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Original Research Article

Ziziphus mauritiana leaf extract emulsion for skin rejuvenation

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Abstract

Purpose: *To formulate stable water in oil (W/O) emulsion containing hydroalcoholic crude extract of Ziziphus mauritiana leaves for skin rejuvenation.*
Methods: *Placebo (base) without any plant extract and formulation with 4 % Ziziphus mauritiana extract were prepared by mixing. Samples of the emulsions were subjected to varying storage conditions, i.e., 8, 25, 40 ^{o}C and 40 ^{o}C + 75 % relative humidity for a period of 4 weeks to predict their stability. During this period, stability parameters, including liquefaction, phase separation, color, electrical conductivity, centrifugation and pH were monitored at specified time intervals. Skin rejuvenation was evaluated using 13 healthy human volunteers over a period of 8 weeks. During this period, various skin parameters such as erythema, melanin level, moisture content, elasticity and sebum content of the skin were evaluated at specified intervals.*
Results: *Both the active formulation and placebo were stable in terms of liquifaction, phase separation and color at all the storage conditions of temperature and humidity. Active formulation showed statistically significant ($p < 0.05$) improvement in skin melanin as well as in skin moisture and sebum levels, whereas these properties were reduced or even absent in the placebo formulation ($p > 0.05$). Both active and placebo formulations changed skin elasticity and erythema significantly ($p < 0.05$).*
Conclusion: *It is evident from the findings that the leaf extract of Ziziphus mauritiana possesses anti-aging properties as well as exert skin lightening, moisturizing and viscoelastic effects on human skin.*

Keywords: *Ziziphus mauritiana, Melanin, Erythema, Sebum, Skin-tlightening, Moistirizing, Anti-aging*

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INTRODUCTION

Skin aging is characterized by wrinkles, dryness of the skin, color changes, loss of elasticity, etc. Besides genetic factors, environmental hazards such as UV irradiation and oxidative stress contribute to skin aging [1]. There is strong evidence supporting that antioxidants may reduce the damage done by oxidative stress in the skin. However, criticism has been raised on the safety of synthetic antioxidants, thus researchers are trying to explore various natural products as potent antioxidants [2,3]. Presently, many plant extracts are used in cosmetics for their antioxidant [7]. Moreover, the consumer attitude toward natural ingredients in cosmetics plays a significant role.

Ziziphus mauritiana commonly known as Indian Jujube and 'Ber' is a tropical fruit found in many

parts of the world especially in Pakistan, India and Africa [5,6]. It possesses anti-diabetic, anti-inflammatory, anti-plasmodial, and anti-microbial, as well as hemolytic, sedative, anxiolytic, diuretic, analgesic and antioxidant properties [7]. The leaves of *Ziziphus mauritiana* are eaten with catechu as an astringent. They are considered as diaphoretic and are prescribed for typhoid in children. They are also used as poultices. A decoction of the bark of *Ziziphus mauritiana* is used for the treatment of diarrhoea and dysentery. The bark is also used as an astringent in gingivitis. *Ziziphus mauritiana* root is used as bitter and cooling, cures headache. Decoction of roots is used in fever, and as powder applied to old wounds and ulcer. Methanolic extract of *Ziziphus mauritiana* showed a concentration dependent inhibition of the spontaneous pendular movement of the isolated rabbit jejunum and inhibited acetylcholine induced contraction of rat ileum. Fruit extract of *Ziziphus mauritiana*, causes dose-dependent hypotension in rabbits [8,9].

EXPERIMENTAL

Materials

Ziziphus mauritiana leaves were obtained from a local market of Bahawalpur, Pakistan. ABIL EM 90® was purchased from the Franken Chemicals Germany, n. Hexane & paraffin oil was purchased from Merk KGaA Darmstadt (Germany). Ethanol was taken from BDH England. Distilled water was prepared in the Cosmetics Laboratory, Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan.

