Valorization of Bioactive Compounds from Residual Saffron Biomass (*Crocus sativus* L.) to Obtain High Value Added Dermato-Cosmetic Products

Delia Turcov,[a] Ana Simona Barna,[a] Oana Teodora Apreutesei (Ciuperca),[a,b] Adriana Trifan,[c] Adrian Catalin Puitel,[a] and Daniela Suteu [a,*]

The valorization of indigenous flora waste by extraction of biologically active compounds has potential applications in the medical and cosmetic fields. The polyphenols and flavonoids extracted from this waste are valuable compounds for the manufacture of new cosmetic and/or dermato-cosmetic formulas to protect the skin from oxidative stress. This study obtained plant extracts from saffron waste—petals, tepals, and superior portions of stem—using different solid-liquid extraction techniques. The influence of some physical operating parameters was studied (extraction time, solid/liquid ratio, solvent extraction composition). The extraction method performance was assessed by the value of the extraction yields. The obtained extracts were characterized by the content of polyphenols and flavonoids, and the antioxidant activity determined with the DPPH and ABTS methods and the UV-VIS spectrometry. Some emulsions O/W were prepared and preliminarily characterized (pH, sensory analysis, stability after centrifugation and storage). The obtained results showed that the incorporation of this natural extract did not negatively affect the stability of the studied cosmetic formulations and advanced characterization (microbiological control of contamination, rheology studies and *in vitro* and *in vivo* studies) can be continued in order to implement a new product.

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*Contact information: a: "Gheorghe Asachi" Technical University of Iași, Romania, "Cristofor Simionescu" Faculty of Chemical Engineering and Environmental Protection, Blv.D. Mangeran, 73A, Iasi, 700500 Romania; b: Research and Processing Centre for Medicinal Plants "PLANTAVOREL" S.A., 46, Cuza Voda Street, 610019 Piatra Neamț, Romania; c: Faculty of Pharmacy, University of Medicine and Pharmacy "Grigore T. Popa" Iași, 700115 Iasi, Romania; *Corresponding author: danasuteu67@yahoo.com*

INTRODUCTION

Dermato-cosmetic therapies have focused on oxidative stress and the increasing trend of natural extract use. Effective and innovative solutions to combat oxidative stress on cells and tissues are being developed. Various molecules offer protection from oxidative stress and its induced conditions; antioxidants are some of the most studied pharmacological ingredients in the pharmaceutical and dermato-cosmetic industry (Turcov *et al*. 2020a,b; Oreopoulou *et al*. 2021). These ingredients are selected based on strict criteria required by certain standards, therapeutic guides, and pharmacopoeias, for the safety of patients.

The common interest of multidisciplinary teams is to reduce synthetic compounds and to use natural compounds obtained by green extraction from natural verified sources,

thus ensuring the quality of the final product. Plants are a basic source for such ingredients, specifically their primary and secondary metabolites. The basic advantages of the natural compounds are as follows (Turcov *et al*. 2020a, 2020b). Most are non-protein molecules, small, with good penetration. They are obtained by extraction methods with no negative consequences on the extracted ingredients or on the human organism. They are obtained from spontaneous flora (without cultivation costs), or from vegetable waste following the extraction of other ingredients of interest, strengthening the modern and innovative concept of zero-waste.

Several liquid-solid extraction techniques have been used to obtain vegetal extracts that contain biologically active compounds with applications in food, pharmaceuticals, cosmetics, phytosanitary, or in agriculture as biopesticides. These methods include cold extraction (classical maceration), heat reflux extraction (also Soxhlet extraction), extraction assisted by ultrasound, extraction assisted by microwaves, methods of extraction using pressured liquid, pulsatile electric field, supercritical fluids, and high hydrostatic pressure or combinations of such techniques (Sasidharan *et al. 2*011; Chemat *et al*. 2012; Azmir *et al*. 2013; Rahaiee *et al*. 2015; Ameer *et al*. 2017; Khaw *et al*. 2017; Rasul 2018; Stelluti *et al*. 2021). Each method has certain features related to the solvents and physical parameters, which provide a number of advantages and disadvantages to each method. In practice, a method is applied with operating parameters depending on the plant material used but especially depending on the desired compounds to be extracted.

The vegetal extracts thus obtained are qualitatively and quantitatively characterized using physical-chemical methods. Many methods can be applied such as chromatographic analysis (HPTLC, HPLC), HPLC assisted by MS detection, LC/MS-MS quantitative analysis, spectroscopy assay, electron paramagnetic resonance (EPR), HPLC-DAD-MS/MS; ultra-high performance liquid chromatography (UHPLC), and HPLC-PDA (Farcas *et al*. 2018; Mocan *et al*. 2019; Ilina *et al*. 2020; Suteu *et al*. 2020).

Among the plants with distinctive bioactive antioxidants, saffron (*Crocus sativus* L.) stands out as a valuable source of important ingredients with high pharmacological and food potential. *Crocus sativus* L. is a perennial plant that belongs to the Iridaceae family and has over 85 species. Due to the growing demand in the food and pharmaceutical industry, saffron is grown on almost every continent (Europe, Asia, U.S.A., and Australia), but especially in the Mediterranean area, the countries of the Middle East, and India. Saffron is the most expensive spice in the world and a rare one that simultaneously gives color, taste, and aroma to food. The only exploited part of the plant *Crocus sativus* is the flower stigma, and for 1 kg of stigmas, 150,000 to 200,000 flowers are needed (Oreopoulou *et al*. 2021).

