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ORIGINAL ARTICLE

Formulation of herbal cream based on *Ziziphus Mauritiana* leaves extract and evaluation on its physicochemical properties

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ABSTRACT – Plant-derived substances have recently attracted the great interest towards their versatile application, as medicinal plants used in traditional and modern medicine. The aim of the current study was to investigate the antioxidant activity of *Ziziphus mauritiana* (ZM) leaves extract followed with the formulation of herbal creams based on ZM methanolic extract then evaluate the physical characteristic and stability of the creams. After the methanolic extract was obtained by using Soxhlet extraction, the extract was assessed for its antioxidant activity by using 2,2- Diphenyl-1-picrylhydrazyl (DPPH) assay which showed IC50 value of the ZM extract is 10.57 µg/ml, while IC_{50} value for ascorbic acid is 4.19 µg/ml. Four formulations of water in oil (w/o) emulsion based cream in range of 39.9 – 44.9% w/o were formulated based on ZM leaves extract which are F1, F2, F3 and F4. Several physical properties were evaluated such as organoleptic, pH, viscosity, homogeneity, washability and emmolliency which proved that all formulations have good homogeneity, non greasy and under suitable pH and viscosity. Based on thermal stability test (45 °C ± 70 % RH, for 48 hours), it showed that F4 is not stable at high temperature compared to other formulations. Thus, the present study indicated that Ziziphus mauritiana leaves extract has great potential for personal care product development due to its antioxidant property.

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INTRODUCTION

Ziziphus Mauritiana (ZM) commonly known as Indian Jujube and 'Ber' is a fruit tree found in many parts, in tropical and subtropical region of the world especially in Southeast asia, Iran and some regions of Africa [1],[2]. According to previous study, *ZM* has some medical benefits such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and antiulcer properties [3]. Moreover, various parts of *ZM* are used for nutritional and medical purposes such as leaves are utilized to treat some diseases like liver problem, asthma and fever, while its root powder is used for wound healing. These medical benefits are due to the presence of large number of bioactive compounds including flavonoids, alkaloids, glycosides, terpenoides, saponins, tannins and phenolic compounds [3].

These chemical constituents and pharmacological properties present in *ZM* leaves extract can be good potential for cosmetic product development especially in formulation of cream due to its flavonoids content. These flavonoids contain high concentrations of antioxidants that help to neutralize free-radicals present in the environment and possess high affinity for the collagen and elastin fibers, which impact moisturizing effects to the skin [4]. As a result, skin care products that contain flavonoids are capable of contributing towards maintaining the skin's overall quality and appearance.

The natural antioxidant properties found in *ZM* leaves aids in preventing the damage of the skin from free radical. To date, many pharmaceutical innovations are invented from natural resources due to its good quality, reliable and safety for therapeutics. In fact, high demands for natural-plant based products and skin care products with good therapeutic effect in market sales especially from people with sensitive skin. In Malaysia, dryness skin and skin aging are major problems among people due to all days's exposure to ultraviolet rays. Even today, people especially in rural areas, are preferred to choose natural remedies (plant extracts) for traditional cosmetics because these products consist of active ingredients that have medical and drug-like benefits [5]. Despite of its many potential benefits, there is still not much commercial products formulated based on *ZM* leaves until now. In aware of consumer requirements and the lack of *ZM* formulated skin care products in market, this present study focussed on investigating antioxidant activity on *ZM* extract, to formulate cream based on *ZM* extract and to evaluate its physical properties and stability.

MATERIALS AND METHODS

Chemicals and reagents

Methanol, ascorbic acid, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's phenol reagent, sodium carbonate (Na_2CO_3), castor oil, polysorbate 80, stearic acid, beeswax, propyl paraben, essential oil (lavender) were purchased from Sigma Aldrich. The fresh leaves of *Ziziphus Mauritiana* were collected from Setiu, Terengganu.