Preparation of the plant extract

Plant material was identified by Prof. Dr. Saeed Peerzada taxonomist (Cholistan Institute of Desert Studies of the Islamia University of Bahawalpur). The voucher number was assigned and a specimen was preserved at the Pharmacognosy section of The Islamia University of Bahawalpur (Voucher# ZM/L/052013). The leaves were shade dried for two weeks to avoid the degradation of active constituents. Dried leaves were then ground in an electric grinder to fine powder and packed in an air tight polythene bag for further use.

The powdered leaf materials (500 g) were put to a 500 mL glass beaker and 80 % alcohol in water was added until all material was completely soaked in the mixture. The beaker was then covered with aluminum foil and kept at room temperature for 72 h. The mixture was shaken at specific intervals to ensure the complete maceration. After 72 h the mixture was filtered through several layered muslin cloth for coarse filtration. Then, this coarse filtrate was again filtered using Number 1 Whatmann filter paper for fine filtration. The final filtrate was subjected to evaporation at 40 ºC under reduced pressure utilizing rotary evaporator. The evaporation continued until quantity of filtrate reduced to about one third of the original volume.

Evaluation of the antioxidant/radical scavenging activity

The DPPH stable free radical was used for the determination of free radical scavenging activity of extract. In 5 microliter of each ethanolic plant extract, added DPPH to make the volume up to 100 µl in 96 well plates. Mixed the contents and incubated at 37 °C for 30 min. The optical density was measured at 517 nm. The scavenging activity percentage (AA %) was determined according Barkat *et al* [10]. The antioxidant activity was 83 % in comparison to standard ascorbic acid.

Preparation of emulsions

Water in oil (W/O) emulsion was used. Initially, the base (a simple W/O emulsion) was formulated using a mechanical homogenizer. Paraffin oil was used as oil phase and ABIL EM 90® as emulsifying agent to form an emulsion. All ingredients were carefully weighed in separate glass beakers and covered with aluminum foil. The oil phase, constituting the paraffin oil (12 %) and ABIL EM 90 (3 %), was first heated to 75 ± 1 °C using a preheated water bath. Meanwhile, the aqueous phase constituting the distilled water was heated to the same temperature as the oily phase. After heating both phases to desired temperature, the aqueous phase was added to the oily phase with continuous stirring at 2000 rpm. The mixing was continued at 2000 rpm for 15 min and few drops of a fragrant were also added at this time. Next, the stirring speed was reduced to 1000 rpm for 5 min and the mixer speed was again reduced to 500 rpm until complete homogenization was achieved. Finally, emulsion was cooled to room temperature.

The test formulation was prepared in the same way having the same concentrations of all ingredients except that the aqueous phase contained the active ingredient i.e. 4 % *Ziziphus mauritiana* extract.

Evaluation of the prepared emulsions

The stability testing at different conditions were carried out to evaluate the effect of these conditions on the storage of prepared emulsions. This testing was performed on emulsion samples kept at 8 ± 0.1 °C using refrigerator and 25 ± 0.1 °C, 40 ± 0.1 °C, 40 ± 0.1 °C with 75 % relative humidity (RH) and 50 °C using incubators. The testing period lasted 28 days and during this time physical characteristics such as creaming, color change and liquefaction of the emulsion were investigated. The electrical conductivity and pH was measured by Conductivity meter and digital pH meter respectively.

Product evaluation on skin

The prepared emulsion was also evaluated for its effects on the skin of 11 volunteers. All volunteers were healthy non-smoking males with age ranging from 25 to 35 years. Melanin content, skin erythema, skin elasticity, sebum content and skin moisture were evaluated. The *in-vivo* evaluation of skin was done by using Mexameter, Corneometer, Visioscan and Sebumeter MPA 5 (Courage +Khazaka Electronic GmbH, Germany)

Ethical approval

This study was approved by the Board of Advanced Study and Research (BASR) of The Islamia University of Bahawalpur as well as by the institutional ethical committee in compliance with Helsinki declaration [11] and assigned the ref no. Acad/2012/1332.