The origin, growth conditions, and analyzed part of the plant determine its composition of bioactive compounds (Lage and Cantrell 2009; Bukhari *et al*. 2018; Caser *et al*. 2020; Jafari *et al*. 2020). Saffron is characterized by three main compounds: *crocin* (ester of the carotenoid crocetin), which gives the characteristic red-yellow color of the stigmas; *picrocrocin* ($C_{16}H_{26}O_7$) responsible for the aroma of saffron; and *safranal* ($C_{10}H_{14}O$) generated from picrocrocin in the drying process by the simultaneous action of heat and enzymes, which gives the plant characteristic scent (Lage *et al*. 2009; Bukhari *et al*. 2018; Caser *et al*. 2020; Jafari *et al*. 2020; Oreopoulou *et al*. 2021). These compounds can act as antioxidants, antithyrosinase, psychostimulants, anti-inflammatory, antifungal, and antitumor agents. They can also intervene in metabolic disorders (modulation of serum lipid profile), antitumor activities, amelioration of mild to moderate obsessive-compulsive disorder, and improving sleep quality (Zeka *et al*. 2015; Da Porto and Natolino 2018;

Bukhari *et al*. 2018; Jafari *et al*. 2020; Wali *et al*. 2020; Caser *et al*. 2020; Jafari *et al*. 2020; Oreopoulou *et al*. 2021). A series of biological effects represent a viable solution to the ubiquitous therapeutic needs in the most difficult and widespread skin pathologies: rosacea, acne, and premature aging. Thus, (i) crocin, one of the carotenoid components, is primarily responsible for the antioxidant properties of saffron; (ii) kaempferol is an alternative treatment for skin fibrosis due to a significant decrease in the level of mRNA associated with oxidative stress factors such as HO-1 and NOX2, as well as inflammatory and pro-fibrotic cytokines, including IL.-6, TGF-β and TNFα in sclerotics (Sekiguchi *et al*. 2019). In addition, kaempferol, as a flavonoid, inhibits the activity of collagenase in the photo-aging process of the skin induced by UVA (Kashif *et al*. 2017) and brings an additional benefit in the management of skin diseases. This last action is due to the direct antiallergic activity and the inhibitory action against the edema induced by allergic contact dermatitis (Shirley *et al*. 2016; Kashif *et al*. 2017).

Given the high market value, the high price of saffron, as well as its pharmacological potential, using the flower residues capitalizes on the full potential of the plant. Thus, the petals and stem of *Crocus sativus* can be tested as potential sources of biologically active compounds, with antioxidant activity often higher than that of some vegetables and fruits (Caser *et al*. 2020).

A variety of liquid-solid extraction techniques are used to obtain extracts from the stigmas of saffron plants. These techniques can be extended to recover saffron flower residues, which contain significant amounts of biologically active compounds. These liquid-solid extraction techniques include cold maceration, hot reflux extraction (including Soxhlet percolation), and ultrasound- and/or microwave-assisted extraction (Suteu *et al*. 2020; Stelluti *et al*. 2021).

This study investigated the floral residues of *Crocus sativus* L.—a mixture of petals, tepals and the upper part of the stem—as a source of high quality and relatively cheap antioxidant compounds with applications in dermato-cosmetic formulas. Plant extracts were obtained by maceration (M), hot reflux extraction (R), and ultrasound assisted extraction (US), using an extraction solvent containing hydro-alcoholic solutions with different concentrations of ethylene alcohol. The effectiveness of each method was assessed by the value of the extraction degree. The plant extracts were characterized by their antioxidant activity, UV-VIS spectra, and the content of polyphenols and flavonoids. A series of emulsions based on some of these extracts were characterized.

EXPERIMENTAL

Plant Materials and Chemicals

Saffron (*Crocus sativus* L.) plants were collected from Suceava county (Romania), in June through July of 2020. After harvesting the stigmas, the plant parts (petals, tepals, and the upper part of the stem) were placed in a single layer and dried in a well-ventilated room in indirect sunlight. The dried plants were crushed using a food mill (particles size between 2-5 mm) and then stored in a clean, dry laboratory container, protected from the action of solar radiation until later use. The humidity of 9% was determined with a Kern thermobalance.

All other used reagents or reference standards were of analytical quality (Sigma Aldrich and Merck Co.).

Vegetal Extract Preparation

Liquid-solid extraction (*e.g.*, maceration-M and simple heat reflux extraction-R) and a "green" technique (ultrasound-assisted extraction-US) were performed in the presence of solvents based on 96% ethyl alcohol and water in different percentages (Fig. 1). The 96% ethyl alcohol solvent is approved by the cosmetic and dermato-cosmetic industry. The variable parameters (contact time, ratio S/L and extractant concentration) whose influence the extraction process, will be studied and can be identified in Fig. 1. Each plant extract was separated and stored in cold and dark room space until further use for its characterization and for its following processing.

