

CHARACTERIZATION OF THE ALCOHOLIC PHYTOEXTRACTS FROM WILD CARROT (*Daucus carota* L.) WITH POTENTIAL APPLICABILITY AS ACTIVE IN COSMETICS

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Abstract: The paper addresses the case of *Daucus carota* L. extract, obtained by liquid-solid extraction methods (heat reflux, Soxhlet extraction and maceration) using ethanol solution of different concentrations as extraction reagent. The performance of the extraction methods was assessed based on the extraction yield value. The extracts obtained were characterized qualitatively by HPLC and quantitatively by determining the content of polyphenols and flavonoids. Two O/W emulsions based on *Daucus carota* L. extract were also prepared and were preliminarily characterized in terms of stability and homogeneity.

Keywords: *cosmetic emulsion, Daucus carota* L., *hydroalcoholic phytoextract, polyphenol determination, solid-liquid extraction*

INTRODUCTION

Recent research highlights the growing interest in plant-derived bioactive molecules in modern cosmetic formulations, with the cosmetic industry increasingly focusing on sustainable development and green technologies and showing a renewed interest in natural resources [1, 2].

Traditional medicine is a good starting point for research into new bioactive compounds with potential benefits for dermatocosmetics in the context of the growing demand for new and more effective therapeutic solutions.

Daucus carota L. (*D. Carrota*), known as wild carrot, is a species of the *Apiaceae* family that was first officially described in 1753 by Carl Linnaeus in his work "Species Plantarum", with the sequencing of the entire genome of *Daucus carota* in 2016 [3]. The cultivated carrot, *D. carota* ssp. *sativus*, originates from the selective breeding of an ancestral wild form of the carrot, *D. carota* ssp. *carrot* [4]. The wild carrot is widespread in various habitats such as meadows, roadsides and fallow land. In certain situations, the wild carrot can be considered an invasive or problematic plant for agricultural crops or pastures, so its use would represent a sustainable and ecological source for research into new bioactive substances that can be used in cosmetic products, in line with the principles of sustainability and environmental protection [5]. It is important to mention the similarity of *Daucus carota* with *Conium maculatum*. The latter is an extremely poisonous plant, and the red dot in the center of the inflorescence is specific only to the wild carrot [6].

The traditional use of wild carrot in Romania for dermatological diseases is described as anti-inflammatory, antibacterial, analgesic, purifying, skin regenerating, anti-ageing, keratolytic for wounds, burns, eczema, aphthous stomatitis, ulcers, boils, impetigo, tinea corporis, abscesses, inflammatory processes, senile keratosis, wrinkles and skin hugs [7 – 9]. Hepatoprotective, diuretic and detoxifying properties have been described in various cultures [10], an effective remedy for numerous digestive, kidney, bladder and menstrual problems, as well as dropsy, flatulence and edema [11, 12]. In the Middle East, the plant is used to protect against liver diseases and to treat diabetes, stomach ulcers, muscle pain and cancer, as a vermifuge, diuretic, antidote for snakebite, antiseptic and anti-inflammatory therapy for prostatitis and cystitis [13].

The wild carrot is considered an aromatic plant, with the inflorescences with the flowers and seeds being the source for the extraction of the essential oils of carrot, which are used as raw material in various industries. Although both the root and the leaves contain active compounds, the seeds are most commonly used for extraction. The essential oil from carrot seeds is a valuable source of fatty acids, antioxidants and UV-protective compounds, making it a promising ingredient for cosmetic applications such as skin moisturizing, anti-aging and sun protection [14]. In a study, Arianto *et al.* reported anti-aging effects through the achieved sun protection, reduction of wrinkles and pigmentation spots after the application of an emulsifying gel containing carrot seed essential oil [15]. In another study, Gilca *et al.* provide an overview of ethnobotanical dermatological practices based on the use of medicinal plants in Romania and other Eastern European countries, including indications, ethnopharmacological activities, parts used and administration of 106 medicinal plants [7]. In the study, *Daucus carota* L. ranks 4th in this ranking with an RDI (Relative Dermatological Index) of 72.28, after cabbage, chamomile and burdock. This index evaluates the efficacy or importance of

plants used in dermatology and indicates how common or significant they are for dermatological treatments, as ethnomedicine in this context.