Data analysis

Changes in the individual values of the various parameters in volunteers, taken every week, were calculated as in Eq 1.

$$\text{Change (\%)} = \{(A - B)/B\}100 \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where A = individual value of any parameter of 1st, 2nd, 3rd, 4th, 6th, or 8th week, and B = zero hour value of the parameter.

The measured values obtained for different parameters (skin moisture, sebum, melanin, erythema, elasticity and pH) were analyzed statistically using SPSS 12.0 (paired sample t-test for variation between the two preparations; two-way ANOVA for variation between different time intervals with post hoc analysis using Least significant difference (LSD) test).

RESULTS

Physical properties of emulsions

The base (control) was white whereas the formulation was of greenish color due to the presence of extract at the time of preparation. During the study period of 28 days, there was no change in the color of base as well as of the formulation in any samples at any storage condition.

No liquefaction was seen in the base and tested formulation kept at 8 °, 25 ° and 40 °C during the entire study period, whereas slight liquefaction was seen in both samples kept at 40 °C 75 % RH on 28th day.

No phase separation was observed in any sample kept at different storage conditions during the entire study period. In addition, no phase separation was seen in any sample in the centrifugation test.

Table 1: The pH values of base (control) and test formulation at varying storage conditions

Time (days)	**8** °C		**25** °C		**40** °C		**40** °C **+ 75% RH**	
	B	F	B	F	B	F	B	F
0	5.96	5.93	5.96	5.93	5.96	5.93	5.96	5.93
0.5	5.91	5.88	6.04	5.98	5.81	6.04	5.88	5.85
1	5.90	5.73	5.83	5.85	6.07	5.71	5.81	5.61
1.5	5.77	5.97	5.53	5.72	5.67	5.21	5.58	5.84
2	5.54	5.59	5.48	5.61	5.87	5.51	5.80	5.36
3	5.61	5.19	5.73	5.13	5.62	5.29	5.57	5.58
7	5.53	5.65	5.42	5.81	5.74	5.47	5.26	5.07
14	5.85	4.84	5.83	4.98	5.67	4.81	5.79	5.21
21	5.27	5.18	5.02	5.12	4.93	4.93	4.87	4.87
28	4.96	4.91	5.21	4.87	5.34	5.21	4.62	5.14

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

Electrical conductivity

The conductivity test was accomplished for every sample of both base and formulation kept at different storage conditions at regular time intervals for the entire period of study. The value of the conductivity test for formulation and base was zero for all measurements.

pH testing

The pH of freshly prepared base and formulation was 5.96 and 5.93, respectively. During the study period there was slight decrease of pH values over time in both, the base and formulation in all samples kept at different storage conditions. The pH of base and active formulations after zero time and 28 days was shown in Table 1). pH change for base (control) samples was significant over time ($p < 0.05$), but insignificant with respect to temperature, while pH change for the test formulation samples was significant with respect to both time and temperature ($p < 0.01$).

Erythema

Both, the base and test formulations, decreased the erythema scoring of the skin. The reduction induced by tested formulation was significantly higher and more regular throughout the study period than the reduction produced by the base. ANOVA test showed that decrease of the skin erythema by both formulation and base was significant ($p < 0.05$). Based on LSD test it was found that base showed significant change only at 1st and 2nd week. However, tested formulation caused significant change in erythema at all visits except 6th week.

Melanin content

The base (control) tended to increase melanin content, whereas the active formulation gradually decreased it (Figure 1). Change in skin melanin content caused by test formulation was significant ($p < 0.05$), whereas the change produced by base was insignificant with respect to time. According to paired sample t test, the variations in skin melanin value were also found to be significant with respect to formulation and base at 1st, 2nd, 3rd, 4th, 6th and 8th week of the study ($p < 0.05$).