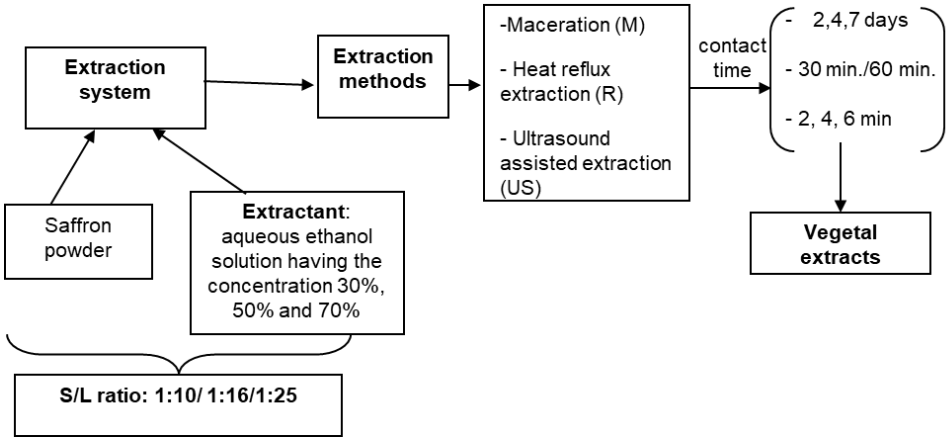


Fig. 1. The stages and parameters involved in obtaining plant extracts

Determination of Extraction Degree

A sample of 5 mL from each plant extract was evaporated to dryness at constant temperature up to 50 °C using a thermostatic oven. The extraction degree was calculated with Eq. 1,

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

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

Qualitative Characterization of Vegetal Extracts

The characterization was based on tracing and interpreting the spectra of the obtained extracts using 1:50 dilutions and a Shimadzu UV-VIS spectrophotometer (model 1280, Kyoto, Japan).

The Quantitative Characterization

The analyses of plant extracts determined two categories of compounds: (1) The total polyphenols content was obtained using the Folin-Ciocalteu method (Pavun *et al*. 2018). The results were expressed in μg of gallic acid equivalent (GAE) per g (μg GAE/g) 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 was determined using the

spectrophotometer-based method with 2% $AlCl_3$ solution in the presence of methanol (Pavun *et al*. 2018). Results were expressed in mg or µg of quercetin equivalent (QE) per g (mg QE/g) taking into account sample dilution. Analyses were performed in duplicates.

Assessment of Antioxidant Activity

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

The assay was performed as previously described (Grochowski *et al*. 2017) with slight modifications. Thus, 50 µL of sample was added to 150 µL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) 0.004% methanol solution. After 30 min of incubation at room temperature in the dark, the absorbance was read at 517 nm. DPPH radical scavenging activity was expressed as milligrams of Trolox equivalents (mg TE/mL extract).

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

The assay was performed using the previously described method (Grochowski *et al*. 2017) with minor changes. $ABTS^{\bullet+}$ was generated by mixing 7 mM 2,2′-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid (ABTS) solution with 2.45 mM potassium persulfate (1:1, *v/v*). The mixture was incubated for 12 to 16 min in the dark at room temperature. In the beginning of the assay, ABTS solution was diluted with methanol to reach an absorbance of 0.700 ± 0.02 at 734 nm. Then, 30 µL sample was added to 200 µL ABTS solution and vigorously mixed. After 30 min incubation at room temperature, the absorbance was read at 734 nm. The ABTS radical scavenging activity was expressed as milligrams of Trolox equivalents (mg TE/mL extract).

Saffron Extract Used in the Formulation of Emulsions

Three series of commercial emulsions oil / water (O/W) have been developed according to an initial base emulsion formulated with different type of emulsifier, co-emulsifier, and the stabilizing agent (Fig. 2). These three bases were tested in order to identify the formula that could offer the best compatibility with the skin (quick and even absorption and penetration), pleasant sensorial properties after application, and the best compliance for the patient. The emulsions contain 1 mL of saffron extract (S/L=1:16, solvent concentration 50% EtOH), and hyaluronic acid (HA): HA oligo and HA HMW.

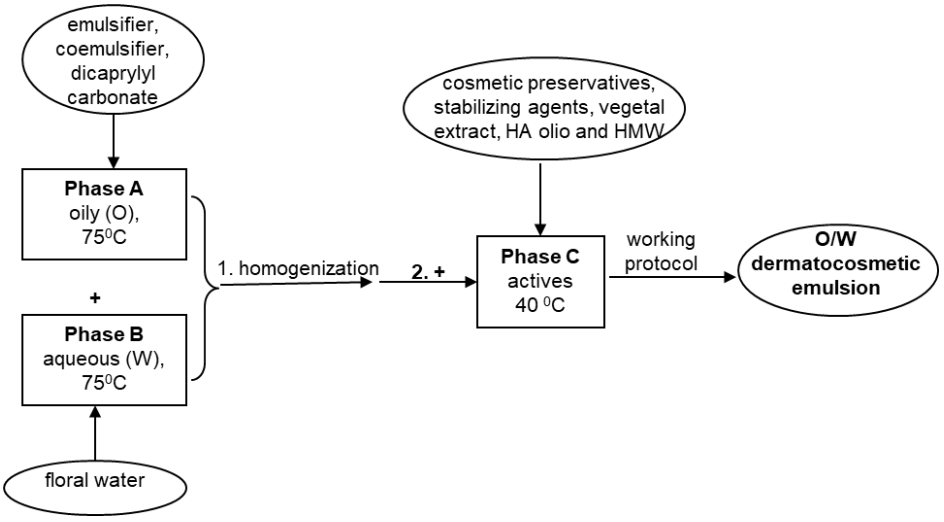


Fig. 2. Component phases and main stages involved in the formation of a dermato-cosmetic emulsion with Saffron extract