Recent studies confirm the value of wild carrot in dermatological applications, showing antifungal, anti-inflammatory, antioxidant and apoptosis-inducing activities in skin cancer [13, 16 – 18].

The choice of extraction method affects the recovery of specific compounds, extraction yield and purity of the extract and also has a significant impact on production costs, the environment and the quality of the final product.

The aim of this article is to determine the conditions for the application of maceration (M), heat reflux extraction (R), extraction in Soxhlet (Sx) in the case of *Daucus carota* L. in order to obtain a hydroalcoholic extract with phytotherapeutic activity. In this context, the studies focused on determining the factors influencing the extraction in the case of using the aerial parts of the plant *Daucus carota* L., as well as on the preliminary characterization of the extracts.

MATERIALS AND METHODS

Plant

The plants of the wild carrot (*Daucus carota* L.) were collected from Popricani - Iasi county (Romania) in June and July 2022. After harvesting, the plants were laid out in a single layer and dried in a well-ventilated room in indirect sunlight. Using a food mill, the dried plants were crushed into particles of 2 to 5 mm in size. They were then stored in a dry, clean laboratory container protected from sunlight until they were needed.

Methods

Extraction methods

The extraction solvent was ethyl alcohol (recognized and approved by the cosmetic and dermatocosmetic industry) in the form of a hydroalcoholic solution in different concentrations (30 %, 50 % and 70 % (v/v)).

Solvents based on ethyl alcohol and water in different ratios were used in liquid-solid extraction processes, such as maceration (M), simple heat reflux extraction (R) and Soxhlet extraction (Sx). The variables affecting the extraction process - contact time, solid/liquid ratio (S/L) and extractant concentration - are investigated. Each plant extract was separated and stored in a dark, cool chamber (5 - 15 °C) until needed for characterization and subsequent processing.

The efficiency of the extraction process carried out under different conditions was assessed by calculating the extraction yield (eqn.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.

Every other reagent or reference standard that was utilized was of analytical quality (Sigma Aldrich and Merck Co.).

The qualitative extract characterization

For the qualitative characterization of the alcoholic extracts of wild carrot (*Daucus carota* L.), the HPLC method was used with an Agilent 1200 high performance liquid chromatograph equipped with a G1322A degasser and an Eclipse XDB-C18 column (150 mm x 4.6mm, 5 μ m) and a multidiode detector (DAD). The separation was performed with a mobile phase (in a concentration gradient) - A) Mixing 50 volumes of acetonitrile with 950 mL of water brought to pH 2 with phosphoric acid; B) Mixing 95 volumes of water brought to pH 2 with phosphoric acid with 905 volumes of acetonitrile.

To identify the peaks, both the retention time values from the chromatogram of the extract from the reference material were compared with those of the reference substances and the absorbance spectra of the peaks obtained were compared with those of the reference substances analyzed under the same chromatographic conditions at two wavelengths, 270 nm and 350 nm [19].

The quantitative extract characterization

For analysis of the plant extracts, it was followed to determine two types of compounds: (1) The total polyphenols content using the Folin-Ciocalteu method [20]. The results were expressed in μ g of gallic acid equivalent (GAE) per mL (μ g GAE $\cdot$ mL $^{-1}$) taking into account the sample dilution. The analyses were done in duplicates. The standard calibration curve was obtained with the same standard methodology applied for different concentrations of gallic acid.

(2) The content of flavonoids, which was determined using the spectrophotometer-based method with 2 % AlCl₃ solution in the presence of methanol [20]. The results were expressed in mg or mg of quercetin equivalent (QE) per mL (mg QE $\cdot$ mL $^{-1}$) taking into account the sample dilution. The analyses were performed in duplicates.