Skin moisture

Using a corneometer, a steady increase in stratum corneum moisture was shown after application of the test formulation. In contrast, the effect of the base was much less pronounced and irregular (Figure 2). Increase in the skin moisture content by test formulation was significant ($p < 0.01$), whereas the base exerted an insignificant effect. Increase of skin moisture after formulation application was significant at all study visits. Moreover, skin moisture following application of test formulation was significantly higher than for the base.

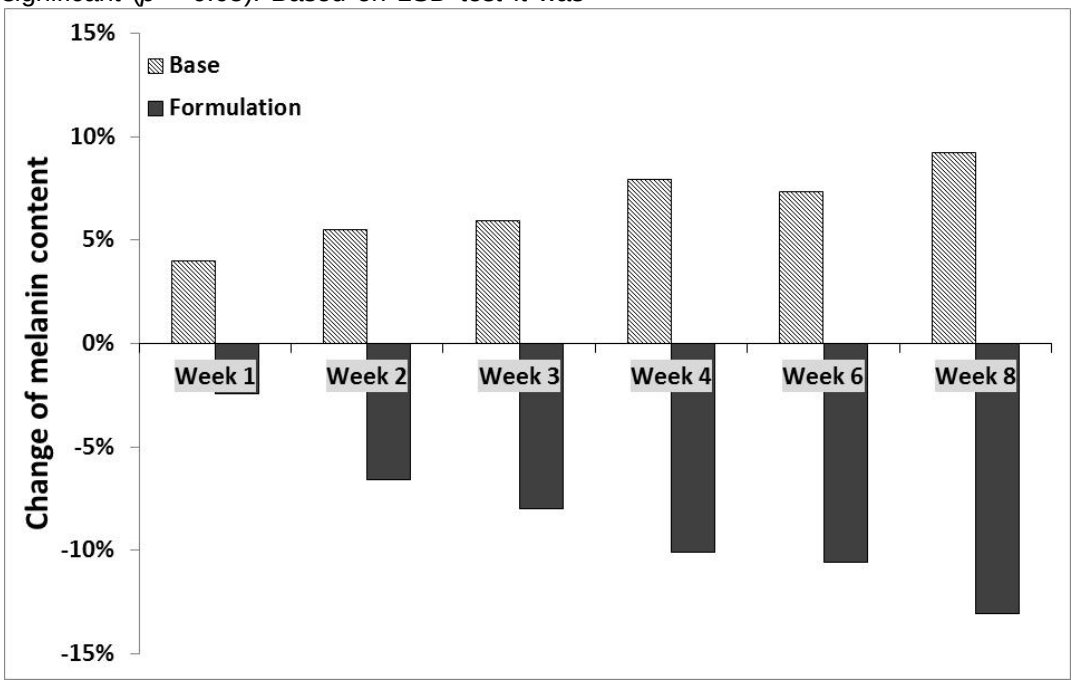

Figure 1: Change of skin melanin content after application of base and test formulation

