

Moisturizing effect of stable cream containing *Crocus sativus* extracts

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Abstract: The present study is about to prepare stable cream of water-in-oil emulsion containing extracts of *Crocus sativus* against its base (without extracts) taken as control, to determine its stability on different storage conditions and effects on skin moisture contents and transepidermal water loss. The formulation contains 3% *Crocus sativus* (Saffron) concentrated extracts, and the base containing no extract, were formulated. Different stability tests were done on samples, which placed at 8°C, 25°C, 40°C and 40°C with 75% relative humidity, for 4 week period. These formulations (Creams) were applied on the cheeks of human volunteers for 8week period. To evaluate any effect produced by these formulations different skin parameters were monitored every week. The significant results of this study explored the fact that water-in-oil emulsion topical cream of saffron formulated from *Crocus sativus* extract has absolute physical stability at different storage conditions. The increase in skin moisture contents and changes in transepidermal water loss were significant ($p \leq 0.05$) with respect to base and formulation respectively. Topical cream of *Crocus sativus* showed significant moisturizing effects on human skin.

Keywords: *Crocus sativus*, oil in water emulsion, physical stability, skin moisture, transepidermal water loss.

INTRODUCTION

An emulsion is a thermodynamically unstable system which contains at least 2 immiscible liquid phases one is dispersed as globules in the other liquid phase and this system is stabilized by an emulsifying agent. These are widely used by the pharmaceutical, food and cosmetic industry. Their advantages include: complete protection of the incorporated drug, increased therapeutic characteristics and spread ability of its constituents, more controlled drug absorption and penetration, prolonged action and greater emollient effects compared to other preparations, and use of water as an inexpensive diluent and good solvent for many drugs and flavors (Akhtar *et al.*, 2012). Both O/W and W/O type emulsion are used to prepare effective creams and lotions for topical use. Topical emulsions possess more patient acceptance due to high degree of elegance, and are easy to wash. Topical emulsions are more effective if are thixotropic in nature. Microemulsions and multiple emulsions are of prime importance in the field of cosmetics and dermatology. Microemulsion of different steroids and non-steroidal anti-inflammatory drugs showed prolonged action as compare to conventional method (Mestress and Nielloud, 2002). *Crocus sativus* is a valuable herb due to its traditional uses in folk medicine. It has been used as spice, food colorant, perfume and as textile dye (Cavusoglu *et al.*, 2009; Abdullaev and Espinosa, 2004; Hadizadeh *et al.*, 2003; Mofleh *et al.*, 2006; Moghaddasi MS, 2010; Soeda *et al.*, 2007; Golmohammadzadeh *et al.*, 2009). *Crocus sativus* exhibit strong antioxidant activity. It is due to the carotenoids and flavonoids constituents.

Crocin of saffron has many pharmacological uses (Arasteh A *et al.*, 2010; Li *et al.*, 2004; Li and Wu, 2002; Hadizadeh *et al.*, 2003; Chen *et al.*, 2008). It increases the skin moisture contents due to phenolic compounds quercetin and kaempferol present in it (Halvarsson and Loden, 2007).

MATERIAL AND METHOD

Material

The plant material was dried stigmas of *Crocus sativus* linnus. The identification of dried stigmas was done at Department of Pharmacy, Khawaja Freed Campus, The Islamia University of Bahawalpur, Bahawalpur, Pakistan. For emulsion preparation extracts of dried stigma of *Crocus sativus* were used. The emulsifying agent; ABIL-EM90 from Franken Chemical (Germany), Paraffin Oil from Merk KgaA Darmstadt (Germany), Ethanol from Merk KgaA Darmstadt (Germany), was purchased. Distilled water was taken from the Department of pharmacy, IUB, Pakistan.