Formulation of an emulsion based on *Daucus carota* L. extract

To create an O/W emulsion with phytochemical qualities to shield the skin from oxidative stress, the extract with the highest polyphenol content (obtained by Soxhlet extraction, Sx5 sample) was utilized. The prepared cosmetic emulsion is based on the following phases and chemical compounds:

- *Lipophilic phase* in which it is found: emollients (*Amaranthus Spinous* Seed Oil, *Psoralea corylifolia* Seed Oil, *Psoralea corylifolia* Seed Oil, *Malus domestica* Seed Oil, *Solanum Lycopersicum* Seed Oil) and an emulsifier: Behenyl Alcohol, Glyceryl Behenate and Lecithin
- *Hydrophilic phase*, containing *Daucus carota* ssp. *carota* Flower Water as solvent (72.7 % from total composition) and Glycerin as conditioning agent;
- *Additives*: Lecithin, Sclerotium Gum, Pullulan, Xanthan Gum with the role of viscosity agent;

- *Preservatives*: Benzyl Alcohol, Dehydroacetic Acid.

The final emulsion is an O/W (oil in water) emulsion obtained following a protocol similar to that described in our previous work [21]. The samples were stored at room temperature (20 - 25 °C) and then allowed to reach room temperature before any analysis.

Preliminary evaluation of cosmetic emulsions containing *Daucus carota* L. extracts

To evaluate the stability of the created emulsion, a series of physicochemical analyses were performed, such as:

- assessment of conductivity using a portable Hanna Instruments type conductometer;
- analysis of microscopic pictures using a binocular microscope (Optika B-159, OPTIKA S.R.L., Ponteranica (BG) - Italy), magnification - 1000x;
- sensory evaluation (odor, color, texture, and overall aspect);
- centrifuge and vortex tests, using the centrifuge model XC-Spinplus operated for thirty minutes at 25 °C and 3000 revolutions per minute and a Multi Speed Vortex MSV-3500 (Grant Instruments Ltd., Cambs, England) 30 minutes at 25 °C and 3000 rpm for the vortex test;
- pH determination using a digital pH meter (Hanna Instrument);
- microbiological determination has been realized by following an adapted protocol from ISO 18415:2007 [21], for the total aerobic mesophilic microorganisms (total aerobic microbial count and total yeast and mold count) using the protocol described in detail in our previous paper [22]. Microorganisms isolated from the Petri dish were grown on selective media: Mannitol Salt Agar, MacConkey Agar, Eosin Methylene Blue, Cetrimide Agar and Brilliant Green Phenol Red Agar in order to examine pathogenic strains: *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa* or *Salmonella*. The total microbial count is reported as CFU·g⁻¹, accounting for the dilution factor, and the detection parameter is reported as present or absent/g.

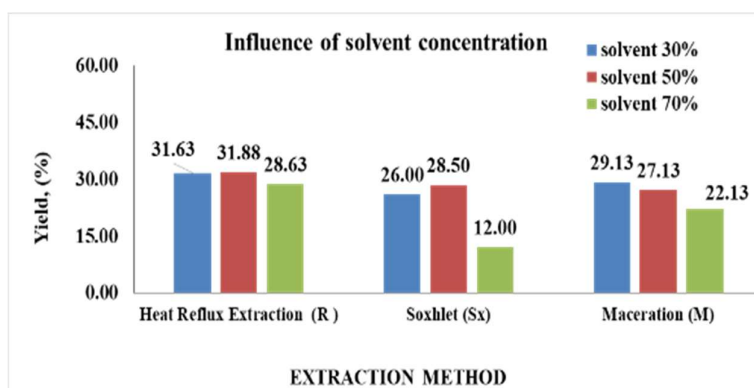
Next, a protocol established in our previous work [23] was followed after consulting the specialized literature [24, 25].

RESULTS AND DISCUSSION

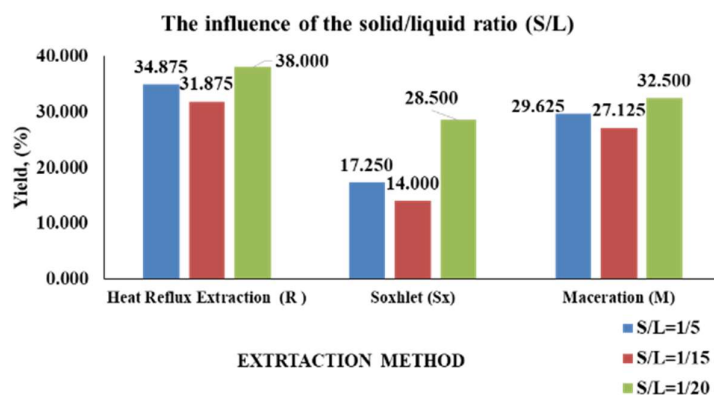
Evaluation of extraction methods depending on the extraction yield

Depending on the work technique and the established extraction conditions, the yield of extraction along with the amount of polyphenols and flavonoids recovered were used to assess the extraction methods' efficiency. The results are presented in Figures 1-3.

