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IN VITRO EVALUATION OF THE DERMATOCOSMETIC EMULSIONS BASED ON SAFFRON (*CROCUS SATIVUS*) ALCHOOLIC EXTRACTS

BY

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Abstract. The dermatocosmetic emulsion at the centre of this work simultaneously responds to the multiple needs of patients, related to the protective effects provided by the ingredients, but also to the comfort and pleasure in using the product. At the same time, the biologically active ingredients have a unique source, by capitalizing on the floral residues of saffron rich in biological compounds with significant antioxidant activity. The aim of this article is to test in vitro the behavior of some emulsions based on Saffron *(Crocus sativus)* plant extract. The results of the in vitro tests complete our previous studies and underline the fact that these emulsions can be made into new dermato-cosmetic formulations with a perspective in combating skin oxidative stress.

Keywords: dermatocosmetic emulsion; Franz cell; human health; in vitro evaluation; Saffron; vegetal extract.

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1. Introduction

Any new dermatological formula with potential success is created based on a need or opportunity, so the end result invariably adds value to either the consumer or the manufacturer. The chances of success increase when both benefits. The dermo-cosmetic emulsion at the centre of this work simultaneously responds to the multiple needs of patients, related to the protective effects provided by the ingredients, but also to the comfort and pleasure in using the product. At the same time, the biologically active ingredients have a unique source, by capitalizing on the floral residues of saffron.

Saffron (*Crocus sativus* L.), a perennial plant that belongs to the Iridaceae family in over 85 species and growing on almost every continent, is the source of what is considered the most expensive spice in the world. For that end-product used for colour, taste and aroma the only part exploited from the plant is the stigma, so that, after this procedure, residues (petals, tepals and the upper part of the stem) from 150,000 to 200,000 flowers are wasted when reaching only 1 kg of stigmas (Oreopoulou *et al*., 2021). These residues are considered to have important antioxidant activity (Caser *et al*., 2020).

Oxidative stress is a phenomenon extensively studied in recent years as a health threatening factor by maintaining or aggravating conditions throughout the body. There is evidence about the involvement of oxidative stress in neurological, cardiovascular, oncological, metabolic and dermatological diseases (Dasgupta and Klein, 2014; Baek and Lee, 2016; Chen *et al.,* 2021; Papaccio *et al*., 2022). Oxidative stress is an imbalance in the body's homeostasis, based on an overproduction of free radicals under the influence of factors that have multiplied as modern lifestyle brings changes in diet, sleep-wake rhythm, medication and mental state of people (Shankar and Mehendale, 2014).

The dermatocosmetic emulsions are oil/water type and is based on a combination of ingredients with a rigorously established role. Considering the pharmacological effects of saffron extract, the paper aims to highlight the process of diffusability through the membrane, essential in ensuring the biological effect from active ingredients. These tests will help confirm the status of dermato-cosmetic for the formula.

Permeation and penetration studies are an essential step in establishing dosage and adequate utilisation of a dermato-cosmetic formula and are usually realised with cellulose (dialysis membrane) or biological membranes (e.g. chicken skin).

The purpose of this article is to test *in vitro* the behavior of two emulsions, prepared based on the extract of the Saffron plant (*Crocus sativus*) based on preliminary permeation studies using a Franz cell with a cellulose membrane. The purpose of this article is to test in vitro the behavior of two emulsions, prepared based on the extract of the saffron plant (*Crocus sativus*) based on preliminary permeation studies using a cellulose membrane Franz cell. These studies are

intended to be a continuation and deepening of our previous studies (Turcov *et al.*, 2022a) regarding the use of alcoholic saffron extract in the formulation of dermatocosmetic products.

2. Experimental methodology

Materials

An alcoholic extract from *Crocus sativus*, obtained by liquid-solid extraction using Soxhlet equipment (followed the previously established extraction protocol (Turcov *et al*., 2022a)) using dry plant material with the following parameters: S:L ratio = 1:25; extraction time = 90 minutes; EtOH 70% extraction reagent. These extracts are characterized by the 2.03434 μg/mL concentration of polyphenols.

Crocus sativus extract was used as active compound to prepare two types of emulsions, differentiated by the type of base used in the formulation (A or B). The two bases differ by the nature of emulsifier and co-emulsifier, respectively by the presence or absence of a compound with the role of thickening agent, emulsion stabilizer (Table 1).