To obtain the final emulsion (Fig. 2), the aqueous (B) and oily phases (A) were heated to 75 °C, after which the oily phase (A) was added over the aqueous phase (B) by mixing using a rotor-stator homogenization (ESGE Zauberstab M 160 G Gourmet) operating at 15.000 rpm. The resulting mixture was cooled to 40 °C (using an ice bath), and afterwards the active ingredients and preservative (C-phase) were added. Finally a quantity of gel was obtained by dissolving HA oligo and HMW in Hamamelis flower water. For further studies on emulsions, weighed samples (15 g) were packed in brown glass containers with a capacity of 15 g. The samples were kept in cool rooms until the analysis was performed and for a maximum period of 90 days. After preparation, the emulsions were characterized by organoleptic analyses, pH, and stability after centrifugation. Prior to any analysis, the samples were allowed to return to room temperature.

RESULTS AND DISCUSSION

Determination of Extraction Degree

The extraction efficiency by maceration, heat reflux extraction, and ultrasound assisted extraction was evaluated under the established working methodology and extraction conditions (Table 1). For all vegetal extracts, residues were obtained after the complete evaporation of 5 mL of plant extract sample in a thermostatic oven (at the constant temperature of 50 °C). The results are presented in Table 1.

Table 1. Realized Extraction Degree by Liquid-Solid Extraction Methods

Extraction Method	Sample	Characteristics			Extraction Degree (%)
		Time	Ratio S:L	Solvent Concentration (%)	
Maceration (M)	M1	7 days	1:10	30	66.929
	M2			50	61.377
	M3			70	56.688
	M4	7 days	1:16	30	69.254
	M5			50	58.730
	M6			70	59.141
	M7	7 days	1:25	30	67.618
	M8			50	61.158
	M9			70	60.674
	M10	2 days	1:16	50	59.333
	M11	4 days	1:16	50	61.477
Heat Reflux Extraction (R)	R1	60 min	1:16	30	56.739
	R2			50	63.532
	R3			70	50.266
	R4	30 min	1:16	50	59.410
	R5	60 min	1:10	50	45.703
	R6		1:25		61.158
Ultrasound Assisted Extraction (US)	US1	4 min	1:16	30	46.660
	US2			50	45.600
	US3			70	39.286
	US4	4 min	1:10	50	48.307
	US5		1:25		48.701
	US6	2 min	1:16	50	47.856
	US7	6 min			46.077

The data from Table 1 indicated that the extraction process depends on the extraction method type, extraction time, composition of the extraction solvent, and solid/liquid ratio. Extraction efficiency was considered as a criterion for assessing the efficiency of the extraction methods studied. Its value was assessed depending on the method, S/L ratio, extraction time, and extraction solvent concentration. For maceration (M), maximum yields of 69.3% to 66.9% were obtained in conditions of: the operating time of 7 days and the concentration of the extractant (extraction solution) of 30%. Regarding the S/L ratio, high but very close values were obtained in the order of 1:16; 1:10, and 1:25, the choice of a certain ratio being dictated in this case strictly by economic conditions. For hot reflux extraction (R), yields of 63.5% to 61.2% were obtained at an extraction time of 60 min using a 50% hydro alcoholic solvent and at solid/liquid ratios of 1:16 and 1:25, respectively. In ultrasonic assisted extraction (US), the yields obtained were much lower (48.3% to 48.7%), but the highest was obtained for an extraction time of 4 min, solvent concentration of 50%, working with the solid/liquid ratios of 1:10 and 1:25.

In the following step, the best results obtained in each of the methods were compared. Regardless of the extraction method used, working with a solid/liquid ratio of 1:16 and with a hydro alcoholic solvent of 50% concentration resulted in optimal process yields.

Quantitative Characterization of the Vegetal Extract

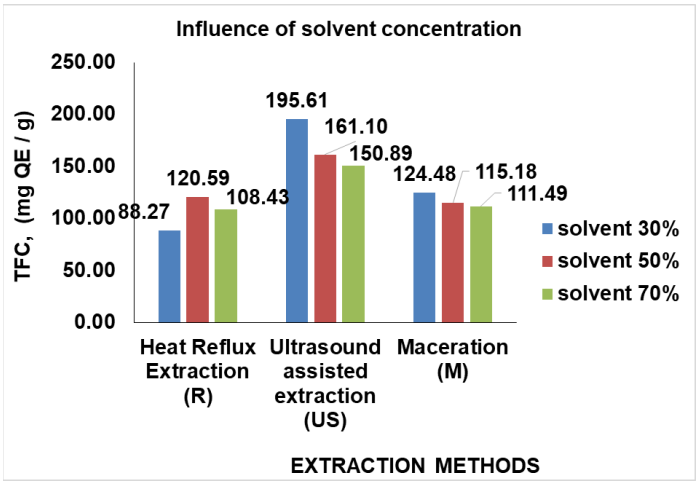
Determination of the total polyphenolic compounds and flavonoids concentration.