Apparatus

Cutometer MPA 580 (Courage and Khazaka, Germany), Conductivity meter WTW COND- 197i (Germany). Centrifuge machine EBA 20 by Hettich (Germany), Digital humidity meter TES Electronic Corp. (Taiwan), Cold incubator MIR-153 by Sanyo (Japan), WTW 197i PH-Meter (Germany), and SPSS 15.0 is used for the analysis of results.

Formulation development

In this study, Water-in-oil emulsion was prepared by adding the aqueous phase to the non-aqueous phase under continuous agitation (Naveed *et al.*, 2011).

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Study design

A single-blind study was used for comparison of these two creams. The creams were named as A (Active formulation) and B (Base) and given to the volunteers with complete knowledge of their application.

10 volunteers with the age limit of 25 to 40 years were selected for this study. The temperature conditions of Patch tests were 25°C and 40% relative humidity and the formulation is applied on the forearms of each volunteer, on the first day of sampling. On the second day, two creams were provided to each volunteer, one of the creams was base and the other was formulation containing the active ingredients. Each cream was marked with “right” or “left” which indicate the application of that cream to the respective cheek. Every volunteer applied these creams for a period of 8 weeks.

Mathematical analysis

The percentage changes of different parameters in all individuals were taken each week and were calculated by following formula;

$$\text{Percentage Change} = [(A - B) / B] \times 100$$

Where,

A = Individual value of any parameter of 1st, 2nd, 3rd, 4th, 6th, or 8th week.

B = Zero hour value of that parameter

RESULTS

Emulsion Stability

The product is stable at different storage conditions i.e. 8°C, 25°C, 40°C and 40°C with relative humidity of 75%. No change in color, Liquefaction and phase separation were shown by formulation until 28 days as shown in Table 1. The Centrifugation test was done for both creams (base and formulation) placed at different storage conditions.

pH test

Average changes in pH values of both creams from the time of preparation up to week 4 of the study period on different storage conditions were determined and shown in table 2. The pH values were measured immediately after preparation, then after 12, 24, 36, 48 and 72 hrs, then up to 4 weeks on weekly basis.

Evaluation of transepidermal water loss

The change in transepidermal water loss after application of base and formulation on 10 volunteers was determined and shown in table 4. Significant results were obtained by saffron formulation containing *Crocus sativus* extracts, due to phenolic compounds of saffron and due to terpenoids present in saffron (Golmohammadzadeh *et al.*, 2009) as shown in table 3.

Evaluation of skin moisture contents

After application of base and formulation on 10 volunteers the percent change in skin hydration level was measured. The significant increase in skin moisture contents in the skin of volunteers was observed with respect to base and time as given in table 4.

DISCUSSION

Optimum stirring speed during formation of emulsion is essential as because over-emulsification can happened due to high speed stirring. Homogenizers with stirrer, stripper and rotor-star are more productive with ABIL[®] EM90. In this study careful stirring with varying stirring speed (2500, 1000 and 500 rpm) was performed to avoid over-emulsification.

The normal range of skin pH is 4.5 to 6.0. Therefore topical creams must be in this range (Jennifer *et al.*, 2003). In this study the base samples were placed at special storage conditions i.e. 8°C, 25°C, 40°C and 40°C± 75% Relative Humidity shown minor decrease in pH in week 3rd and 4th. While samples of formulation shown decline in pH at all test conditions and time intervals. The statistical evaluation of creams showed that changes in pH of base samples were insignificant with respect to time but significant results obtained by the formulation.

The outward transmission of water through the skin is known as Transepidermal water loss (TEWL). An increase in TEWL indicates an impaired water barrier. The anatomical site, sweating, temperature of skin surface, inter and intra-individual variations, air convection, ambient air humidity and instrument related variables effect the TEWL measurements (Akhtar *et al.*, 2012). During in-vivo study of 8 weeks with base and formulation samples, formulation decreased more promptly skin water loss as compared to the base.