Table 1
The main components of the studied dermato-cosmetic emulsions

Compounds	**Bases involved**	
	A	**B**
Emulsifier	Polyglyceryl-6 Stearate (and) Polyglyceryl-6 Behenate	Metil Sesquistearat glucose
Coemulsifier	Glyceryl Stearate	Alcohol Cetilic
Thickening agent, emulsion stabilizer	Xanthan Gum	
Aqueous phase	Hamamelis Virginiana Rosa Damascene	
Cosmetic preservative	Cosgard benzyl alcohol, salicylic acid, glycerin, sorbic acid)	
Penetration enhancer of the vegetable oil into the skin and to improve the sensory performance of the final formulation	Dicaprylyl carbonate	
Oil phase	Calophyllum Inophyllum Seed Oil (Tamanu Oil)	

The purpose of using these different compositions was to increase the performance of the product through high penetration, obtaining a pleasant texture and excellent sensory characteristics for patients.

Before these *in vitro* tests, the stability of the emulsions was characterized based on a previously described protocol (Turcov *et al*., 2022b), study including organoleptic analyses, pH determination, phase stability after centrifugation, conductivity determination and microbiological control. Also, the total amount of polyphenols was determined using a standardized protocol based on the Folin - Ciocalteu reagent and Na_2CO_3 (Grochowski *et al.,* 2017; Pavun *et al.,* 2018) according to a detailed protocol in our previous studies (Turcov *et al*., 2022c).

Methods

In vitro diffusion study

Preliminary permeation studies were developed using a Franz cell equipped with a dialysis membrane (from Sigma and with 12400 Da porosity) in order to investigate as appropriately the application of emulsions with *Crocus sativus* extract as active ingredient by cutaneous route and to establish the necessary data for extending this study to a real skin (i.e. chicken skin) in order to investigate a behavior close to the real case.

The dialysis membrane (prepared by boiling for 60 minutes) ensures a penetration diameter of 1 cm^2 between the donor compartment (where a precise amount of emulsion, weighed, at room temperature, was introduced) and the receiver chamber (where a volume was ensured of approximately 5 mL bidistilled water). The working protocol took into account the results from the literature (Bujor *et al*., 2020; Abla and Banga, 2013) and from our previous studies (Turcov *et al*., 2022a,b).

The Franz cell was continuously maintained at room temperature (250C), and with an active magnetic stirrer in the receiving chamber. Samples of 150 μL emulsion were taken from the receptor chamber at predetermined intervals (ranging from 5 minutes to 24 hours), with the removed volume being replaced with the exact same volume of distilled water so that the volume in the compartment remained constant.

At every established timescale (from 5 minutes to 24 hours) samples of 150 μL emulsion were collected from the receptor chamber, the removed volume being replaced with the exact same amount of distilled water, such that the volume in the compartment to remain constant.

Total polyphenolic and flavonoids content

In the samples taken, the amount of polyphenols and flavonoids transferred through the dialysis membrane of the Franz cell was determined. For this, the spectrophotometric method with Folin-Ciocalteu reagent was used. The concentration was determined using the absorbance of the complex formed read at the characteristic wavelength of 765 nm using a Shimadzu UV-VIS spectrophotometer (model 1280, Kyoto, Japan) (Grochowski *et al*., 2017; Pavun *et al.*, 2018).

3. Results and Discussions

It was chosen to express the results in three different forms with different connotations. They are as follows:

a) the polyphenols released in the 5 mL of the receptor chamber, expressed in μg PF /mL versus time (Fig. 1);

b) the polyphenols releasing speed, expressed in μg PF /mL/t (Fig. 2);

c) the polyphenols permeation efficiency through membrane (Fig. 3).

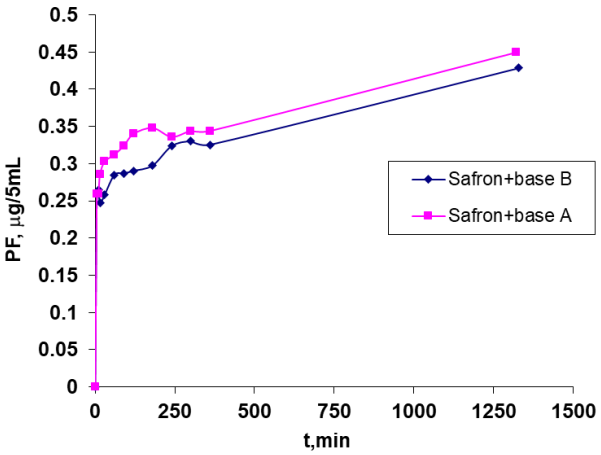

Fig. 1 – The total amount of polyphenols (PF) released in the receptor compartment (5mL) vs time.