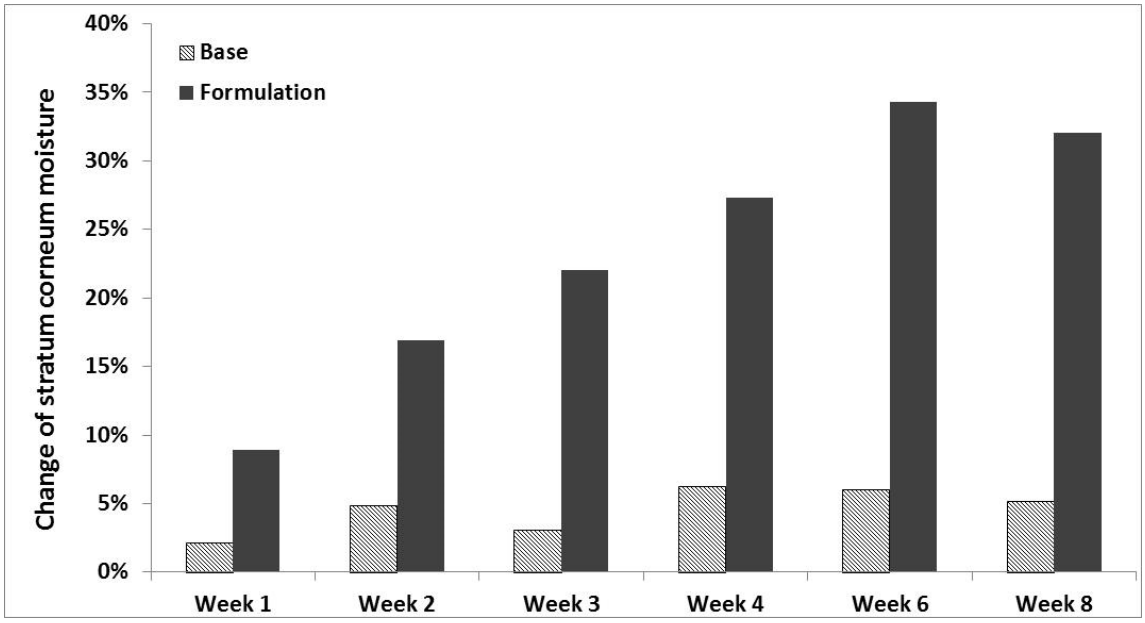

Figure 2: Change in skin moisture after application of base (control) and test formulations

Skin elasticity

Figure 3 shows increase in skin elasticity for the test formulation, while the base decreased the elasticity of the skin. The ANOVA test revealed that variations of skin elasticity by the base and formulation were significant with respect to time ($p < 0.05$). With paired sample t test it was shown that the skin elasticity was significantly higher after treatment with formulation compared to the base at 3rd, 4th, 6th and 8th week of the study ($p < 0.01$), while in the 1st and 2nd weeks, the difference was not statistically significant.

Sebum content

Figure 4 shows an increase of the skin sebum content after application of the base and formulation. According to ANOVA, the increase of the sebum content by the formulation was significant throughout the study period ($p < 0.05$), while the change after base treatment was not significant, except 8th week of the study ($p < 0.05$).

