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Research Article

FORMULATION AND EVALUATION OF ANTIAGING CREAM CONTAINING MANGIFERIN

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ABSTRACT

Skin aging can be described as irregular pigmentation, increased wrinkling, loss of elasticity, dryness and roughness. *Phaleria macrocarpa* is known as *Mahkota Dewa* in Malay and commonly available in Malaysia. The fruits contain large quantity of mangiferin which possess medicinal benefits including anti-diabetic, antioxidant, anti-proliferative, immunodulatory, cardiotonic and diuretic properties. However, there is no formulation has been developed though it was reported for many biological properties. The aim of our study is to formulate and evaluate anti-aging cream containing mangiferin as an active ingredient. Two formulations with different concentrations of mangiferin were formulated by mixing oil and aqueous phase using homogenizer. Both the formulations showed significant antioxidant activity and tyrosinase inhibition. The formulated creams were consistent in quality and safe to be used on the skin. These results demonstrate that mangiferin isolated from *P. macrocarpa* has a good potential for cosmetic product development.

Keywords: *Phaleria macrocarpa;* Mangiferin; Antiaging; Antioxidant; Tyrosinase inhibition

INTRODUCTION

Skin aging is a complex process induced by constant exposure to ultraviolet (UV) irradiation and damages human skin. Melanogenesis is the complex process which is responsible for the formation of pigment melanins by melanocytes while skin wrinkling is associated with collagen deficiency resulting from reactive oxygen species that is generated from UV[1]. Skin aging is associated with many factors and when doing researches on the topic, one will definitely come across to terms like ROS (reactive oxygen species) which generally a free radical that contributes to skin aging. ROS is basically generated during cellular metabolism, but the excessive production of ROS can lead to OS (oxidative stress). OS can be a precursor to a lot of health damaging issues including skin aging[2].

Phaleria macrocarpa is traditionally known as Mahkota Dewa that grows in tropical countries including Malaysia. This plant is called simalakama in Sumatra (Malay) and Depok (West Java, Indonesia). *P. macrocarpa* is a well-known medicinal plant and extensively used in traditional medicine. The fruits are used for treating various diseases like hypertension, kidney disorder, cancer and diabetes[3,4]. Studies have also found that some beneficial bioactivities of this plant include anti-cancer, anti-oxidant, anti-cancer anti-bacterial, anti-hyperglycemic and anti-hyperlipidemia[5]. Mangiferin is a pharmacologically active compound present large amount in *P. macrocarpa.* Mangiferin showed antidiabetic[6], anticancer[7-8], antioxidant[9], anti-inflammatory[10], antiviral[11] and antimicrobial[12] activities.

However, so for there is no formulation have been developed though it was reported for potent antioxidant properties. Hence in the present study we are interested to formulate an antiaging cream using mangiferin obtained from *P.macrocarpa*. The tyrosinase inhibition activity and antioxidant activities of the formulated creams were tested to check the potency of anti-aging effect.

MATERIALS AND METHODS

Collection and Extraction of *P. macrocarpa* Fruits

P. macrocarpa fruits were collected from Kg Paya Resak, Terengganu, Malaysia and authenticated by a botanist. The fruits were cut into small slices and shade dried. Then, they were grinded into course powder using blender. 250 g of plant powder was extracted with 1.5 L of methanol using soxhlet apparatus for 18-20 hours. The extract obtained then was concentrated in vacuo by rotary evaporation at reduced pressure and controlled temperature. After evaporation, the concentrated extract was kept it in refrigerator and stored at 4ºC until further use (Yield 5.5 g, 2.2%).

Isolation of Mangiferin

The concentrated mass (5.5 g) was suspended in 50 ml of 50% of ethanol then partitioned with 100 ml of dichloromethane for 4 times. The aqueous ethanolic phase was then hydrolysed by reflux with 2N sulphuric acid at pH 3 for an hour with continuous stirring. After cooled to room temperature, it was partitioned with ethyl acetate for 3 times. Subsequently, the ethyl acetate layer was combined and dried at 40 ºC using vacuum rotary evaporator. The dried ethyl acetate fractions were dissolved in ethanol and left in a refrigerator overnight. After that, the precipitate was isolated by filtration. The crystallization was done by dissolving in aqueous ethanolic solution and again left in refrigerator overnight. Lastly, pale yellow needle-shaped crystals of mangiferin was isolated (2.15 g, 0.86%) and dried[13]. It was found to be homogenous by HPTLC when separated using the solvent system ethyl acetate: methanol: water: formic acid (6:2:1:1, Rf = 0.76). It was

characterized by comparing its melting point, IR, NMR and MS data with literature values[14]. The structure of mangiferin was shown in Fig. 1.