The results of all experiments performed in duplicate are presented in Figs. 3 and 4. The plant extracts obtained by any method and subsequently analyzed by physical-chemical methods contained a much higher amount of flavonoids than phenolic compounds. The analysis of the results presented in these two figures allowed a series of conclusions necessary to approach further studies.

In the case of flavonoids, taking as a criterion the concentration of the extraction solvent, the best results were obtained in the case of US (30%) - 195.61 mg QE/g; 161,1 mg QE/g (50%); 150.89 mg QE/g (70%) followed by M and R methods, with approximately equal values for all three concentrations of extraction solvent, respectively M (30%) - 124.48 mg QE/g and R (50%) -120.59 mg QE /g. Considering the value of the solid/liquid ratio as a comparison criterion, the best results were recorded in the case of R (1:10) - 197.84 mg QE/g, US (1:16) -161.19 mg QE/g and M (1:10) - 162.37 mg QE/g. In the case of extraction time, the results differed for US at 6 min with values of 201.99 mg QE/g, followed by R with 163.4 mg QE/g (60 min), US at 4 min with 161.1 mg QE/g, US at 2 min with 157.03 mg QE/g and the M variants and R at 30 min led to approximately equal values in the range (106.0 to 115.2) mg QE/g.

In conclusion, the best flavonoids results were obtained by US (conditions: S/L ratio of 1:16, solvent concentration 50% and extraction time of 6 min), US (conditions: S/L ratio 1:16, solvent concentration 30% and extraction time 4 min), and R (conditions: 1:10 S/L ratio, 50% solvent concentration and 60 min reflux time).

In the case of polyphenols, depending on the concentration of the extraction solvent, the best results were the ultrasonic-assisted extraction-US in case of all three concentrations, respectively 55.76 μg GAE/g, 51.69 μg GAE/g and 50.10 μg GAE/g followed by R - 30.61 μg GAE/g (30%) and the three variants of maceration with values in the range (27.11-28.23) μg GAE/g.



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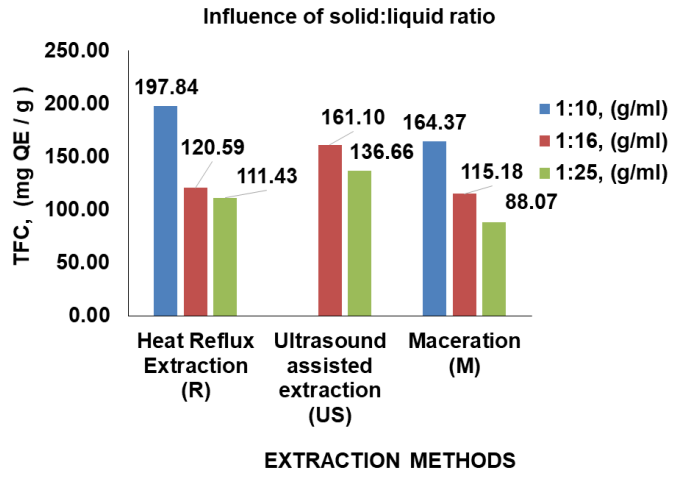

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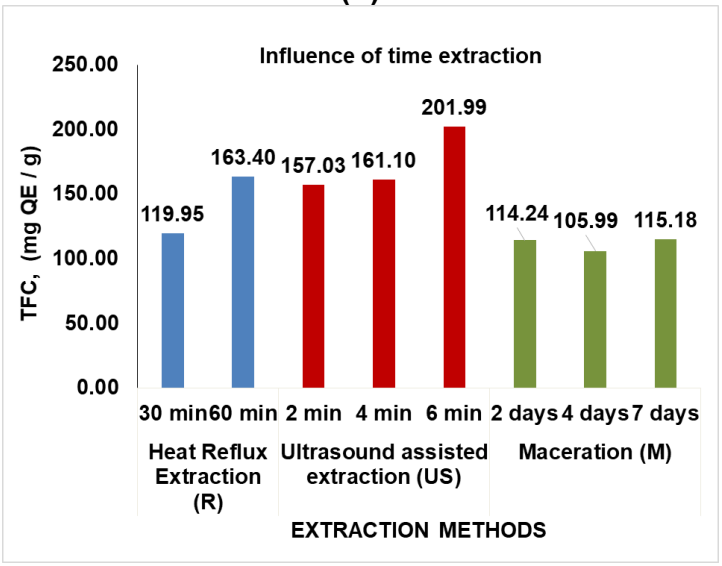
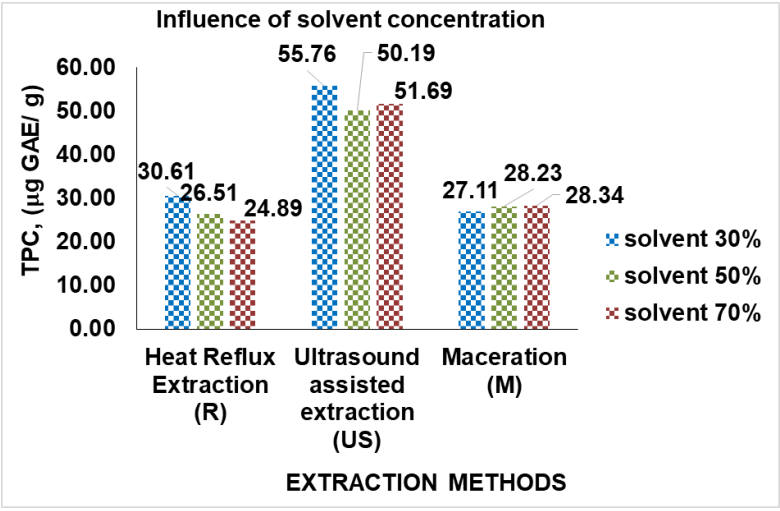
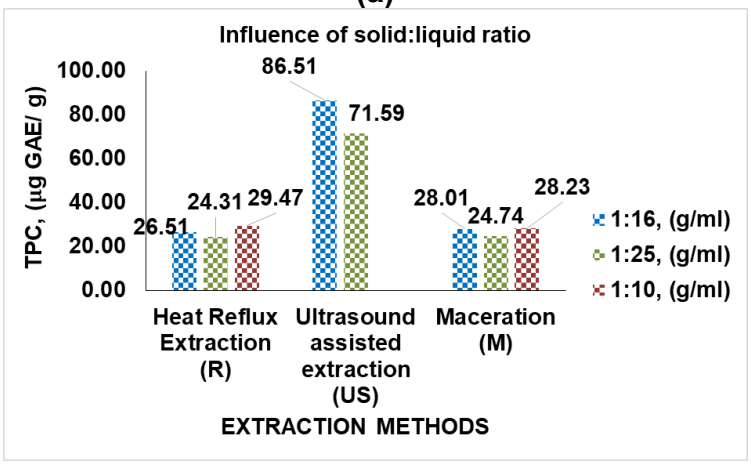