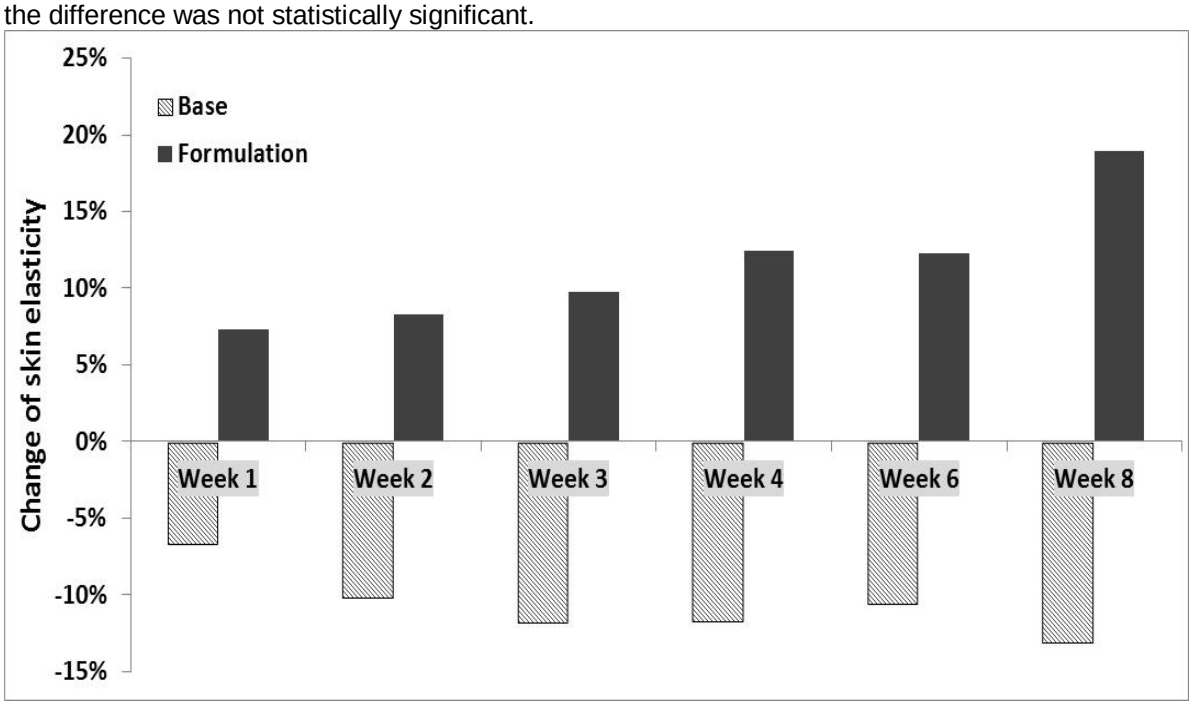

Figure 3: Percentage change of skin elasticity after application of base and formulation

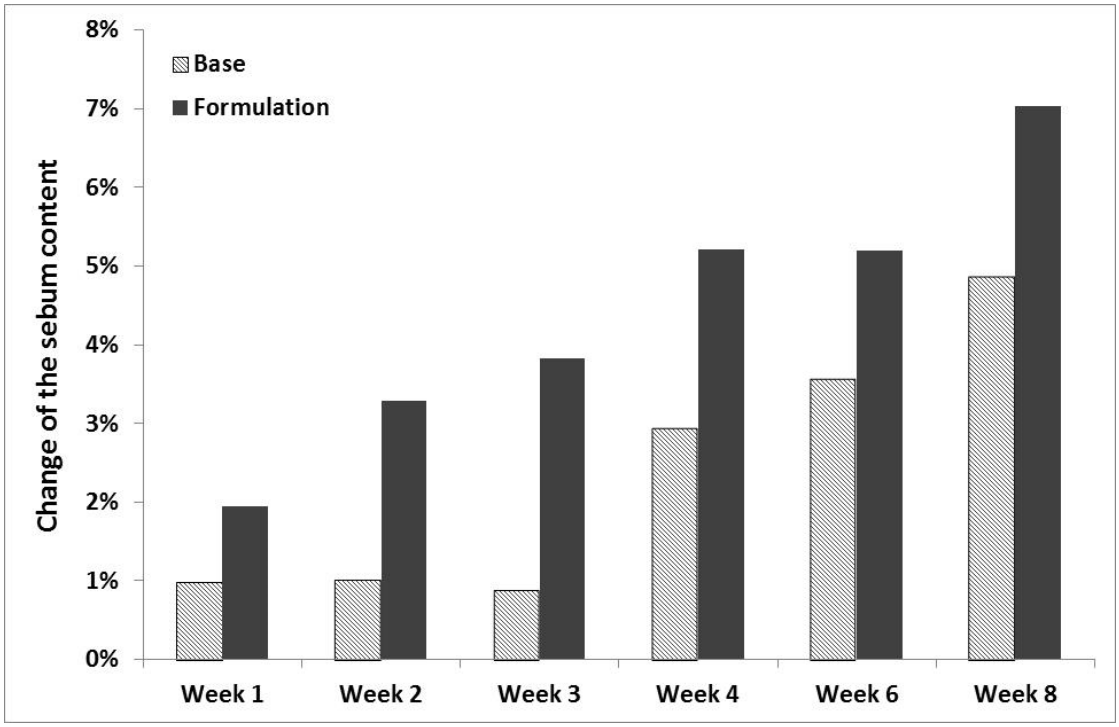

Figure 4: Change in skin sebum content after application of base (control) and test formulations

DISCUSSION

Stability testing showed no change in the color of both different formulations. This may be attributed to the fact that the constituting components of preparations (paraffin oil and ABIL EM 90) are both colorless liquids [10,11]. Moreover, *Ziziphus mauritiana* has well documented antimicrobial activity [12], which may stabilize the test formulation. Thus the plant extract prevented microbial growth in the formulation and hence minimized microbial degradation of the product.