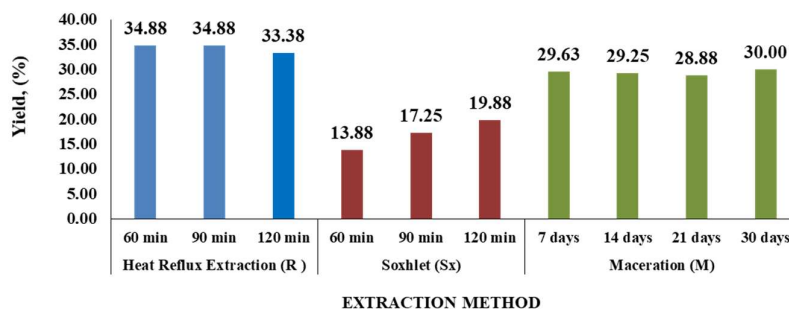
Figure 1 shows that regardless of the extraction conditions chosen, heat reflux extraction (R) is the method with the best yield (34.88 %). Thus, if we consider as evaluation criteria the high efficiency of the extraction process and the degree of economy, then we can consider the heat reflux extraction with a hydroalcoholic solvent of 50 % concentration, an extraction time of 60 minutes and considering a solid-liquid ratio of 1:15.



(a)



(b)



(c)

Figure 1. The extraction yield (%) depending on the extraction method used and the physical parameters considered

Conditions: (a) R- S/L= 1:5; extraction time 60 min; M- S/L= 1:5; extraction time 7 days; Sx-S/L=1:20, extraction time 90 min; (b) R - 50 % and extraction time - 60min; M -50 % and extraction time - 7 days; Sx-50 % and extraction time - 90min; (c) R , M, Sx- 50 % and S/L=1:15

The total polyphenols (TPC) compound content determination from hydro alcoholic vegetal extracts

From the analysis of Figures 2 (a-c), the total amount of polyphenols (TPC) extracted by the selected methods depends on the selected physical parameters as follows:

- Considering the concentration of the extracting agent, it is observed that in the case of maceration, the performances are similar for all three concentrations. Soxhlet extraction emphasizes an extractant concentration of 50 %, while the concentration for the hot reflux method is 70 %. However, it should be noted that all these values are close to the values obtained in the case of maceration. The choice of a method and concentration of extractant depending on this parameter is therefore based on economic criteria of cost and benefit in relation to the purpose of the determination.

