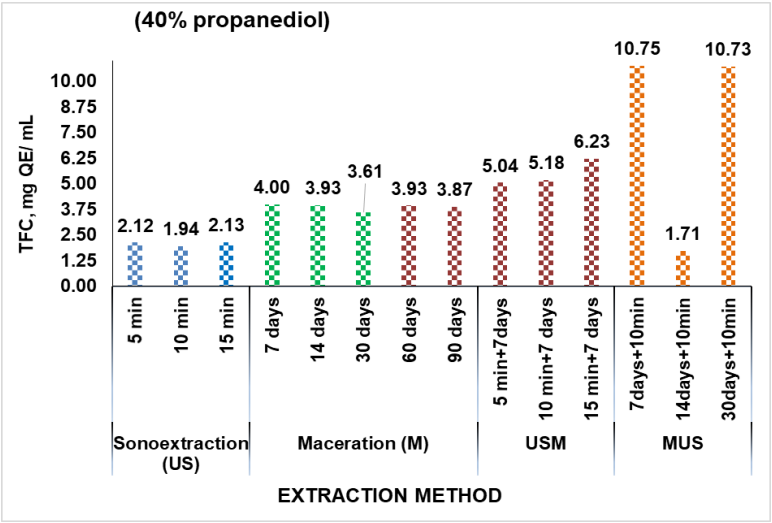


Figure 11. The flavonoids content (TFC) in mg QE/mL determined depending on the influence of extraction time, when 40% propanediol was used as extraction agent. *Conditions*: M, US, USM- S/L=1:5; MUS – S/L=1:20.

When analyzing the results according to the S/L ratio (Figures 8 and 9), it can be seen that the best results were obtained with the combined MUS method (10.73 mg QE/mL) under the conditions S/L=1:20; propanediol at 40% concentration; an extraction time of 30 days+10 min. were obtained, followed by US (7.74 mg QE/mL) under the conditions S/L=1:5; propanediol at 40% concentration; an extraction time of 5 min. and the combined USM method (7.48 mg QE/mL) under the conditions: S/L=1:15 propanediol with 60% concentration and an extraction time of 5min.+7 days).

If the amount of extracted flavonoids is analyzed as a function of the extraction time, the best results for both concentrations of propanediol are obtained with the combined methods as follows: (■) 11.94 mg QE/mL or 11.43 mg QE/mL in the case of USM performed under the following conditions: S/L = 1:5; 60% propanediol concentration and an extraction time of 5 min+7 days and 10 min+7 days, respectively; (■) 10.75 mg QE/mL and 10.73 mg QE/mL, respectively, in the case of the MUS method performed under the following conditions: S/L =1:20; 40% concentration of propanediol and an extraction time of 7 days + 10 min and 14 days + 10 min, respectively.

In conclusion:

- the best results in terms of polyphenol content were recorded in the case of the extracts obtained by maceration (3,386 mg GAE/mL) under working conditions of: S/L = 1:5, extraction time of 7 days and using 60% concentration popanediol as extraction solvent.

- the best results regarding the content of flavonoids were recorded in the case of the extracts resulting from the USM combined method (11.94 mg QE/mL) under conditions of S/L ratio of 1:5, an extraction time of 5 min. + 7 days and extraction solvent - popanediol with concentration 60%.

There are a number of studies in the specialized literature that stipulate that the use of organic solvents in the form of aqueous or alcoholic solutions to obtain plant extracts is much more beneficial than hydro alcoholic solutions [21-23]. One such organic solvent is propanediol, and recently attention is focused on the one of natural origin (propanediol 1,3).

Propanediol is increasingly used in cosmetic formulations as a texturing or humectant agent [24] or to stabilize and preserve the product [25]. Natural extracts in carrier solvents, such as glycerin or glycols, the most common being propylene glycol and 1,3 propanediol, are preferred to classic solvents, so that in the Coptis database, which includes over 15,000 cosmetic raw materials, one out of two extracts natural products sold on the market of cosmetic ingredients is diluted in glycerin or propanediol [26].

This type of extracts are much more combatable with the subsequent introduction in cosmetic formulations of the Oil in Water (O/W) or Water in Oil (W/O) type.