(c)

Fig. 3. The total flavonoids compounds (TFC) content (mg QE/mL) depending on the extraction method used and the physical parameters considered. Conditions: (a) solid/liquid ratio= 1:16; R-60 min, M-7 days and US 4 min; (b) solvent concentration = 50%; R-60 min, M-7 days and US 4 min;(c) solvent concentration = 50%; R-60 min, M-7 days and US 4 min



(a)



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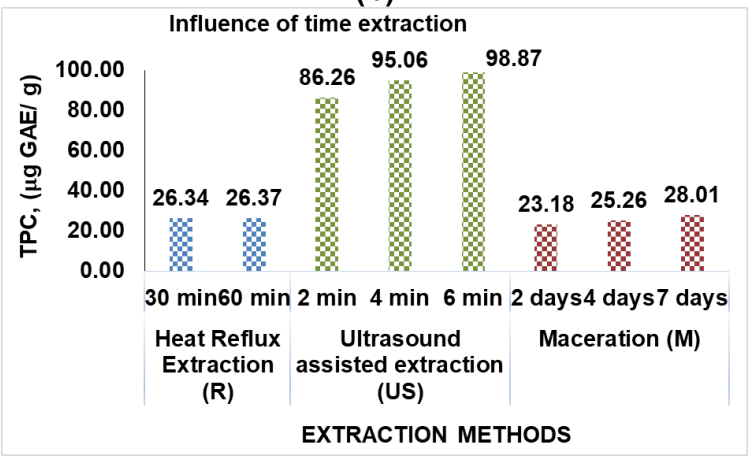


(c)

Fig. 4. The total polyphenols (TPC) compounds content (mg GAE/mL) depending on the extraction method used and the physical parameters considered. Conditions: (a) solid/liquid ratio= 1:16; R-60 min, M-7 days and US 4 min; (b) solvent concentration = 50%; R-60 min, M-7 days and US-4 min; (c) solvent concentration = 50%; R-60 min, M-7 days and US- 4 min

In the case of the solid/liquid ratio, the best results were recorded in the case of US: (1:16) - 86.51 and (1:25) -71.59 μg GAE/g followed by R and M variants with values obtained in the range (24.31- 29.47) μg GAE/g. Depending on the extraction time, the best values were obtained for the US variants: (6 min) - 98.89 μg GAE/g, (4 min) - 95.06 μg GAE/mL, (2 min).) - 86.26 μg GAE/g, followed by R and M variants with values obtained in the range (23.18-28.01) μg GAE/g.

In conclusion, the best polyphenols results were obtained by US in the following conditions: S/L ratio of 1:16, solvent concentration 50%, and time of 4 and 6 min. It was followed by R (S/L ratio of 1:16, solvent concentration 30%, and time of 60 min).

Depending on the type of bioactive compounds to be extracted, the method of ultrasound-assisted extraction or hot reflux extraction can be applied, using as conditions: extraction solvents based on ethyl alcohol 30% to 50%, solid/liquid ratio of 1:16, and an extraction time specific to each extraction procedure.

Evaluation the Antioxidant Activity

The antioxidant capacity the samples R2 and M5 were determined, as presented in Table 2. The extract obtained by R method had a higher antioxidant activity than that obtained by maceration (M). The values of the calculated sizes were not significantly different, which is in agreement with the content of polyphenols and flavonoids determined in their composition (Figs. 3 and 4).