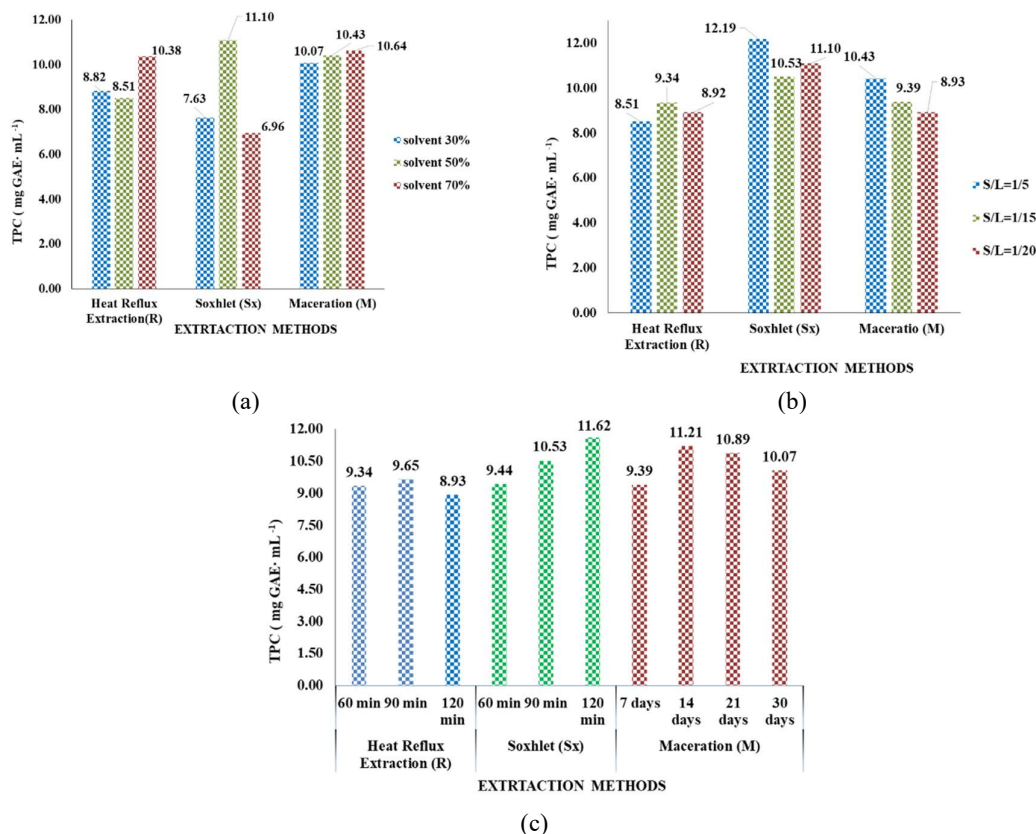


Figure 2. The total polyphenols (TPC) compounds content ($\text{mg GAE} \cdot \text{mL}^{-1}$) depending on the extraction method used and the physical parameters considered

Conditions: (a) R- S/L=1:5, extraction time 60 min; M- S/L=1:5, extraction time 7 days; Sx- S/L=1:20, extraction time 90 min.; (b) R- 50 %, extraction time 60 min; M- 50 %, extraction time 7 days; Sx-50 %, extraction time 90 min.; (c) R, M, Sx- 50 %, S/L = 1:15

- Taking into account the solid/liquid ratio (S /L) when using the extraction reagent with concentrations of 50% (Figure 1b), the performances of the methods can be arranged in descending order as follows: Sx with $12.19 \text{ mg GAE} \cdot \text{mL}^{-1}$ at S/L ratio of 1:5, followed by Sx with $11.1 \text{ mg GAE} \cdot \text{mL}^{-1}$ at S/L ratio of 1:20 and M with $10.43 \text{ mg GAE} \cdot \text{mL}^{-1}$ and S/L ratio of 1:5 and Sx with $10.53 \text{ mg GAE} \cdot \text{mL}^{-1}$ at S/L ratio of 1:15.

- Depending on the extraction time, Figure 2c shows that the best values are achieved with a Soxhlet extraction of 90 minutes and maceration of 14 days (50 % extractant concentration and S/L=1:15).

Taking into account all the results obtained in the case of polyphenols (shown in Figure 2 and not listed in the paper), it can be emphasized that the highest amount after *Soxhlet extraction method* was $12.192 \text{ mg GAE} \cdot \text{mL}^{-1}$ (50 % concentration, S/L=1:5 and 90 minutes extraction time) and $11.62 \text{ mg GAE} \cdot \text{mL}^{-1}$ (50 % concentration, S/L=1:15 and 120 minutes extraction time), followed by maceration with $11.202 \text{ mg GAE} \cdot \text{mL}^{-1}$ (50 % concentration, S/L=1:5 and 14 days extra extraction time).

The total flavonoids (TFC) compound content determination from hydro alcoholic vegetal extracts