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Extraction method

The collected leaves were rinsed with tap water for several times to eliminate the unwanted matters and were dried at 45 ℃ for 24 hours. The dried leaves were cut into small pieces and crushed into powder form mechanically by using an electrical grinder. Those powdered leaves were tightly packed in a polyethylene bag for further use. 10 g of powdered leaves extracted with 360 mL of 80% methanol solvent at 70 ℃ and extraction time of 6 hours. The extract solution was concentrated to dryness using a rotary evaporator under a controlled temperature and pressure. The extract was stored in the airtight container at cool and dark place.

DPPH Radical Scavenging Activity

The free radical scavenging activity of extract was estimated by using DPPH according to a previous method [6]. Ascorbic acid was used to prepare standard reference curve with different concentrations (0.98 to 62.5 µg/mL). 1 mg/mL test samples of *Ziziphus Mauritiana* methanolic leaves extract was serially diluted to lower concentrations. 1.5 mL of each extract solution was mixed with 1.5 mL of 0.004% (w/v) DPPH stock solution. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. Then, the absorbance was measured at 517 nm against methanol as blank in UV-Vis spectrophotometer (Shimadzu UV-1800). The percentage of inhibition was calculated using the following equation:

$$\text{Inhibition (\%) of DPPH radicals} = \frac{\text{Ac} - \text{As}}{\text{Ac}} x\ 100\% \quad (1)$$

Where Ac is absorbance of control (reaction in which all reagents participated except plant extract) and As is absorbance of sample (plant extract).

Formulation of herbal creams

Ziziphus Mauritiana methanolic leaves extract was used as an active ingredient to prepare the herbal cream. Several formulations of herbal cream with w/o emulsion type were formulated based on Table 1.

Table 1. Composition of herbal cream.

	Ingredients/ formulations	F1	F2	F3	F4
Oil Phase	**Stearic acid (g)**	4	4	3	6
	Beeswax (g)	5	4	2.5	5
	Castor oil (mL)	40	32	24	48
	Polysorbate 80 (mL)	10	8	6	12
Water Phase	**Distilled water (mL)**	20	16	12	24
	Propyl paraben (g)	0.04	0.04	0.02	0.02
	***Z. mauritiana* extract (mL)**	3.5	5.5	2.5	4.5
	Essential oil (fragrance)	Qs	Qs	Qs	Qs

Qs: quantity sufficient

Physical Properties Evaluation of formulated creams

Organoleptic properties

The formulated creams were observed for their colour, odour and appearance by visual observation. The appearance of the creams was evaluated by its colour pearl essence, roughness and graded.

Determination of the pH value

About 1 g of the cream was weighed and dissolved in 100 mL of distilled water. The pH was measured by using digital pH meter (S20 Mettler Toledo, Germany).

Determination of viscosity

Viscosity of all the formulated cream were measured with FungiLab Viscometer (Viscolead Adv, Spain) by using spindle no. R6. Samples of the creams were allowed to settle over 30 min at the temperature of test (25±1 °C) before the measurements were taken.

Homogeneity, Washability, Immediate Skin Feel

The formulations were tested for its homogeneity by visual appearance and touch and the result obtained were ranked based on Table 2. Immediate skin feels such as stiffness, grittiness, and greasiness were evaluated as well. For the washability test, a small amount of formulated cream was taken and applied on hand and later was washed under running tap water.

Table 2. Ranking of cream based on homogeneity

Criteria	Ranking
Excellent	5
Very Good	4
Good	3
Poor	2
Very Poor	1

Emolliency Test

One gram portion of each cream formulation was smeared onto the surface of white sheets of paper over approximately 4 cm^2 surface area and left to stand on the incubator chamber for 24 hours at temperature of 29 ± 1°C and humidity 78 ± 2% [7]. The degree of translucency of cream formulation was compared with a control and was graded into a three-level rankings: mild, moderate, or strong translucency.