Table 2. Antioxidant Activity of Investigated Extracts

Sample	DPPH (mg TE/mL)	ABTS (mg TE/mL)
R2 (R: 1:16; 50%; 60 min)	7.38 ± 0.41	28.23 ± 0.60
M5 (M: 1:16; 50%; 7 days)	5.74 ± 0.13	23.60 ± 0.52

Data are presented as mean ± standard deviation (SD) of three determinations. Abbreviations: ABTS - 2,2′-azino-bis(3-ethylbenzothiazoline) 6-sulfonic acid; DPPH - 1,1-diphenyl-2-picrylhydrazyl; TE - Trolox equivalents.

Physical-Chemical Characterization of Saffron Extracts Using UV-Vis Spectra Analysis

The qualitative and quantitative information presented above demonstrate that hydro alcoholic extraction by maceration had greater efficiency than the hot reflux or ultrasound-assisted extraction, and the amount of biologically active compounds extracted was higher. The wavelengths for the three main components, pure constituents of saffron, were the values of: 310 nm for saffron; 254 nm for picrocrocin, and 440 nm for crocin. These wavelength values may change slightly depending on the origin of the plant, the specific characteristics of the plant's cultivation and pre-processing (drying operation is important due to the high content of volatile oils in the plant), and the extraction technique applied. Also, the major metabolites may be present in the form of their derivatives (*e.g.*, trans and/or cis esters of crocetin) or of their partial transformation (such as those resulting from the conversion of picrocrocin to saffron) (Atyane *et al*. 2017). For this reason, two extracts obtained after maceration and hot reflux were analyzed by UV-Vis spectrophotometry, as illustrated in Fig. 5.

Analysis of the UV-Vis spectra of hydro-alcoholic saffron extracts can provide a number of preliminary information about the nature of the extracted compounds. A general feature of these extracts is the presence of a high content of flavonoids (Table 3). There were used for the UV-VIS analysis the extracts obtained by extraction with reflux at temperature (solid-liquid ratio = 1/10, extraction time = 60 min; 50% solvent concentration) and maceration (solid-liquid ratio = 1/10, extraction time = 7days; 50% solvent concentration). The extract resulting from the heat reflux extraction method is richer in extracted compounds than that obtained from maceration. Along with the main metabolites, there were a number of other secondary compounds, some with valuable antioxidant properties. Thus, the extraction method influences the classes of extracted bioactive substances, such as flavonoids, polyphenols, or carotenoids, as well as the different types of compounds present in saffron extracts.

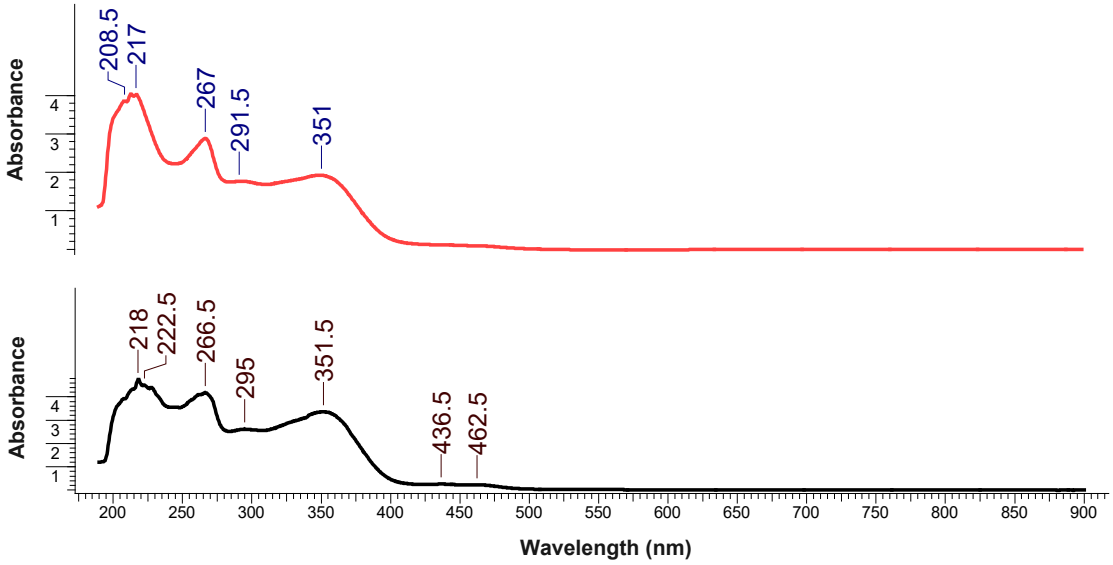


Fig. 5. UV-Vis spectra of Saffron extracts prepared by heat reflux extraction (black line) and maceration (red line)

Table 3. UV-Vis Characteristics of the Plant Extracts

Extraction method	λ_{max} (nm)	Possible compounds
Maceration solid-liquid ratio = 1/10, extraction time = 7 days; 50% solvent concentration	208.5; 217; 291.5; 351	Main component: picrocrocin (254-325 nm); safranal (325-425 nm) and crocin around 440 nm. Other secondary compounds: rosmarinic acid (218 nm); quercetin (351 nm); kaempferol (266.5 nm);
Heat reflux extraction solid-liquid ratio = 1/10, extraction time = 60 min; 50% solvent concentration	218; 222.5; 266.5; 295; 436.5; 462.5	

Preliminary Characterization of Emulsions Obtained on the Basis of Saffron Extracts

The stability of the cosmetic emulsions was preliminary evaluated through a series of physical analyses, such as pH, centrifuge test, evaluation of the sensory analyses (odor, color, texture, and general aspect), and study of microscopic images (Mahmood and Aktar 2013; Kim *et al*. 2020). The results obtained are summarized in Table 4.