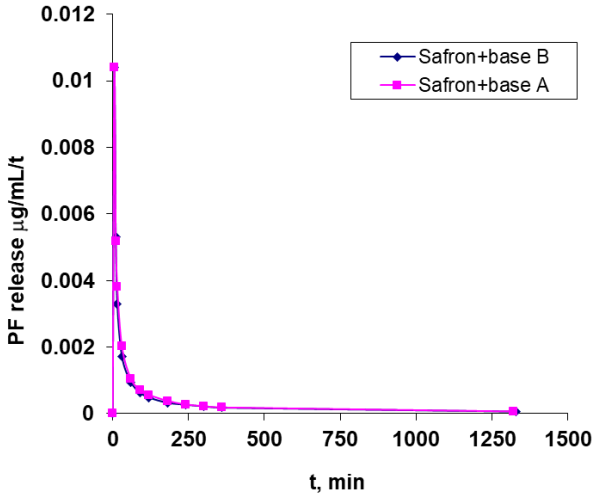

Fig. 2 – The rate of release of polyphenols expressed as PF μg/mL/t vs time.

From Fig. 2, it is clearly observed that the release rate of polyphenols is high at the beginning of the release process when the concentration gradient is maximum, after which it decreases to the minimum value after about 5-6 hours. The behaviour is similar for all 2 samples (with bases A and B) analysed.

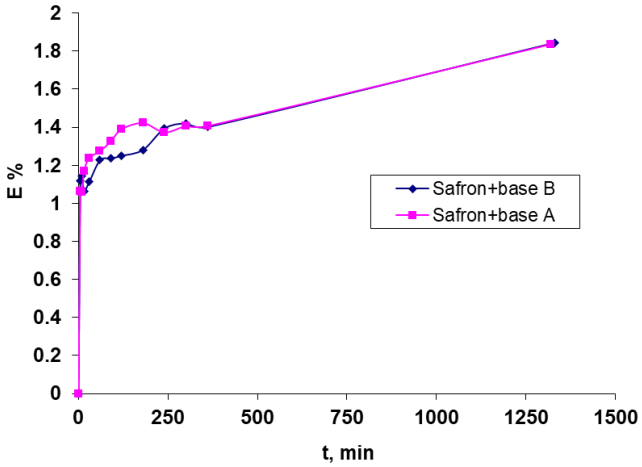

Fig. 3 – Efficiency of polyphenols (a) and flavonoids (b) permeation through the dialysis membrane.

Taking into account the fact that the system as a whole has relatively limited dynamics, especially in the donor compartment, but also the fact that the formulations used are relatively viscous, the low release efficiency is explained by the fact that the active principles are released mainly from the superficial layers, those located towards the dialysis membranes. It should also be taken into account that a massive passage of polyphenols through the membrane is not pursued, their effect being desirable on the surface of the membrane (dermis). Analysis of the resulting data (Figs. 1-3) suggests that the type of base used to create the two forms does not significantly influence how the active principle is released from them. The role of assets remains that of printing a certain product texture.

4. Conclusions

The *in vitro* test findings complete our earlier research and demonstrate that these emulsions can be turned into novel dermato-cosmetic formulations with the aim of reducing oxidative stress at the skin's surface. The goal of emphasizing the diffusion system's functionality in the context of using a new emulsion has been accomplished, and this study serves as an essential first step for future research (*in vivo*).

These outcomes will also enable the study's continuation into its last phase, which includes skin testing and patient follow-up.

REFERENCES

Abla M.J., Banga A.K., *Quantification of skin penetration of antioxidants of varying lipophilicity*, Int. J. of Cosmetic Sci., **35**(1), 19-26, https://doi.org/10.1111/j.1468-2494.2012.00728.x (2013).

Baek J., Lee M.G., *Oxidative stress and antioxidant strategies in Dermatology*, Redox Report - Communications in Free Radical Research, **21(4),** 164-169 (2016).

Bujor A., Ochiuz L., Sha`at M., Stoleriu I., Stamate Iliuta M., Luca S.V., Miron A., *Chemical, antioxidant and In vitro permeation and penetration studies of extracts obtained from Viburnum opulus and Crataegus pentagyna*, Farmacia, **68(4**), 672-678 (2020).

