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IN VITRO CHARACTERIZATION AND ASSESSMENT OF COSMETIC POTENTIALS OF W/O EMULSION CREAM CONTAINING 2% *PROSOPIS CINERARIA* EXTRACT

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Abstract: In recent years, cosmetic research shifted towards natural products. Attempts are made to formulate stable topical preparations containing natural bioactive constituents but instability of topical emulsion is still a challenge for researchers. The aim of this study was to formulate stable W/O emulsion cream loaded with pre-concentrated 2% *Prosopis cineraria* extract and to explore its *in vitro* characteristics as compared to base (without extract). Moreover, stable W/O emulsion cream was applied to healthy human volunteers to assess its cosmetic potentials. The samples of base and formulation were placed at 8, 25, 40°C and 40°C at 75% RH (relative humidity) in different stability chambers. Stability parameters i.e., color, liquefaction, phase separation by centrifugation, conductivity, pH were monitored for 28 days for both base and formulation by using mechanical instruments. Healthy human volunteers (n = 11) were used for panel test. Results were analyzed by using SPSS 15.0. It was found that both base and formulation were stable with respect to color, liquefaction and phase separation with time. Furthermore, zero conductivity was found by all the samples of base and formulation kept at 8, 25, 40°C and 40°C at 75% RH in different stability chambers for 28 days. The pH of base and formulation kept at 8°C was 5.64 ± 12.6 and 5.54 ± 7.92, at 25°C was 5.77 ± 14.5 and 5.27 ± 13.5, at 40°C was 5.17 ± 16.1 and 5.13 ± 11.4 and at 40°C at 75% RH was 5.8 ± 7.3 and 4.92 ± 9.2, respectively. Statistically significant panel test results were obtained. It is concluded that the formulation W/O emulsion cream is stable with respect to *in vitro* evaluation and results of panel test coined that it can be used cosmetically.

Keywords: W/O emulsion cream, *in vitro* characterization, pH, panel test

Emulsions are metastable colloids made out of two immiscible fluids, one being dispersed in other, in the presence of surface active agents. They are obtained by shearing two immiscible fluids leading to the fragmentation of one phase into the other. They are thermodynamically unstable and revert back to separate oil and water phases until kinetically stabilized by third component, the emulsifying agent. Because the shelf life of emulsions may become significant (more than a year), they become good candidates for various commercial applications (1).

In cosmetology, the quality of a product is determined by high merit of its stability (2). Stability testing of cream is one of the most important quality control measures of topical preparations. Shelf lives, temperature, humidity, sunlight, shipment, abrasion are some keen factors which directly affect the stability of creams (3). Water-in-oil emulsion systems do have desirable properties. However, one drawback to the use of such systems for commercial products is the difficulty associated with maintaining such systems stable against separation. World consumers are now focused on their health and well-being more than before. Different words such as ‘natural’, ‘organic’, ‘no artificial preservatives’ and ‘no animal ingredients’ are drawing alarming attention. This trend increase the demand

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for products formulated as cosmeceuticals with natural and nutraceutical ingredients. Active ingredients and new delivery systems are driving the new product development ground (4).

According to these trends, researchers struggle to develop highly differentiated products with center of treatment as well as aesthetics. A significant number of novel products are based on new natural bioactive ingredients. With these promising actives, come a variety of formulation challenges that includes stability control and the complications of combining several actives into a sole cosmetic product (5). The present study aimed to formulate stable emulsion cream containing natural plant extract and to ensure its aesthetic potentials.

MATERIALS AND METHODS

The dried bark of plant (*Prosopis cineraria*) was collected from a field owned by Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Bahawalpur, Pakistan. The identification of dried bark was done at CIDS and voucher no. was 125/.I.U.2012. Paraffin oil was of Merck KGaA Darmstadt, Germany, emulsifying agent ABIL® EM 90 (Cetyl-PEG/PPG-10/1 Dimethicone) was purchased from Franken Chemicals, Gebinde, Germany and 70% ethanol was from Merck KGaA Darmstadt, Germany. The bees wax and deionized water was obtained from Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur Pakistan.