The skin becomes dry with aging but it is normalized by moisturizers. In this study skin moisture contents were increased by formulation due to phenolic compounds quercetin, and kaempherol which proved to be potent moisturizers and antisolaragents (Golmohammadzadeh *et al.*, 2009). It shows that base samples, increased the skin moisture contents irregularly but formulation gradually increased the skin moisture contents. According to ANOVA two way analyses for skin moisture contents, both base and formulation showed significant effects.

Skin moisturizers enhanced the moisture contents of skin, which leads to hydration of stratum corneum. Lipids are used mainly in formulation of moisturizers due to their hydrophobic nature (Akhtar *et al.*, 2010).

Exposure to UV rays results in loss of body water, which is known as total epidermal water loss. Stratum corneum

Table 1: Stability study profile of both base and formulation with time

		Fresh		After 12 hrs		After 24 hrs		After 7days		After 14days		After 21days		After 28days	
		B	F	B	F	B	F	B	F	B	F	B	F	B	F
Color	A	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW
	B	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW
	C	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW
	D	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW	W	YW
Liquefaction	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve
Phase Separation	A	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	B	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	C	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
	D	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

B=base, F= formulation, YW=YELLOWISH, W= White, -ve= No change, +ve= little change; A= At 8°C; B= At 25°C; C= At 40°C; D= At 40°C +75% Relative Humidity (RH).

Table 2: pH value (mean $\pm$ standard error of mean) at different storage conditions

	8°C	25°C	40°C	40°C $\pm$ 75% RH
Base	6.777 $\pm$ 0.102	6.996 $\pm$ 0.517	6.869 $\pm$ 0.125	6.822 $\pm$ 0.51
Formulation	6.274 $\pm$ 0.059	6.175 $\pm$.081	6.170 $\pm$.069	6.154 $\pm$.032

RH= Relative Humidity

Table 3: Percentage Changes in Values of Transepidermal Water Loss (TEWL) after Application of Base and Formulation (Mean $\pm$ standard error of mean) with Time

	1st Week	2nd Week	3rd Week	4th Week	6th Week	8th Week
Base	28.781 $\pm$ 1.861	24.102 $\pm$ 5.215	36.832 $\pm$ 5.549	37.979 $\pm$ 4.520	45.905 $\pm$ 7.046	29.028 $\pm$ 5.667
Formulation	-2.891 $\pm$ 8.573	-3.954 $\pm$ 8.285	-4.896 $\pm$ 10.246	-5.28 $\pm$ 11.1	-5.874 $\pm$ 11.51	-7.105 $\pm$ 8.444

Table 4: The Percentage of Change in Values of Skin Moisture after application of Base and Formulation (Mean $\pm$ standard error of mean) with Time

	1st Week	2nd Week	3rd Week	4th Week	6th Week	8th Week
Base	-26.396 $\pm$ 18.280	-15.506 $\pm$ 11.614	-18.346 $\pm$ 7.119	-28.796 $\pm$ 13.970	-16.792 $\pm$ 14.329	-24.351 $\pm$ 17.124
Formulation	0.081 $\pm$ 11.728	1.662 $\pm$ 12.498	2.81 $\pm$ 14.49	3.04 $\pm$ 14.05	6.476 $\pm$ 12.51	8.104 $\pm$ 14.43

water affinity of skin controls this change. When there is excessive loss of water and water level of stratum corneum decreased then 10% it leads to drying of skin, reduction in flexibility of skin (Abdullaev and Espinosa, 2004).

CONCLUSION

It is concluded from above results that oil-in-water emulsion topical cream of saffron containing *Crocus-sativus* extracts has significant effects on skin. The cream formulated from *Crocus sativus* extract is stable and has good cosmetic appeal, absolute physical stability, complete absence of color changes and phase separation. Also it is concluded from the results that formulation reduces Transepidermal water loss, and enhances the skin

hydration level. It exhibits depigmenting, anti-aging and skin hydration effects. More research is needed on saffron and its constituents to explore it cosmetically.

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