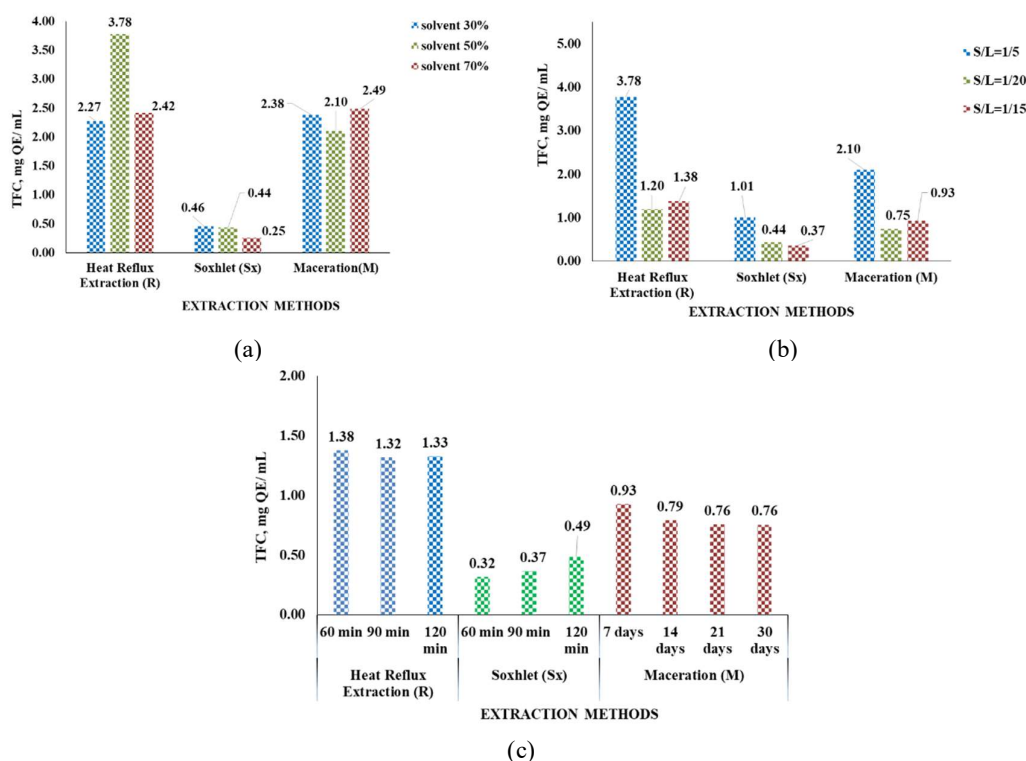


Figure 3. The total flavonoids compounds (TFC) content ($\text{mg QE} \cdot \text{mL}^{-1}$) depending on the extraction method used and the physical parameters considered

Conditions: (a) R- S/L=1:5, extraction time 60 min; M- S/L=1:5, extraction time 7 days; Sx- S/L=1:20, extraction time 90 min.; (b) R- 50 %, extraction time 60 min; M- 50 %, extraction time 7 days; Sx-50 %, extraction time 90 min.; (c) R, M, Sx- 50 %, S/L = 1:15

From the analysis of Figures 3 (a - c), the total amount of flavonoids (TFC) extracted by the used methods depends on the selected physical parameters as follows:

- Considering the concentration of the extracting agent (Figure 3a), it is observed that in the case of maceration, the performances are similar for all three concentrations. In the case of heat reflux extraction, an extractant concentration of 50 % is emphasized, while the concentration in the heat reflux treatment is 70 %. In the case of heat reflux extraction, the best performance was recorded at $3.78 \text{ mg QE} \cdot \text{mL}^{-1}$ when using the 50 % concentration extractant.
- Taking into account the solid/liquid ratio (S /L) (Figure 3b) when using the extraction reagent with concentrations of 50 %, the best performances ($3.78 \text{ mg QE} \cdot \text{mL}^{-1}$) was

recorded in the case of heat reflux extraction in condition of S/L = 1:5; 50 % extractant concentration and 60 min extraction time.

- Depending on the extraction time, Figure 3c shows that the best performances were obtained in the case of heat reflux extraction, values approximately equal for all the times studied.