Instruments

Centrifuge machine (Hettich EBA 20, Germany), cold incubator (Sanyo MIR-153, Japan), conductivity-meter (WTW COND-197i, Germany), digital humidity meter (TES Electronic Corp., Taiwan), electrical weight balance (Precisa BJ-210, Switzerland), homogenizer (Euro-Star, IKA D 230, Germany), hot incubator (Sanyo MIR-162, Japan), pH-Meter (WTW pH-197i, Germany), rotary evaporator (Eyela, Co. Ltd., Japan), refrigerator (Orient, Pakistan), water bath (HH .$S_{21\ 4}$, China) and SPSS 15.0 was used for statistical analysis.

Preparation of formulation

The oily phase used in the preparation of formulation comprises of paraffin oil, bees wax, cetyl PEG/PPG-10/1 Dimethicone with HLB value 4-6 and heated up to75 ± 1°C. Meanwhile, aqueous phase (distilled water) was also heated up to 75 ± 1°C. The 2% preconcentrated *Prosopis cineraria* bark extract was added in the aqueous phase. After heating, aqueous phase was added to the oily phase drop by drop. Stirring was continued at 2000 rpm by the mechanical mixer for 15 min until all aqueous phase was added; 2 to 3 drops of rose water were added during this stirring time to give good fragrance to the cream. After complete addition of aqueous phase, speed of mixer was reduced to 1000 rpm for homogenization, for a period of 5 min and then speed of mixer was reduced to 500 rpm for further 5 min for complete homogenization; until cooled to room temperature.

Preparation of base

The procedure adopted for the preparation of base was the same as for formulation except that no extract was incorporated in aqueous phase of base cream (Table 1).

Participants

Eleven human volunteers were chosen whose ages were between 25 and 40 years. Only male volunteers were included in this study. Volunteers were examined for any serious skin disease or damage especially on cheeks and forearms. Every volunteer was provided with a volunteer protocol before continuation of study. Every volunteer signed the terms and conditions of the protocol for testing individually.

Table 1. Composition of emulsion (creams).

Emulsion phase	Ingredients	Emulsion base (g)	Extract emulsion (g)
Oily	Paraffin oil	14	14
	Cetyl-PEG/PPG-10/1 Dimethicone	3.5	3.5
	Bees wax	2	5
Aqueous	Extract	——	2
	Distilled water	100	100

Table 2. Stability characteristics of base and formulation at 8°, 25°, 40°C and 40°C ± 75% RH.

		Fresh		After 12 h		After 24 h		After 7 days		After 14 days		After 21 days		After 28 days	
		B	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**	**B**	**F**
Color	**A**	W	LP	W	LP	W	LP	W	LP	W	LP	W	LP	W	LP
	B	W	LP	W	LP	W	LP	W	LP	W	LP	W	LP	W	LP
	C	W	LP	W	LP	W	LP	W	LP	W	LP	W	LP	W	LP
	D	W	LP	W	LP	W	LP	W	LP	W	LP	W	LP	W	LP
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
Conductivity	**A**	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	B	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	C	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N

B = base, **F** = formulation, LP = light pink, W = white, -ve = no change, +ve = little change, N = zero electrical conductivity; **A** = at 8°C; **B** = at 25°C; **C** = at 40°C; **D** = at 40°C + 75% RH (RH = relative humidity).

Ethical approval

This study was approved by the Board of Advanced Study and Research (BASR), The Islamia University of Bahawalpur, and the institutional ethical committee, Department of Pharmacy, The Islamia University of Bahawalpur, Bahawalpur, Pakistan, in compliance with NIH Principles of Laboratory Animal Care, 1985. The reference no. is Pharm 1991/2013.

RRSULTS AND DISCUSSION

In vitro evaluation of W/O emulsion creams

For *in vitro* evaluation of both creams, formulation (having *Prosopis cineraria* extract) and base (without extract) were placed at different temperatures i.e., at 8, 25 and 40°C and 40°C at 75% RH (relative humidity) in stability chambers for 28 days (Table 2). No change in color, liquefaction and phase separation was observed; furthermore, the electrical conductivity test was also negative for each sample of creams. The samples of formulation evaluated at 8, 25 and 40°C and 40°C at 75% RH after of study period are shown in Figure 1.

In this study, the light pink color of the formulation was due to the presence of significant amount of tannins and tryptamines in the extract of plant. In formulation extracts were used as active ingredient and it is a rich source of natural antioxidants. These antioxidants have a potential of natural preserving

Table 3. pH value (mean ± standard error of mean) at different storage conditions of temperature.