Evaluation of antioxidant activity

The results obtained were reported as mean ± standard deviation (SD) of three independent determinations in Table 1.

Table 1. DPPH and ABTS radicals scavenging activity of investigated extracts

Sample	DPPH (mg TE/mL)	ABTS (mg TE/mL)	TPC (mg GAE/ml)
M (S/L=1:5; propanediol 60%, extraction time of 7 days)	0.56 ± 0.03	2.18 ± 0.04	3.386
US (S/L=1:5; propanediol 60%, extraction time of 5 min.)	0.53 ± 0.02	2.22 ± 0.05	1.773
USM (S/L=1:5; propanediol 40%, extraction time of 15`+7days)	0.15 ± 0.04	2.05 ± 0.10	2.267
MUS (S/L=1:20; propanediol 60%, extraction time of 30 days+10 min.)	0.95 ± 0.01	2.41 ± 0.02	1.791

Legend: ABTS - 2,2′-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid; DPPH - 1,1-diphenyl-2-picrylhydrazyl; TE - Trolox equivalents.

The data presented in Table 1 made it possible to select the most suitable extract to proceed to the next phase of the formulation of a new dermatocosmetic product. Thus, the extract resulting from maceration (M in conditions: S/L=1:5; propanediol 60%, extraction time of 7 days) was chosen because it has the greatest amount of polyphenols (needed in the final product), although it is second in terms of antioxidant activity.

Preliminary analyses for emulsions based on *Acmella oleracea* extract

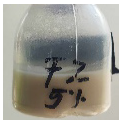
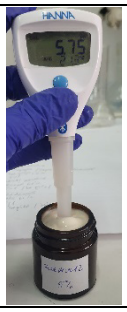
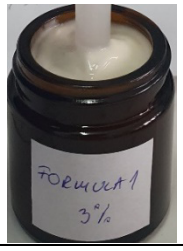
Using the extract of *Acmella oleracea* obtained after extraction by maceration 7 days in following conditions: S/L=1:5, time of extraction = 7 days, solvent: 60% aqueous solution of propanediol, characterized by a total polyphenol content of 3.386 mg GAE/ml, we proceeded to obtain two types of emulsions using the same base. Their composition are presented in Table 4.

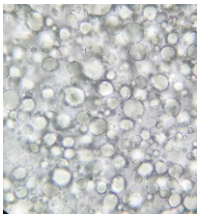
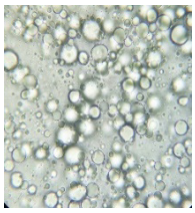
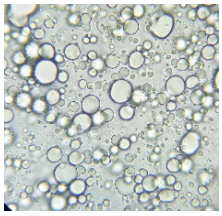
Before a product is placed on the market, it must undergo a series of analyzes according to quality standards to determine its stability and the best storage and conservation practices. With this in mind, a series of initial analyzes on the quality and stability of the manufactured preparations were taken into account. Based on the results obtained, the studies will continue with *in vitro* and *in vivo* evaluations.

An important aspect to consider when evaluating the quality of dermatocosmetic preparations is their stability in different environments and under different conditions. To evaluate the stability of the resulting dermatocosmetic emulsions, three physical evaluations were performed at two different time points: immediately after preparation at room temperature and 24 hours after formulation. Table 3 briefly summarises the results of the preliminary analyses that were performed, such as pH and conductivity measurements, sensory analysis, centrifugal and vibrational forces and microscopic image analysis.

Table 3 presents the results of two stable products whose analysis will continue with investigations of structure morphology, rheological behavior, microbiological stability, diffusion through membranes, and in vitro and/or in vivo analyses.