Stability test

Thermal stability test

The bottle was filled up with formulated cream to two-third capacity, insert plug and tighten the cap. The filled bottles were stored upright inside the stability chamber at 45 ℃ ± 1 ℃ for 48 hours. After 48 hours, the bottles were removed from the incubator. If it shows no oil separation or any other physical degradation in the sample, it shows the cream has successfully passed the thermal stability test.

Long Term Stability Studies

The formulated creams were kept in a closed container and stored at temperature of 25 ℃ ± 2 ℃ with 60 ± 5% relative humidity (RH) for long term stability studies and were not exposed to light. The creams were observed and analyzed for 30 days. Any changes in color, appearance and phase separation were carefully observed.

RESULTS AND DISCUSSION

The present study was performed by extracting the *ZM* leaves extract before formulating the herbal creams based on the extracts. The extraction method used in this study is Soxhlet extraction method with methanol as extraction solvent to obtain yield of extract prior to formulation.

Determination of DPPH radical scavenging activity

The antioxidant activity of the methanol extract of *ZM* leaves was assessed by using DPPH radical scavenging activity. DPPH is a stable free radical containing an odd electron in its structure and widely used for detection of the radical scavenging activity in chemical analysis. From this study, IC_{50} value for ascorbic acid and *ZM* extract which defined as the concentration of sample that scavenged 50% of the DPPH were obtained.

Table 3 indicates the *Ziziphus Mauritiana* leaves extract was able to reduce the stable, purple-colored radical DPPH to yellow colored DPPH-H reaching 50% of reduction with IC_{50} value 10.57 µg/ml, while IC_{50} value for ascorbic acid is 4.19 µg/ml. It shows the IC_{50} value of extract was near to the inhibition capacity of the ascorbic acid. Thus, it can be proved that the methanolic extract of *ZM* possess remarkable antioxidant property and has similar result reported by [8]. As can be seen in Figure 1, it showed that when the concentration increased, the percentage of scavenging activity also increased. It proved that the reducing power of *Ziziphus Mauritiana* extract increased with the increasing of their concentration.

Table 3. IC_{50} value of ascorbic acid and *Ziziphus Mauritiana* extract to DPPH assay.

Samples	IC_{50} (µg/mL)
Ascorbic acid	4.19
ZM methanolic extract	10.57

The antioxidant activity shown by the extracts may be due to the presence of various phenolic acid and flavonoid contents of the plant [9]. Previous studies have shown that phenolic acid and flavonoid are the major ingredients in *ZM* leaves extract in addition to alkaloids, glycosides, terpenoides, saponins, and tannins [10] [11] [12]. In fact, phenolic compounds represent one of the major groups of compounds acting as a primary antioxidant mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals [13].