	8°C	25°C	40°C	40°C ± 75% RH
F	5.54 ± 7.92	5.27 ± 13.5	5.13 ± 11.4	4.92 ± 9.2
B	5.64 ± 12.6	5.77 ± 14.5	5.17 ± 16.1	5.8 ± 7.3

F = formulation, B = base, RH = relative humidity

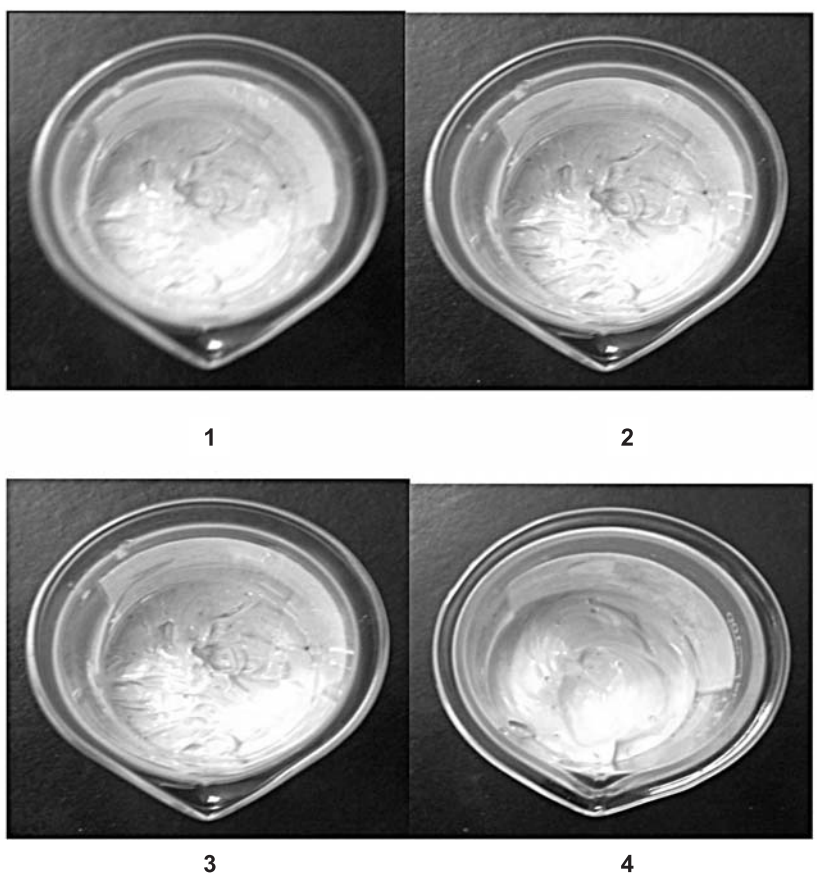

Figure 1. Samples of formulation. **1** = 8°C, **2** = 25°C, **3** = 40°C, **4** = 40°C ± 75% RH

Table 4. Panel test for base and formulation.

	Average points for base ± SEM	Average points for formulation ± SEM
Ease of application	4.21 ± 0.004	4.86 ± 0.003
Sense just after application	3.22 ± 0.004	4.02 ± 0.003
Sense in long term	3.68 ± 0.005	3.98 ± 0.005
Spreadability	4.01 ± 0.003	5.02 ± 0.004
Irritation	0.00 ± 0.000	0.00 ± 0.000
Shine on skin	3.47 ± 0.006	3.22 ± 0.006
Sense of softness	4.42 ± 0.005	4.54 ± 0.004