Table 3. Preliminary Characterization of Emulsions with Acmella oleracea

Parameters	**Emulsions**		
	Base emulsion	**Formula 1 with 3% active**	**Formula 2 whis 5% active**
Emulsion stability after the action of centrifugal force			
	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
Emulsion stability after the action of vibrational force			
	Homogeneous compact texture and appearance		
Measuring pH with a Hanna pH meter			
	Initial emulsion pH value: 6.01	The pH value 5.86 and 5.75 respectively, are compliant value and it does not require adjustment	
Organoleptic analysis			
	Compact, homogeneous texture. Characteristic, pleasant smell. Yellowish, glossy color, derived from the raw materials used		

<table>
<tr><th rowspan="2">Parameters</th><th colspan="3">Emulsions</th></tr>
<tr><th>Base emulsion</th><th>Formula 1
with 3% active</th><th>Formula 2
whis 5% active</th></tr>
<tr><td rowspan="2">Microscopic images after 24 hours of emulsion preparation storage 25 °C</td><td>  </td><td>  </td><td>  </td></tr>
<tr><td colspan="3">Uniform particles of different sizes, without cremation, flocculation or sedimentation phenomena</td></tr>
<tr><td>Conductivity measurement (mS) after 24 hours from emulsion preparation, storage at 25 °C</td><td>  </td><td>  </td><td>  </td></tr>
<tr><td></td><td></td><td colspan="2">The conductivity value of 0.48 and 0.62 respectively, is a compliant value and it does not require adjustment</td></tr>
</table>

CONCLUSIONS

When the aqueous propanediol solution was used as an extractant, maceration (for a maximum content of polyphenols of 3.386 mg GAE/mL) and the combined sonoextraction-maceration method (for a maximum content of flavonoids of 11.94 mg QE/mL) proved to be the most effective methods for obtaining plant extracts from *Acmella oleracea*. Of the two concentrations of propanediol studied, the 60 % concentration proved to be the more efficient. The choice of working parameters leads to extracts rich in the desired biologically active compounds and with antioxidant activity. Due to the synergy of the compounds in the extracts, they can be used for the preparation of dermato-cosmetic emulsions to combat/treat oxidative stress

on the skin. The stability of the two emulsions prepared with the propanediol extract of Acmella oleracea was preliminarily evaluated during preparation and during storage. The results were encouraging and suggest further in vivo and in vitro analysis to produce a market-ready topical preparation.

EXPERIMENTAL SECTION

Reagents

Acmella oleracea (Regn Plantae, Order Asterales, Fam. Asteraceae, Gen.Acmella) plants come from a culture acclimatized in Romania by one of our team members, the culture located Popricani village, Iasi county, Romania (Figure 12). After harvesting the whole plant, its aerial parts were separated from the roots and dried in cool and shaded areas for six to seven weeks and then stored in brown glass containers to prevent moisture and UV light until needed.

Figure 12. Culture of *Acmella oleracea* from Popricani locality, Iasi county (Romania).

The **extraction solvent**, 1,3 propanediol, was obtained from the Elemental SRL company from Oradea (Romania). Obtained by fermentation of glucose, it is considered a natural and biodegradable solvent and is often studied as an alternative extraction solvent as a donor of hydrogen bonds in deep eutectic solvent, it is able to extract a large amount of polyphenols, compared to hydroethanolic extracts [26]. Due to the function of an ingredient in a cosmetic product, the incorporation of the solvent in the final formulation eliminates the subsequent processes of separating the solvent compounds [27].

Extraction methods

The extraction methods used are: Maceration at room temperature (20 ^{0}C) - (M), ultrasound-assisted extraction (sonoextraction – US) and two combined methods: ultrasound-assisted extraction + maceration (USM) and maceration + ultrasound-assisted extraction (MUS) to obtain plant extracts from the Acmella oleracea plant with the highest content of active compounds. An aqueous solution of 40% and 60% propanediol was used as extracting agent. The variables whose effects on the extraction process were studied included the S/L ratio (1:5; 1:15 and 1:20), the concentration of the extractant (40% and 60%) and the extraction time (M= 60 min, 90 min and 120 min; US= 5 min, 10 min and 15 min; USM= 5 min+ 7 days, 10 min+ 7 days and 15 min+ 7 days; MUS= 7 days +10min; 14 days +10 min; and 30 days +10 min).

The total polyphenol content (TPC) was determined using the Folin-Ciocalteu method [19]. The results were presented as mg Gallic acid equivalent (GAE) per g (mg GAE/mL extract), taking into account the sample dilution. The analyses were performed twice. Using the same standard methods, the standard calibration curve (y=0.849x-0.01) was constructed for different gallic acid concentrations.