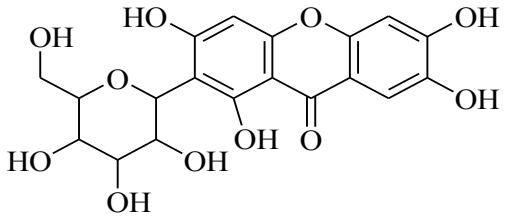

Fig. 1. Structure of Mangiferin

Formulation of Antiaging Cream Containing Mangiferin

The oily phase and aqueous phase were heated up to 70ºC and mixed using homogenizer by addition of methyl of methyl paraben, mangiferin and fragrance. With constant mixing, remaining distilled water was added and stirring was continuing until mixture cools [15]. Cream was formed when the consistency of the mixture was viscous and opaque. The composition of formulations 1 and 2 were shown in Table 1.

Table 1. Composition of Antiaging Cream

Ingredients	Composition of formulations (%w/w)	
	F1	F2
Active Ingredient		
Mangiferin	**1%**	**3%**
Oily Phase		
Stearic acid	10%	10%
Cetyl alcohol	6%	6%
Liquid paraffin	6.6%	6.6%
Aqueous Phase		
Glycerin	5%	5%
Methyl paraben	0.05%	0.05%
Propylene glycol	30%	30%
Distilled water	q.s.	q.s.

The study is carried out as per International conference of Harmonization-Good Clinical Practices Guidelines (ICH-GCP) or as per Declaration of Helsinki guidelines.

Evaluation of Antiaging Cream[15-17]

The following parameters were used to evaluate the antiaging cream. The standard procedure was followed to evaluate all the parameters.

Determination of Type of Emulsion (Dye Method)

A scarlet red dye was mixed with the cream. A drop of the cream was placed on a microscopic slide and examined under a microscope. If the disperse globules appear red the continuous phase colourless, the cream is water-in-oil (w/o) type. The reverse condition is occurring in oil-in-water (o/w) type cream i.e. the disperse globules appear colourless and the continuous phase red.

pH of the Cream: The pH meter was calibrated using standard buffer solution. About 0.5 g of the cream was weighed and dissolved in 50 ml of distilled water and its pH was measured.

Homogeneity: The formulation was tested for homogeneity by visual appearance and touch.

Appearance: The appearance of the cream was judged by its color, pearlescence and roughness and graded.

After Feel: Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream were checked.

Type of Smear: After application of cream, the type of film or smear formed on the skin was checked.

Removal: The ease of removal of the cream applied were examined by washing the applied part with tap water.

Irritancy test: An area (1sq.cm) was marked on the left-hand dorsal surface of human volunteers. The cream was applied to the specified area and time is noted. Presence of irritancy, erythema and edema were checked at regular intervals up to 24 h and reported.

Accelerated Stability Testing: Creams were divided into four parts and stability test was performed at 8°C ± 0.1°C in refrigerator and at 25°C ± 1°C, 40°C ± 1°C and 40°C ± 1°C in incubator with 75% relative humidity (RH), and the above parameters were observed for 8 weeks at weekly intervals.

***In vitro* antioxidant activity by DPPH radical scavenging method**

A 100 µl aliquot of the different concentrations of mangiferin and its formulations along with standards were added to 2 ml of DPPH in methanol solution (100 µM) and incubated at 37ºC for 20 min. After that, the absorbance of each solution was determined at 490 nm using UV-visible spectrophotometer[18]. The percentage inhibition was calculated as follows

Percentage inhibition = [(Abs Control –Abs Sample) X 100)/ (Abs control)]

Tyrosinase Inhibition Activity

Different concentrations of mangiferin and its formulations were assayed for tyrosinase inhibition by measuring their effect on tyrosinase activity. The reaction was carried out in 100 mM sodium phosphate buffer (pH 6.7) containing 1 mM L-tyrosine and 80 unit/mL mushroom tyrosinase at 37°C. The reaction mixture was pre-incubated for 10 min before adding mangiferin. The change of the absorbance at 475 nm (sometimes 490 nm) was measured[15]. The percent inhibition of tyrosinase was calculated as follows:

Percentage inhibition = [(Abs Control –Abs Sample) X 100)/ (Abs control)]

RESULTS AND DISCUSSION

Mangiferin was isolated from the fruits of *P. macrocarpa,* found to be homogenous by HPTLC (Fig. 2) when separated using the solvent system ethyl acetate: methanol: water: formic acid (6:2:1:1, R_f = 0.76). Obtained as pale-yellow needle shaped crystals, mp 270-272ºC; yield 2.15 g, 0.86%; IR υ_{max} (KBr) cm^{-1}: 3365 (OH), 1651 (C=O), 1621 and 1594 (aromatic C=C), 1200 and 1096 (C–O), 1050, 828, 585 (Fig. 3); ^{1}H NMR (400 MHz, DMSO) δ : see Table 2; negative ESI-MS: m/z calculated for 422.08, Found : 444 for [M +Na – H].