Caser M., Demasi S., Stelluti S., Donno D., Scariot V., *Crocus Sativus l. cultivation in alpine environments: stigmas and tepals as source of bioactive compounds*, A*gronomy*, **10**(10), 1473, doi: 10.3390/agronomy10101473 (2020).

Chen J., Liu Y**.,** Zhao Z**.,** Qiu J**.,** *Oxidative stress in the skin: Impact and related protection*, Int J Cosmet Sci., **43**, 495–509, doi: 10.1111/ics.12728 (2021).

Dasgupta A., Klein K., *Combating Oxidative Stress with a Healthy Lifestyle*, In Antioxidants in Food, Vitamins and Supplements, Dasgupta A. and Klein K. (Eds), Elsevier. Inc., 317-333, https://doi.org/10.1016/B978-0-12-405872-9.00016-1 (2014).

Grochowski D.M., Uysal S., Aktumsek A., Granica S., Zengin G., Ceylan R., Locatelli M., Tomczyk M., *In vitro enzyme inhibitory properties, antioxidant activities, and phytochemical profile of Potentilla thuringiaca*, Phytochem. Lett., **20**, 365-372 (2017).

Oreopoulou A., Choulitoudi E., Tsimogiannis D., Oreopoulou V., *Six common herbs with distinctive bioactive, antioxidant components. a review of their separation techniques*, Molecules, **26**(10), 2920, doi:10.3390/molecules26102920 (2021).

Papaccio F., D'Arino A., Caputo S., Bellei B., *Focus on the Contribution of Oxidative Stress in Skin Aging*, Antioxidants, **11**, 1121, https://doi.org/10.3390/antiox11061121 (2022).

Pavun L., Đikanovic P., Jelikic-Stankov M., Đurdevic D., Uskokovic-Markovic S., *Determination of flavonoids and total polyphenol contents in commercial apple juices*, Czech J. Food Sci., **36**, 233–238 (2018).

Shankar K., Mehendale H.M., *Oxidative Stress*, Encyclopedia of Toxicology (3rd edition), 735-737, http://dx.doi.org/10.1016/B978-0-12-386454-3.00345-6 (2014).

Turcov D., Barna A.S., Apreutesei (Ciuperca) O.T., Puitel A.C., Suteu D., *Preliminary processing of floral bio-residues of saffron (crocus sativus l.) as an innovative resource for development of high added-value cosmetic products*, BioResource, **17(3**), 4730-4744, (2022a).

Turcov D., Peptu A.C., Barna A.S., Zbranca A., Șuteu D., *In vitro evaluation of the dermato-cosmetic emulsions based on Lady's Bedstraw (Galium verum) alcoholic extracts*, The 10th IEEE International Conference on E-Health and

Bioengineering - EHB 2022 “Grigore T. Popa” University of Medicine and Pharmacy, Iași, Romania, November 17-18, 2022 (2022b).

Turcov D., Barna A.S., Blaga A.C., Ibanescu C., Danu M., Trifan A., Zbranca A., Suteu D., *Dermatocosmetic Emulsions Based on Resveratrol, Ferulic Acid and Saffron (Crocus sativus) Extract to Combat Skin Oxidative Stress-Trigger Factor of Some Potential Malignant Effects: Stability Studies and Rheological Properties*, Pharmaceutics, **14**, 2376, doi.org/10.3390/pharmaceutics14112376 (2022c).

EVALUAREA *IN VITRO* A EMULSIILOR DERMATOCOSMETICE PE BAZĂ DE EXTRACTE ALCOOLICE DE ȘOFRAN (*CROCUS SATIVUS*)

(Rezumat)

Emulsia dermatocosmetică aflată în centrul acestei lucrări răspunde simultan nevoilor multiple ale pacienților, legate de efectele protectoare oferite de ingrediente, dar și de confortul și plăcerea de a folosi produsul. În același timp, ingredientele biologic active au o sursă unică, prin valorificarea reziduurilor florale de Șofran bogat în compuși biologici cu activitate antioxidantă semnificativă. Scopul acestui articol este de a testa *in vitro* comportamentul unor emulsii pe baza de extract din deșeuri din planta de Șofran (*Crocus sativus*). Rezultatele testelor *in vitro* completează studiile noastre anterioare și subliniază faptul că aceste emulsii pot fi transformate în noi formulări dermatocosmetice cu perspectivă în combaterea stresului oxidativ al pielii.