The amount of flavonoids (TFC) was determined using a spectrophotometric technique with a 2% $AlCl_3$ solution in methanol [20]. The results were expressed as mg quercetin equivalent (QE) per mL of extract (mg QE/mL). A spectrophotometric method was used in which the standard calibration curve (0.0005x-0.037) was established for different quercetin concentrations.

For **antioxidant assays** were used two methods:

(a) *based on 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH)* conducted in accordance with a previously described method [17]. DPPH radical scavenging activity was calculated as milligrams of Trolox (TE) equivalents (mg TE/mL extract) based on spectrophotometric method ($y = 0.0108x + 0.1167$, $R^2 = 0.998$). Results were reported as the mean ± standard deviation (SD) of three independent determinations;

(b) *based on 2,2'-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid radical scavenging assay* (ABTS) which followed a spectrophotometric methodology previously described in our research [28]. The ABTS radical scavenging activity was calculated as milligrams of Trolox equivalents (mg TE/mL extract) based on spectrophotometric method ($y = 0.0082x + 0.0505$, $R^2 = 0.9998$).

All the results were reported as the mean ± standard deviation (SD) of three independent determinations.

Formulation of emulsions based on *Acmella oleracea* extract

The extract with the highest polyphenol content was used to prepare two W/O emulsions that differed in the amount of extract used as an active ingredient with phytochemical properties to protect the skin from oxidative damage.

The base used to prepare the emulsions contains

- a lipophilic phase consisting of: *Amaranthus spinosus* seed oil, *Psoralea corylifolia* seed oil, *Malus domestica* seed oil and *Solanum lycopersicum* seed oil as emulsifier and the mixture: Cetearyl alcohol (and) glyceryl stearate (and) jojoba ester (and) *Helianthus annuus* (sunflower) seed wax (and) sodium stearoyl glutamate (and) water (and) polyglycerin-3 with the role of emulsifier;
- a hydrophilic phase consisting of: *Acmella oleracea* floral water as solvent and glycerol as conditioning agent;
- additives as viscosity agents: lecithin, sclerotium gum, pullulan, xanthan gum;
- preservatives such as: Benzyl alcohol, dehydroacetic acid.

The final W/O emulsions were prepared according to a method described in our previous work [29] and stored in cool rooms until analysis was completed, which can take up to ninety days. Samples were returned to room temperature before each analysis.

REFERENCES

1. T. K. Lim; *Edible medicinal and non-medicinal plants* (Vol. 1), Springer: Dordrecht, The Netherlands, **2012;** pp. 285-292
2. T. G. G. Uthpala, S. B. Navaratne; *Food Rev. Int.,* **2021**, *37(4*), 399-414.
3. J. Paulraj; R. Govindarajan; P. Palpu; *Advances in Pharmacological and Pharmaceutical Sciences*, **2013**, *2013* (*1)*, Article ID 510298
4. N. K. Simas; E.D.C.L. Dellamora; J. Schripsema; C.L.S. Lage; A. M. de Oliveira Filho; L. Wessjohann; R. M. Kuster; *Phytochem. Lett.*, **2013***,* 6(1), 67-72.
5. R. Sharma; N. Arumugam; *Future Foods*, **2021***,3*, 100022.
6. C. P. S. Martins; M. T. P. Melo; I. C. G. Honório; V. A. D'Ávila; W. G. O Carvalho Júnior; *Revista Brasileira de Plantas Medicinais*, **2012**, *14*, 410-413.
7. M. Rondanelli; F. Fossari; V. Vecchio; V. Braschi; A. Riva, P. Allegrini; G. Petrangolinid; G. Iannelloe; M. A. Falivac; G. Peronic; M. Nichettic; C. Gasparric; D. Spadaccinic; V. Infantinof; S. Mustafag; T. Alalwang; S. Perna; *Fitoterapia*, **2020**,*140*, 104419.
8. E. Spinozzi; R. Pavela; G. Bonacucina; D. R. Perinelli; M. Cespi; R. Petrelli; L. Cappellacci; D. Fiorini; S. Scortichini, S. Garzoli, C. Angeloni, M. Freschi, S. Hrelia, L. Quassinti, M. Bramucci, G. Lupidi, S. Sut, S. Dall'Acqua, G. Benelli, A. Canale, E. Drenaggi, F. Maggi, *Ind. Crops Prod.*, **2021**, *172*, 114027.