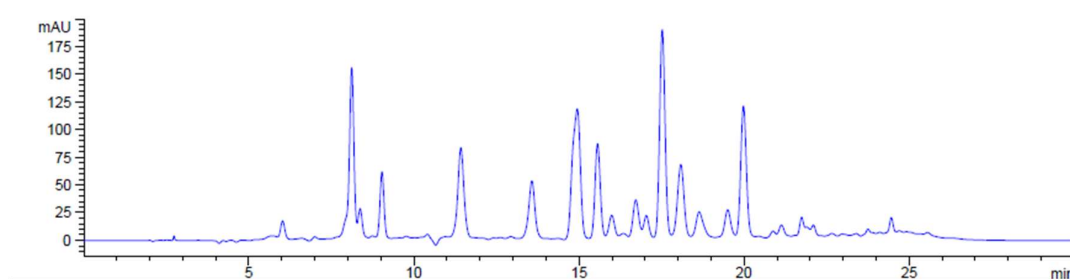
Taking into account all the results obtained in the case of flavonoids (shown in Figure 3 and not listed in the paper), it can be emphasized that the highest amounts of extracted flavonoids were obtained by *heat reflux extraction* method ($3.78 \text{ mg QE} \cdot \text{mL}^{-1}$) under the following conditions: 60 minutes extraction time, S/L=1:5 and 50 % concentration of ethanol solution as extractant agent.

The qualitative extract characterization

Two samples of the extract obtained by Soxhlet extraction were used for this analysis: Sx5 (solvent concentration of 50 %, extraction time of 90 min; S/L ratio of 1:5 and polyphenol concentration (expressed as gallic acid (GAE) content) of $12.19 \text{ mg GAE} \cdot \text{mL}^{-1}$) and Sx6 (solvent concentration of 50 %, extraction time 120 min; S/L ratio of 1:15 and polyphenol concentration of $11.62 \text{ mg GAE} \cdot \text{mL}^{-1}$). The extracts were analyzed to reveal the presence of some polyphenolic compounds, flavonoid glycosides and flavone. The study was based on the HPLC chromatograms shown in Figure 4.

Figure 4 shows that chromatograms with a large number of peaks are obtained, indicating numerous compounds with different retention times. By comparing the retention times with those of the reference substances, it was possible to determine the presence or absence of the compounds of interest in the analyzed extracts. For example, p-coumaric acid and gallic acid were identified for both samples at a wavelength of 270 nm for both Sx5 and Sx6. Analysis at 350 nm led to the identification of rutin, isorhamnetin and luteolin in the case of Sx5 and rutin in the case of Sx6.

A comparison of the chromatograms in Figures 4a and 4b shows that they correspond to the characteristic polyphenol content of the two samples analyzed, Sx5 ($12.19 \text{ mg GAE} \cdot \text{mL}^{-1}$) and Sx6 ($11.62 \text{ mg GAE} \cdot \text{mL}^{-1}$). For example, if we compare the peak areas obtained, we find higher amounts of p-coumaric acid and gallic acid in the Sx5 sample. We can say that the longer extraction time did not lead to better results in terms of the chemical composition of the plant extract.



(a)

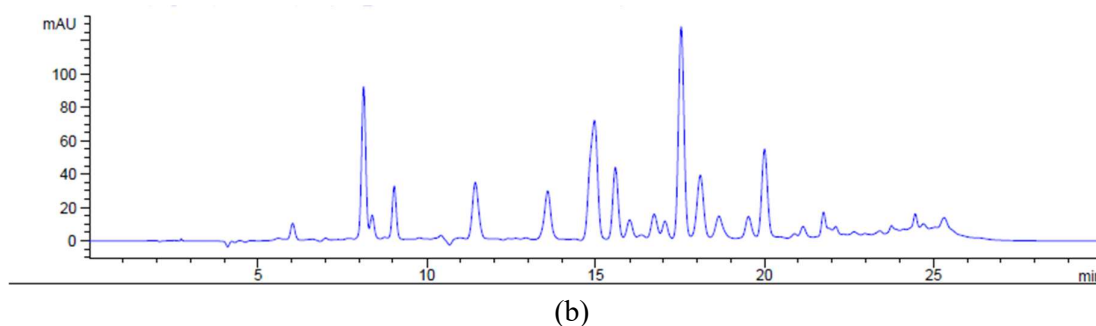


Figure 4. Chromatograms obtained for the identification of polyphenolic compounds in wild carrot (*Daucus carota* L.) extracts: (a) Sx5 sample vegetal extract; (b) Sx6 sample vegetal extract

Preliminary analysis of emulsions based on *Daucus carota* L. extract

A cosmetic emulsion was prepared using as active ingredient the *Daucus carota* L. extract obtained following extraction by the Soxhlet method - sample Sx5 (50 % concentration, S/L=1:5 and extraction time 90 minutes), characterized by a total polyphenol content of 12.19 mg GAE·mL⁻¹.