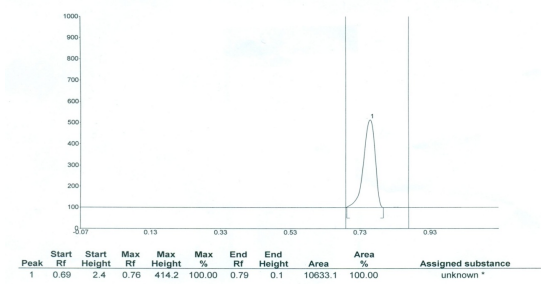

Fig. 2. HPTLC Spectrum of Mangiferin

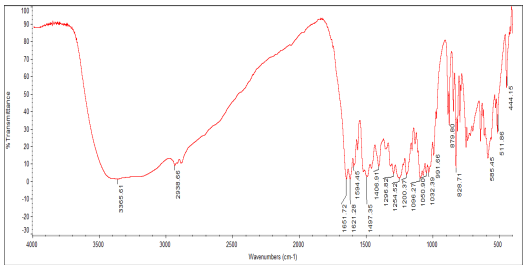

Fig. 3. IR Spectrum of Mangiferin

Table 2. ^{1}H NMR Spectrum of Mangiferin

Proton	Literature Value[14]	MGN
1-OH	13.76 (1H)	13.50 (1H)
6-OH	10.55 (1H)	10.80 (1H)
7-OH	10.55 (1H)	10.80 (1H)
3-OH	9.86 (1H)	9.80 (1H)
3′,4′-OH	4.86 (2H)	4.90 (2H)
6′-OH	4.49 (1H)	4.49 (1H)
2′-OH	3.87 (1H)	4.60 (1H)
8-H	7.38 (1H)	7.40 (1H)
5-H	6.86 (1H)	6.90 (1H)
4-H	6.37 (1H)	6.40 (1H)
1′-H	4.60 (1H)	4.60 (1H)
2′-H	4.05 (1H)	4.05 (1H)
6′a-H	3.69 (1H)	3.69 (1H)
6′b-H	3.42 (1H)	3.41 (1H)
3′-H	3.18 (1H)	3.18 (1H)
4′-H	3.18 (1H)	3.18 (1H)
5′-H	3.18 (1H)	3.18 (1H)
Total number of protons	18	18

Table 3: Type of Adverse Effect of Formulations

Formulation	Irritant	Erythema	Edema
F1	Nil	Nil	Nil
F2	Nil	Nil	Nil

Table 4. Physiochemical Evaluation of Formulated Cream

Parameter	Formulation 1	Formulation 2
Homogenity	Good	Good
Appearance	No change in colour	No change in colour
Odour	Good	Good
Spreadability	Good	Good
After feel	Emollient	Emollient
Type of semar	Non greasy	Non greasy
Removal	Easy	Easy
Stability	Stable for 3 months	Stable for 3 months

Table 5. *In-vitro* Antioxidant Activity by DPPH Method

Concentration (µg/ml)	% inhibition		
	Mangiferin	Formulation 1	Formulation 2
1000		51.42 ± 3.73	68.67 ± 1.57
500		32.35 ± 2.58	45.39 ± 3.52
250		23.52 ± 1.80	37.76 ± 1.74
125		--	25.45 ± 2.00
62.5	92.28 ± 2.92	--	19.97 ± 2.06
31.25	91.39 ± 1.13	--	16.35 ± 1.85
15.60	89.25 ± 1.00	--	--
7.80	81.45 ± 5.91	--	--
3.90	65.05 ± 9.98	--	--
1.95	47.65 ± 3.06	--	--
Ascorbic Acid (10 µg/ml)	**92.45 ± 3.82**		
Rutin (10 µg/ml)	**48.25 ± 2.50**		

-- No inhibition. Values are expressed as mean±SD (n=3).