Sensory Characterization

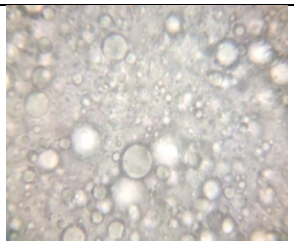
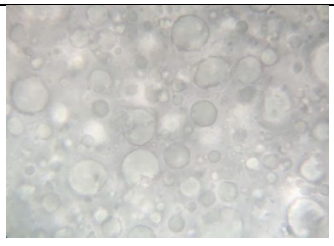
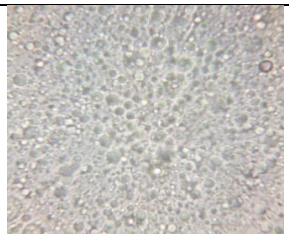
The color was bright white and slightly shiny. The texture was homogenous, firm, light/non-greasy, with no lumps detected after more than 72 h to 7 days. The substance was odorless, or with a very discreet, nonspecific odor.

pH Determination

The pH value of cosmetic emulsions was determined using a digital pH Meter (Hanna Instrument), as follows: 0.5 g of emulsion were dissolved in 50 mL of purified water and stored for two hours. The pH value was measured by inserting the electrode directly into the sample solution at 24.0 ± 2.0 °C. Following dilution with distilled water for pH test, emulsions presented a milky aspect and remained homogenous.

The pH of the cosmetic emulsion ranged from 4.99 to 5.04, which is considered adequate for topic application and acceptable to avoid the risk of irritation upon application onto the skin.

Table 4. Preliminary Characterization of Emulsions with Saffron Extract

Parameters	Emulsions		
	A2	B2	C2
Image after centrifugation			
pH	5.004	5.001	5.002
Stability after centrifugation	Intact texture and appearance	Thin upper layer of light foam	Intact texture and appearance
Organoleptic analyse	Colour: bright white, slightly shiny. Texture: homogenous, firm, light/non-greasy, with no lumps detected after more than 72 h to 7 days. Weak odor specific to emulsifier and floral water.	Colour: bright white, slightly shiny. Texture: homogenous, firm, light/non-greasy, higher viscosity than A2 and C2, with no lumps detected after more than 72 h. Weak odor specific to emulsifier and floral water.	Colour: bright white, slightly shiny. Texture: homogenous, firm, light/non-greasy, with no lumps detected after more than 72 h. Weak odor specific to emulsifier and floral water.
Microscopic image after 7 days of storage			

Centrifugation Test

The centrifugation test assesses potential instabilities of the emulsion, which may occur during precipitation, separation of phases, caking, or coalescence. The sample is

stressed during testing, which simulates the increase in the force of gravity and enhances the mobility of the particles. The test was performed by subjecting 5 g of sample to a cycle of 3000 rpm for 30 min at 25 °C (model XC-Spinplus). Once the centrifugation was completed, the formulations were examined for phase separation as an indicator of instability. The measurements of pH, conductivity, and the assessment of stability after centrifugation are presented in Table 6. Phase separation was not observed in any of the formulations tested during the centrifugation test.

Microscopic images

Microscopic images were taken from samples of the emulsions prepared after 7 days of storage under normal temperature conditions, using a binocular microscope Optika B-159 (OPTIKA S.r.l., Ponteranica (BG) - Italy), magnification - 1000x. The images (Table 4) show the uniformity of the sample, suggesting the maintenance of compatibility and phase homogeneity during the storage period.

All formulations showed a good physical and chemical stability. The incorporation of the natural product did not negatively impact the stability of the studied cosmetic formulations.

CONCLUSIONS

1. Hydro alcoholic plant extracts from saffron (*Crocus sativus* L.) bio waste (petals, tepals, and superior portion of the stem) were prepared using three solid/liquid extraction methods: maceration, heat reflux extraction, and ultrasonic assisted extraction.
2. Depending on the type of bioactive compounds extracted, the method of ultrasound-assisted extraction or hot reflux extraction can be applied, in the following conditions: extraction solvents based on ethyl alcohol 30% to 50%, solids/liquids ratio 1:16, and an extraction time specific to each extraction procedure.
3. The UV-VIS spectra of saffron extracts showed that the extraction method is very important in the extraction of certain classes and types of bioactive compounds.
4. The study of the saffron extract obtained was continued with its introduction in new dermato-cosmetic formulas with a role in combating the effects of oxidative stress on the skin or to protect it from the action of free radicals.
5. The three types of emulsions obtained (differentiated by the composition of the bases used in the formulation) were preliminarily analysed, by the pH value, the centrifugation test, the sensory analyses and microscopic images. The results showed that the incorporation of the natural extract did not negatively affect the stability of the studied dermato-cosmetic formulations.

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