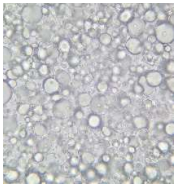
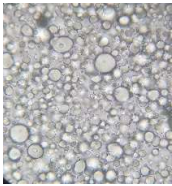
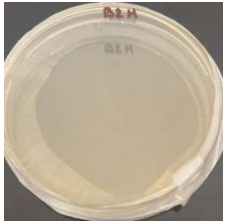
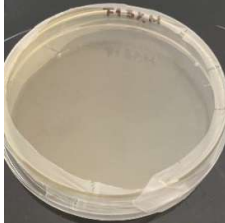
A number of analyses pertaining to product stability and storage and preservation conditions must be conducted before a product is put on the market. In this regard, we took into consideration a series of initial analyses pertaining to the stability and quality of the final cosmetic emulsion. The experiments could be continued with in vitro and in vivo assessments based on the outcomes.

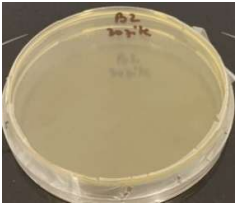
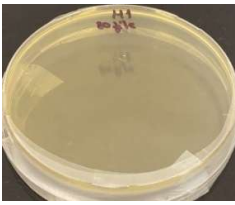
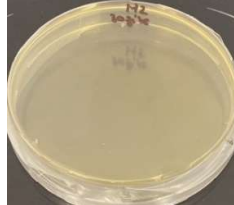
The results of the preliminary analysis about the stability of cosmetic emulsion carried out (pH and conductivity determinations, sensory analysis, the action of centrifugal and vibrational forces, obtaining microscopic images) are presented concisely in Table 1.

Table 1. Physical characterization of emulsions with *Daucus carota* L.

Analysis method	Emulsions		
	Base emulsion	Formula 1 with 3 % active	Formula 2 with 5 % active
Centrifugation and vortex test			
			
	Homogeneous compact texture and appearance		

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Measuring pH			
	Initial emulsion pH value: 5.13	The pH value 5.10 is a compliant value, it does not require adjustment	The pH value 5.03 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)			
	0.05, value which indicates an emulsion without phase separation phenomena	The conductivity value increases to 0.09 after adding the percentage of 3 % actives. The emulsion is stable	The conductivity value increases to 0.10 after adding 5 % actives. The emulsion is stable
Microbiological evaluation (after 24 hours of emulsion preparation, stored at 25 °C)			
	Microbiological analysis revealed the presence of a single colony-forming unit	Microbiological analysis revealed 0 CFU after incubation, indicating a	Microbiological analysis revealed 0 CFU after incubation, indicating a sterile

	(CFU) after incubation	sterile sample	sample
Microbiological evaluation (after 30 days of emulsion preparation, stored at 25 °C)			
	Microbiological analysis revealed 0 CFU after incubation, indicating a sterile sample	Microbiological analysis revealed the presence of a 2 colony-forming unit (CFU) after incubation	Microbiological analysis revealed 0 CFU after incubation, indicating a sterile sample

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

CONCLUSIONS

Experimental results regarding the characterization of *Daucus carota* L. vegetal extracts lead to two distinct ideas: (i) when the polyphenol content is of interest, then the most relevant extract is the one obtained by *Soxhlet extraction* for which a content of 12.192 mg GAE·mL⁻¹ was recorded. The method was practically applied under the following conditions: 50 % concentration of hydroalcoholic solution, S/L=1:5 and 90 minutes extraction time; (ii) when the flavonoids content is of interest, then the most relevant extract is the one obtained by *heat reflux extraction* for which a content of 3.78 mg QE·mL⁻¹ was recorded. The method was practically applied under the following conditions: 60 minutes extraction time, S/L=1:5 and 50 % concentration of ethanol solution as extractant agent.

In order to prevent or treat oxidative stress on the skin, phytocosmetic emulsions were prepared using the extracts' constituents, which worked synergic. A preliminary analysis of the stability of these two emulsions based on *Daucus carota* L. extract throughout preparation and storage yielded encouraging results for more thorough *in vitro* and *in vivo* analyses.

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