The emulsion quality is dependent on many factors. One of the main factor is its viscosity [11]. When an emulsion is prepared, different temperature and time dependent processes happen affecting phase separation which decreases its viscosity. This may eventually result in increased liquefaction of the emulsion [13]. During the study we did not observe any liquefaction (except the sample kept at 40 °C + 75 % RH which showed slight liquefaction at the end of the study) further confirming the stability of the test formulation.

Creaming is the settling down of globules of denser phase under the influence of gravitational force. Creaming is thought to be the major reason for phase separation in an emulsion [14]. Basically, emulsions are thermodynamically unstable systems and their droplets tend to merge and form bigger droplets. This eventually increase the rate of coalescence of the emulsion and coalescence is one of the possible reasons for the breaking of an emulsion [15]. However, no phase separation was observed in any of the sample of our formulation. We may suggest that proper homogenization during the preparation of emulsion prevented the breaking of emulsion during the accelerated stability studies [15].

As far as the effectiveness of the cream is concerned, pH is a significant parameter to be kept in mind. The pH of the human skin ranges from 4.5 to 6.0 [16]. pH 5.5 is considered the average for human skin. Thus, preparations for topical application should be close to this pH.

We have shown that the test formulation had a pH of 5.93 and this value decreased over time. This result was close to the average pH of human skin. The decrease of formulation pH at different storage conditions may be attributed to the absorption of CO_2 from air by the formulation or changes in the extract of *Ziziphus mauritiana* [17].

In our study we have also shown, that *Ziziphus mauritiana* extract may decrease skin reddening (erythema). However, as we only tested healthy volunteers, this observation has to be confirmed in patients with inflammatory skin diseases. The reduction of the skin erythema may be attributed to the anti-inflammatory effects of the *Ziziphus mauritiana*.

The skin melanin content result showed that the base tended to increase the melanin level

whereas the formulation gradually decreased it. The increase in melanin content by the base may be attributed to presence of paraffin oil. Preparations containing paraffin oil were found to increase the melanin level in the skin when applied topically [18]. In contrast, the reduction of the skin melanin content by the formulation may be credited to the antioxidant activity of polyphenols and flavonoids, which are present in significant quantities in the *Ziziphus mauritiana* [12]. Moreover, reduction of skin melanin content may also be due to linoleic acid present in the *Ziziphus mauritiana* [7]. Linoleic acid is involved in the degradation of tyrosinase which is a key enzyme in the biosynthesis of melanin in the skin. The presence of linoleic acid lead to the degradation of tyrosinase which eventually results in the diminished melanin production [19].

Test *Ziziphus mauritiana* formulation also increased the skin moisture. The increase in the skin moisture content after formulation application may be due to the presence of flavonoids in *Ziziphus mauritiana*. Flavonoids possess high affinity for the collagen and elastin fibers which impact moisturizing effects to the skin [20,21]. There was also an increase of the skin elasticity upon treatment with formulation. The increase of the skin elasticity may be credited to the presence of ascorbic acid present in the plant extract of *Ziziphus mauritiana* leaves [7], which is very important component in the collagen biosynthesis. Moreover, the anti-oxidant activity of the phenolic compounds in the extract may also contribute to the elasticity of the skin which may prevent photoaging of the skin by UV irradiations. Finally, increase of the sebum content of the volunteers may be attributed to the oily nature of the creams used in the study. Paraffin oil used as oil phase of both creams might increase the sebum content of the skin [10].

CONCLUSION

The W/O emulsion of *Ziziphus mauritiana* extract (4 %) possesses good physical characteristics and pharmaceutical stability and may, therefore, be suitable for topical application of the leaf extract to reverse skin aging and enhance the viscoelastic properties of the skin. It may also be useful in the treatment of hyperpigmentation.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

CONTRIBUTION OF AUTHORS

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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