Table 6. Tyrosinase Inhibition of Mangiferin Formulations

Concentration (µg/ml)	% inhibition		
	Mangiferin	Formulation 1	Formulation 2
1000	93.40 ± 2.10	54.49 ± 7.70	75.65 ± 2.53
500	71.65 ± 1.48	45.63 ± 2.68	64.67 ± 0.85
250	64.65 ± 4.04	37.35 ± 0.90	61.25 ± 1.98
125	56.93 ± 3.50	33.00 ± 2.85	57.57 ± 2.38
62.5	44.45 ± 3.67	20.73 ± 2.66	44.53 ± 2.55
31.25	32.13 ± 1.78	--	24.63 ± 3.90
15.60	25.42 ± 2.69	--	12.30 ± 1.15
7.80	14.67 ± 2.55	--	--
3.90	--	--	--
1.95	--	--	--
Gallic Acid (25 µg/ml)	**52.35 ± 3.25**		

-- No inhibition. Values are expressed as mean±SD (n=3).

Evaluation of Formulated Antiaging Cream

The dye test was confirming that all the formulations were o/w type of emulsion cream. The pH of the formulated cream was found to be in range of 4.80 to 5.60 which is good and recommended pH for the skin. Irritancy test was conducted with 5 healthy volunteers to identify the safety, skin irritation and allergic sensitization were scarce or absent. Both the formulations don't show redness, edema, inflammation and irritation during irritancy studies. The results indicate that both the formulations were safe to be used on the skin (Table 3).

The formulated antiaging creams were evaluated for several physicochemical tests and the results were shown in Table 4. The type of smear formed on the skin was not greasy after the application of all formulated creams. Both the formulated creams (F1 & F2) when applied on skin were easily removable by washing with water. All the formulations were producing a uniform distribution of extracts in the cream. This was confirmed by visual examination and by touch. When formulation kept for a long time, it was found that there were no changes in the colour of the cream. After feel test showed that the formulated creams were emollient and slipperiness.

All the physiochemical parameters were maintained during the accelerated stability studies at temperatures 8°C ± 0.1°C in refrigerator and at 25°C ± 1°C, 40°C ± 1°C and 40°C ± 1°C in incubator for 12 weeks. The results of accelerated stability test showed that there were not any particular changes in the colour of the cream.

***In-vitro* Antioxidant Activity**

Mangiferin showed potent antioxidant activity with percentage of inhibition value 92.28 ± 2.92 at 62.5 µg/ml in DPPH method. The formulation 1 and 2 showed potent antioxidant activity with percentage of inhibition value 51.42 ± 3.73 and 68.67 ± 1.57 at 1000 µg/ml, respectively. Formulation 2 showed higher percentage of inhibition when compared to Formulation 1 in all the tested concentrations. However, standard ascorbic acid and rutin showed higher inhibition at low concentration when compared to mangiferin and its formulations. The results were showed in Table 5.

Tyrosinase Inhibition

Mangiferin showed potent tyrosinase inhibition activity with percentage of inhibition value 93.40 ± 2.10 at 1000 µg/ml. The formulation 1 and 2 showed potent tyrosinase inhibition with percentage of inhibition value 54.49 ± 7.70 and 75.65 ± 2.53 at 1000 µg/ml, respectively. Formulation 2 showed higher percentage of inhibition when compared to Formulation 1 in all the tested concentrations. However, standard gallic acid showed higher inhibition at low concentration when compared to mangiferin and its formulations. The results were showed in Table 6.

The use of antioxidants for a particular topical formulation appears to be an interesting approach to protect skin against oxidative stress caused by different extrinsic agents. To ensure the effectiveness of antioxidants against free radicals, it is essential to stabilize the final formulation as antioxidants are very unstable and can easily oxidize, becoming inactive before reaching its site of action[15].

Our earlier laboratory studies reported that mangiferin showed potent antioxidant activity in DPPH, ABTS, H_2O_2 and nitric oxide methods[18]. It is beneficial effects on the process of skin aging, skin sun protection or skin cancer. There are many studies were reported that an acute exposure of human skin to UV radiation *in vivo* leads to oxidation of cellular biomolecules and that could be prevented by a prior antioxidant treatment. Hence, there is an increased demand for herbal cosmetics in the Malaysian markets. Therefore, the present study was tried to formulate an antiaging cream using mangiferin as an active ingredient.

The formulated cream was o/w type emulsion, hence can easily washed with water and gives better consumer compliance. Our study indicated that, both the formulated creams were stable with no signs of breakdown of emulsion and change in colour of the product. Also maintained constant pH, homogeneity emollient properties; they were not greasy and easily removable after the application. The irritancy test showed that there no severe erythema was occurred in any of the volunteers for formulation containing mangiferin. This indicates that the formulated creams were safe to the consumers.

CONCLUSION

Both the mangiferin formulations showed potent antioxidant activities and tyrosinase inhibition. The results demonstrated that the formulated antiaging creams are safe and usable for the skin and having good potential for cosmetic product development.

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