9. F. Barbira-Freedman; An Amazon Plant in Clinical Trial: Intersections of Knowledge and Practice. In *Collaborators Collaborating*: *Counterparts in Anthropological Knowledge and International Research Relations*, M.Konrad Ed.; Berghahn Books: Oxford, New York, **2012**, Chapter 2, pp. 40-58.
10. V. Prachayasittikul; S. Prachayasittikul; S. Ruchirawat; V. Prachayasittikul; *EXCLI* **2013***, 12*, 291.
11. A. F. Barbosa; M. G. D. Carvalho; R. E. Smith; A.U. Sabaa-Srur; *Revista Brasileira de Farmacognosia*, **2016**, *26*, 128-133.
12. E. Spinozzi; M. Ferrati; C. Baldassarri; L. Cappellacci; M. Marmugi; A. Caselli; G. Benelli; F. Maggi; R. Petrelli; *Plants*, **2022**, 11(20), 2721.
13. L. Veryser; E. Wynendaele; L. Taevernier; F. Verbeke; T. Joshib; P. Tatke; B. De Spiegeleer; *Functional Foods in Health and Disease*, **2014**, *4(6*), 264-275.
14. F. Demarne; G. Passaro, U.S. Patent No. 7,531,193. Washington, DC: U.S. Patent and Trademark Office, **2009.**
15. B. De Spiegeleer; J. Boonen; L. Veryser; L. Taevernier; S. Malysheva; J.D. Di Mavungu; S. De Saeger; *In Stratum Corneum VII*, **2012**, *34(4*), 383-383.
16. R.K. Jansen; *Syst. Bot. Monogr.,* **1985**, *8*, 1–115.
17. P. B. Lalthanpuii; R. Lalawmpuii; K. Vanlaldinpuia; K. Lalchhandama, *Science Vision*, **2016***, 16*(4), 177-83.
18. R. Sulpice; P. C. McKeown; *Annual review of plant biology*, **2015**, *66*, 187-210.
19. L. Pavun; P. Đikanovic; M. Jelikic-Stankov; D. Đurdevic; S. Uskokovic-Markovic; *Czech J. Food Sci.*, **2018**, *36*, 233–238.
20. D. Turcov; A.S. Barna; A. Trifan; A.C. Blaga; A.M. Tanasa; D. Suteu; *Plants-Basel*, **2022**, *11(19*), Article Number 2454.
21. A. Apostolakis; S.Grigorakis; D.P. Makris; *Separation and Purification Technology*, **2014,** *128*, 89–95.
22. N.L. Huamán-Castilla; M.S. Mariotti-Celis; M. Martínez-Cifuentes; J.R. Pérez-Correa; *Biomolecules,* **2020**, *10*, 474.
23. J.R. Nastasi; V.D. Daygon; V. Kontogiorgos; M.A. Fitzgerald; *Metabolites,* **2023**, *13*, 566.
24. F. Chemat; M. Abert Vian; H. K. Ravi; B. Khadhraoui; S. Hilali; S. Perino; A. S. Fabiano Tixier; *Molecules*, **2019***, 24*(16), 3007.
25. A. Kerdudo; F. Fontaine-Vive; A. Dingas; C. Faure; X. Fernandez; *Int. J Cosmetic Sci.*, **2015***, 37*(1), 31-40.
26. V. Vieira; R. C. Calhelha; L. Barros; J. A. Coutinho; I.Cfr Ferreira; O. Ferreira; *Molecules*, **2020***, 25*(11), 2497.
27. M. Ruesgas-Ramón; M. C. Figueroa-Espinoza; E. Durand; *J. Agric. Food Chem.*, **2017***, 65*(18), 3591-3601.
28. S.V. Luca; L. Kulinowski; C. Ciobanu; G. Zengin; M.E. Czerwinska; S. Granica; J. Xiao; K. Skalicka-Wo´zniak; A. Trifan; *Ind. Crops Prod.,* **2022**, 178, 114623.
29. C. Maxim; D. Turcov; A. Trifan; D. Suteu; A. S. Barna; *SCS-ClCBlA*, **2024**, 25 (2), 1679-172.