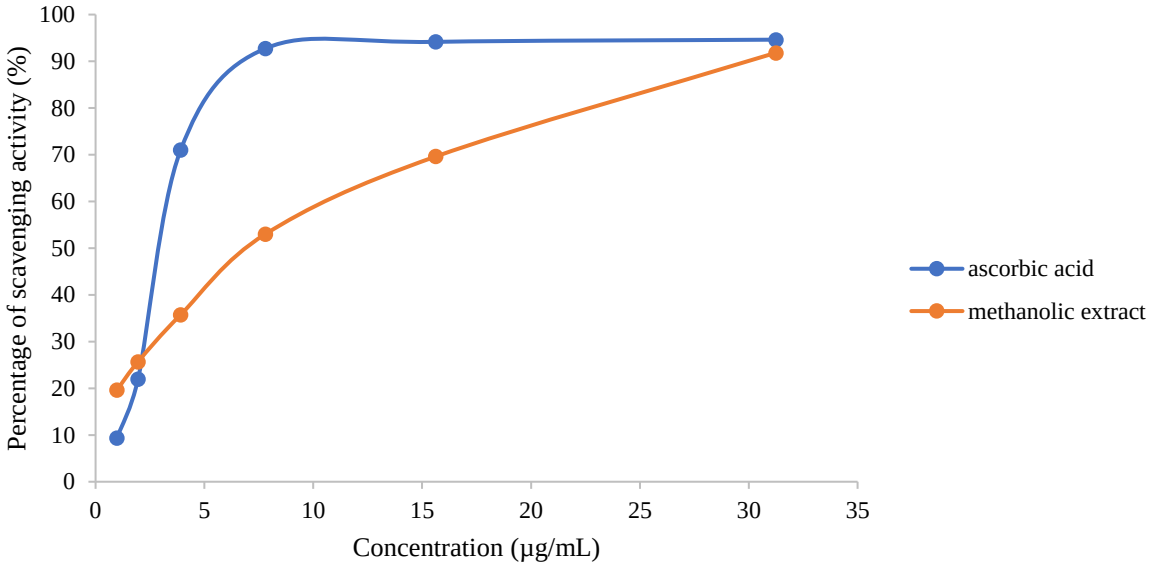

Figure 1. Plot of scavenging activity against concentration of sample.

Formulation and organoleptic properties of herbal cream

The present study was performed to formulate and evaluate the herbal cream. Creams are semisolid dosage forms intended mainly for external use and commonly consist of two immiscible phases, an oily phase and an aqueous phase in range of 39.9 to 44.9% of w/o. In this study, aqueous phase was added into the oil phase which is a combination of polysorbate 80, castor oil, stearic acid and beeswax. Based on previous study, w/o cream is the most suitable formulation of cream in skin care cream product because they give more moisturising effect as they provide an oily barrier which reduces water loss from the outermost layer of the skin [14]. Figure 2 depicted the cream formulation based on the formulation stated in Table 1. All formulated herbal cream showed light green coloured, which was the color obtained from natural leaves extract and produced mildly aromatic odour.

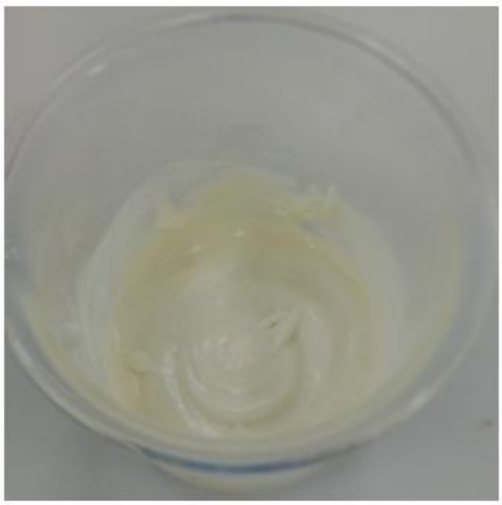

Figure 2. Formulated cream

Organoleptic and physical evaluation properties

The formulated herbal creams from methanolic extract of *ZM* leaves were subjected to the several physical evaluations and the results were shown in Table 4. The pH value of the formulated herbal creams was found to be from 4.63 – 5.75 for F1, F2, F3 and F4, which is recommended as suitable pH in cosmetic skin cream formulations. All formulated creams gave a pH value near to skin pH which normally in the range of 4 – 6 [15].

In addition, the viscosity of herbal creams was in a range of 28,898 – 32,347 cps which indicates that the creams were easily spreadable by small amounts of shear. This observation was supported by previous study [16]. Amongst the formulations, F1 has the highest viscosity (32,324 cps) which also had 39.9% of w/o, the smallest percentage of water in oil as compared to other formulations. Besides, the creams showed an excellent and very good homogenous distribution of cream which was confirmed by visual examination. It shows a uniform distribution of cream in all formulations. Feel

test showed that the formulated herbal creams were strongly emollient and soothing effect. The formulated herbal creams were not greasy after application to the skin and easily removed by washing with tap water. All formulated creams were tested to confirm in w/o type of emulsion by using dilution test when phase changes were observed in cream formulations when diluted with water.

Table 4. Physical properties of herbal creams.