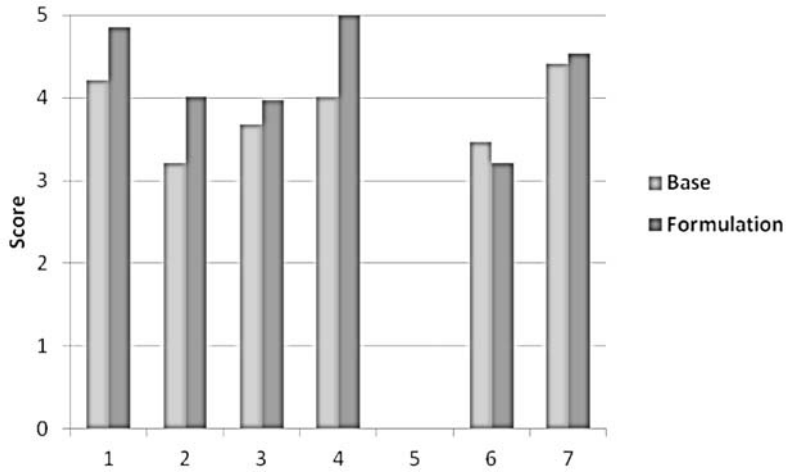

Figure 2. Average values for panel test. 1 = Ease of application, 2 = Sense just after application, 3 = Sense in long term, 4 = Spreadability, 5 = Irritation, 6 = Shine on skin, 7 = Sense of softness

and may protect the cream from microbial growth and of oxidative degradation, which may change the color of cream during shelf life. Many other factors support color stability of formulation and base such as Cetyl PEG/PPG-10/1 Dimethicone -a colorless, non toxic and clear liquid emulsifying agent (6). It acts at interphase of dispersed and continuous phase and properties of emulsion cream heavily depend on its nature and type. Bees wax is naturally produced by bees and used for a long time to control the change in color of creams, paraffin oil is a blend of purified liquid hydrocarbons and it controls the viscosity and stability of creams. Stock's Law reveals the fact that increasing the viscosity of the continuous phase enhances the shelf life of topical preparations (7).

pH test

Skin pH is an important indicator of topical preparation stability especially in case of creams (2). The average pH of human skin ranges from 5.5 to 6. Hence, the pH of topical preparations must be in accordance with skin's pH (7). The change in pH values of both base and formulation kept at 8, 25 and 40°C and 40°C ± 75% RH have been evaluated**.**

The pH of base and formulation at different storage conditions of temperature and humidity were in the range of normal skin pH. The pH of freshly prepared base and formulation was 6.66 and 6.64, respectively. The samples of base and formulation showed gradual decrease in pH from 12 h to 28th day study period. At the end of study (on 28th day) pH of base samples decreased to 5.64 ± 12.6, 5.77 ± 14.5, 5.17 ± 16.1, 5.8 ± 7.3, while in case of formulation pH decreased to 5.54 ± 7.92, 5.27 ± 13.5, 5.13 ± 11.4, and 4.92 ± 9.2, respectively, as shown in Table 3. When the results were manipulated by statistical technique ANOVA at 5% level of significance, in base samples of different temperature; it was found that the change in pH was

insignificant at different time intervals. In case of formulation samples which were placed at different temperatures, the same insignificant results were seen. The paraffin oil produces aldehyde and different organic acids on oxidation at accelerated temperature. This may be a reason in lowering of pH of base and formulation samples (6). Furthermore, Naveed et al. described that at different storage conditions of temperature, the ingredient of both creams decomposed (8). It has been reported that the bark of *Prosopis cineraria* (L.) Druce have significant amount of gallic acid (9), palmitic acid, stearic acid, oleic acid and linoleic acid (10). This significant change in decrease in the pH of formulation with respect to base is due to these acidic secondary metabolites of *Prosopis cineraria* (L.) Druce.

Panel test

The results of panel test showed that there were no difficulty in applying the creams by volunteers, the sense in long term of formulation was greater than base and formulation produces more pleasant feeling than base by applying on skin. It showed that sense just after the application of formulation was more pleasant and has greater spreadability. No skin irritation was produced by any of the creams on volunteer's skin.

The results of panel tests of both base and formulation by volunteers have been presented in Table 4 and Figure 2.

The shine on skin with base was a little bit greater than that with formulation. This was due to more absorption value and spreadability of the formulation than base. The softness of skin by formulation was greater than base. Softness may be produced by paraffin oil which was present in the same quantity in both formulation but formulation containing extract of *Prosopis cineraria* have high amount of polyphenols which increases blood supply to skin (11).

CONCLUSION

It is concluded that the formulation cream is stable with respect to all *in vitro* characterization. There is no color change, no liquefaction, and no phase separation throughout the study period. Beside this, the pH of formulation cream is in accordance with normal skin pH range so it can be used without any skin reaction. The results of panel test clears that formulation cream has marvelous characteristics to be used cosmetically. Further *in vivo* research is needed to evaluate formulation cream for its cosmetic effects on human skin.

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Conflict of interest

The authors declare no conflict of interest in this study.

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