Parameters	Formulations			
	F1	F2	F3	F4
Colour	Light green	Light green	Light green	Light green
Odour	Mildly aromatic	Mildly aromatic	Mildly aromatic	Mildly aromatic
pH	5.75	5.68	5.22	4.63
Viscosity (cps)	32,347	31,027	32,200	28,898
Homogeneity	Excellent	Very good	Very good	Excellent
Emolliency	Strong	Moderate	Strong	Moderate
Washability	Easily removable	Easily removable	Easily removable	Easily removable

Stability studies of cream

Table 5 shows the stability studies for all formulated creams which proved that all physical characteristics were well maintained during the period of long term stability studies at temperature of 25 °C ± 2 °C with 60 ± 5 % RH. Long term stability test showed a stable homogenous appearance for 30 days storage period and no separation phase occurred. However, F4 showed cracking and the globules of the disperse phase become coalesced then phase separation occurred at 45 °C ± 70 % RH (Figure 3). It might be due to high temperature and high humidity condition as well as large composition of oil phase compared to other formulations. In addition, formulations F1, F2 and F3 were stable throughout the thermal stability study for 48 hours. They also showed no changes in appearance after thermal stability test thus indicated that the creams are able to withstand even at exaggerated conditions.

Table 5. Stability studies of herbal creams.

Tests/ formulations	F1	F2	F3	F4
Thermal stability test (45 °C ± 70 % RH) for 48 hours	Stable	Stable	Stable	Unstable (cracking with phase separation)
Long term stability test (25 °C ± 60 % RH) for 30 days	No phase separation	No phase separation	No phase separation	No phase separation

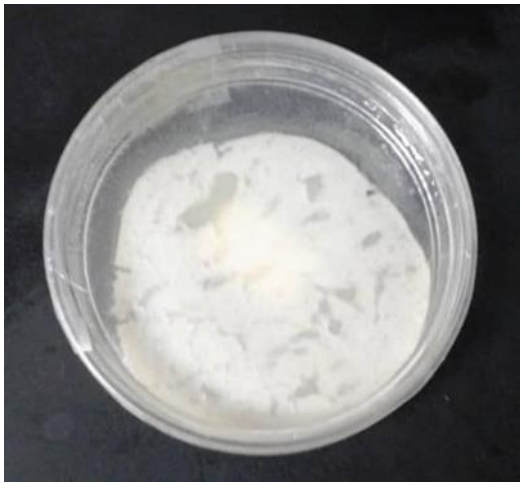

Figure 3. Phase separation of F4 at temperature 45 ºC ± 70 % RH for 48 hours.

CONCLUSION

In this study, the results proved that the methanolic extracts of *Z. mauritiana* possess significant antioxidant properties. Four emulsions based on *ZM* methanolic extract were successfully formulated and several physical characteristics were evaluated to assess the quality of w/o emulsion. All formulated creams showed good organoleptic and physical properties

such as colour, odour, pH, viscosity, non-greasy and strongly emollient when applied on the skin. F1, F2 and F3 were found to be stable formulations during both thermal stability test and long term stability test with no physical degradation observed. However, F4 showed a phase separation at condition 45 °C ± 70 % RH which indicates that F4 is not stable at high temperature and humidity for 48 hours. A further clinical studies are needed to validate the therapeutic potential as well as other biological activity of this cream against all skin disorder.

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REFERENCES

[1] Ghasham, A. A., Muzaini, M. A., Qureshi, K. A., Elhassan, G. O., Khan, R. A., Farhana, S. A.,Hashmi, S., El-Agamy, E., and Abdallah, W. E. "Phytochemical Screening, Antioxidant and Antimicrobial Activities of Methanolic Extract of Ziziphus Mauritiana Lam. Leaves Collected from Unaizah, Saudi Arabia". *International Journal of Pharmaceutical Research & Allied Sciences* 6(3): 33-46. 2017.

[2] Khattak, K., and Rahman, T. "Effect of Gamma Irradiation on the Vitamins, Phytochemicals, Antimicrobial and Antioxidant properties of Ziziphus mauritiana Lam. Leaves". *Radiation Physics and Chemistry* 127: 243-248. 2016.

[3] Gupta, M. K., Bhandari, A. K., and Singh, R. K. "Pharmacognostical Evaluations of the Leaves of Ziziphus mauritiana". *International Journal of Pharmaceutical Sciences and Research* 3(3): 818-821. 2012.

[4] Akhtar, N., Ijaz, S., Khan, HMS., Uzair, B., Reich, A., and Khan, BA. "Ziziphus mauritiana leaf extract emulsion for skin rejuvenation". *Tropical Journal of Pharmaceutical Research* 15(5): 929-936. 2016.

[5] Saheb, SK., Reddy, AP., Rajitha, K., Sravani, B., and Vanitha, B. "Formulation and Evaluation of Cream from Naturally Containing Plant Extract". *World Journal of Pharmacy and Pharmaceutical Sciences*, 7(5): 851-862. 2018.

[6] Muthukumarasamy, R., and Mohd Ideris, N. "Formulation and Evaluation of Antioxidant Cream Containing Methanolic Extract of Piper Betel Leaves". *International Journal of Pharma and Bio Sciences* 7(4): 323 - 328. 2016.

[7] Ayobami, O. O., Ezekiel, O. A., Doyinsola, D. F., and Lara, O. O. "Physicochemical properties and antimicrobial activities of soap formulations containing Sennaalata and Eugenia uniflora leaf preparations". *Journal of Medicinal Plants Research* 11(48): 778–787. 2017.

[8] Abdallah, E. M., Elsharkawy, E. R., and Ed-dra, A. "Biological activities of methanolic leaf extract of *Ziziphus mauritiana*". *Bioscience Biotechnology Research Communications* 9(4): 605-614. 2016.

[9] Perumal, S., Mahmud, R., and Piaru, S. P. "Potential Antiradical and Cytotoxicity Assessment of Ziziphus mauritiana and Syzygium polyanthum". *International Journal of Pharmacology* 8(6): 535-541. 2012.

[10] Ashraf, A., Sarfraz, R., Anwar, F., Shahid, S., and M. Alkharfy, K. "Chemical Composition and Biological Activities of Leaves of Ziziphus Mauritiana L. Native to Pakistan". *Pakistan Journal of Botany* 47(1): 367-376. 2015.

[11] Kumar, R. M., Gayatri, N., Sivasudha, T., and Ruckmani, K. "Profiling of Bioactive Components Present in *Ziziphus Mauritiana* Lam for *In-Vitro* Antioxidant and *In-Vivo* Anti-Inflammatory Activities International". *Research Journal of Pharmacy* 8(9): 19-24. 2017.

[12] Sakna, S. T., Mocan, A., Sultani, H. N., El-fiky, N. M., Wessjohann, L. A., Farag, M. A. "Metabolites profiling of Ziziphus leaf taxa via UHPLC/PDA/ESI-MS in relation to their biological activities". *Food Chemistry* 293: 233-246. 2019.

[13] Zin, Z. M., A. Abdul-Hamid and A. Osman." Antioxidative activity of extracts from Mengkudu (Morinda citrifolia L.) root, fruit and leaf". *Food Chemistry* 78: 227-231. 2002.

[14] Sharma, G. K., Gadiya, J., and Dhanawat, M. Textbooks of Cosmetics Formulation. Page 51-66. 2018.

[15] Xin Chen, M. "Formulations of tropical products with antiviral and antibacterial activity" (Doctoral dissertation). The University of Toledo. 2014.

[16] Nair, S. S., Mathew, M., Sreena., K. "Formulation and Evaluation of Herbal Cream containing Curcuma logan". *International Journal of Pharmaceutical and Chemical Sciences* 1(4): 1362-1368. 2012.