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Research Article

Formulation and Evaluation of Natural Antioxidant Cream Comprising Methanolic Peel Extract of *Dimocarpus longan*

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ABSTRACT

Photo aging is a common problem that occurs in our community due to ongoing exposure to ultraviolet rays. The use of antioxidants is an effective approach to prevent symptoms related to photo-induced aging of the skin. Thus, the present study was to prepare and evaluate the antioxidant cream comprising the methanolic peel extracts of *Dimocarpus longan* for their radical scavenging activity. Antioxidant activity of peels and seeds methanolic extract (by continuous hot percolation-soxhletation) of *D. longan* was assessed by using stable 2,2 -Diphenyl-1-picryl hydrazyl (DPPH). The extract of the *D. longan* fruits contained three major polyphenolic compounds which are corilagin, gallic acid, and ellagic acid which are responsible for the antioxidant properties. Methanolic extracts of both peels and seeds of *D. longan* exhibited high radical scavenging properties, the IC_{50} result revealed that peels extract were having higher antioxidant properties with 23.5 µg/ml compared to the seeds 32.13 µg/ml. Based on the higher scavenging activity the peels was chosen to prepare as formulation. Thus, the cream was formulated with 2.5% of peels extract by fusion method with incorporation of two different emulsifying agents for two formulations (F1 & F2). The evaluation of the formulations was done on different parameters like pH, spreadability, rheological study, non-volatile matter at 105 °C, physical stability of cream and microbial limit test. Both the formulations (F1 & F2) were showed good pH, homogeneity, appearance, ease to remove, good consistency, spreadability and no microbial growth. However, after four weeks of storage formulation of F1 showed cracking and phase separation. The evaluation parameters of the formulated cream F2 showed good results and are safe to use for skin. The present results indicates that the *D. longan* fruit peel extract has a good potential for cosmetic product development.

Keywords: *Dimocarpus longan*, photoaging, antioxidant, evaluation, DPPH.

INTRODUCTION

Malaysia's climate is categorized as equatorial; being hot and humid throughout the year and Malaysian folks will expose more to ultraviolet rays. Ultraviolet rays will facilitate the aging process and will cause the decrease in skin elasticity. For this reason, the investigations of radical scavenging activities are done to know the ability of the extract to combat or delay the photo aging process. *D.longan* is commonly known as dragon eye fruit or longan, which is most prevailed fruits in Southeast Asia. It comes from the Sapindaceae family which is the same group as *Litchichinensis L.* (litchi) and *Nephelium lappaceum L*. (rambutan). The extract of the *D.longan* fruits contained three major polyphenolic compounds which are corilagin, gallic acid, and ellagic acid which are responsible for the antioxidant properties. Despite many research is still ongoing for *D.longan*, however so far the longan fruits extract have not been used as an antioxidant formulation for skin care. Thus, the present study focused to develop a formulation that may scavenge free radicals and protect skin against oxidative damage. The study from Nair et. al., concluded that it is possible to develop creams containing herbal extracts having antioxidant property and they can be used as the alternative as a barrier to protect skin[1]. The litchi pericarps are proven to have high antioxidant cue to its high in phenolic compounds content[2]. The antioxidant activity of rambutan is due to the presence of ellagic acid and gallic acid in the rambutan are responsible for the antioxidant activity in the fruits[3]. The longan seeds exhibited three major polyphenolic compounds which are corilagin, gallic acid and ellagic acid. Longan seed water extract shows the scavenging activity as good as white tea and dried longan pulp have the least scavenging activity[4]. The study conducted to observe the ability of natural products to suppress the production of oxidative stress and increasing enzymatic antioxidants in tissues. The result revealed that water extract longan pericarp can inhibit production of oxidation, elevated antioxidant enzyme activities, and decreased inflammatory response[5]. The longan seeds extracts have the MMPIs activity which probably correlates with the high antioxidant properties in the *D. longan*[6].

MATERIAL AND METHODS

**Author for Correspondence:*

Figure 1: Fruits of *Dimocarpus longan*.

Formulation 1 Formulation 2

Figure 2: Formulated antioxidant creams

Chemicals

2,2 -Diphenyl-1-picryl hydrazyl (DPPH) was obtained from Sigma Aldrich Co, St Louis, USA. Ascorbic acid was obtained from S.D. Fine Chem, Ltd., Biosar, India. All other chemicals used were of analytical grade.

Collection and Identification

10 kg of *D. longan* fruits was purchased from the local market and identified. Care was taken to select healthy fruits, the selected fruits were washed carefully with water to remove dust and foreign materials.

Extraction

The peels and seeds of *D. longan* fruits were separated and dried in the oven at 40 ℃ for 48 hours and grinded to coarse powder using blender. The dried powder of peels (250 g) and seeds (250 g) of the fruits were individually extracted with methanol as solvent using Soxhlet extraction method. Both the extracts were concentrated to dryness under reduced pressure and controlled temperature using rotary evaporator. The percentage yield of the extracts were calculated. The collected extracts were stored in air tight containers in refrigerator at 4 ℃ until further study.

Qualitative Phytochemical Analysis[6]

The stock solution was prepared from the crude extract and was dissolved in 10 ml of its own mother solvent. The obtained stock solution were subjected to preliminary phytochemical screening.

Test for alkaloids: Mayer's reagent, Dragondroff's reagent, Hager's reagent and Wagner's reagent.

Test for carbohydrates: Molisch test, Fehling's test and Benedict's test.

Test for proteins: Biuret test.

Test for glycosides: Keller-Killiani test, Borntrager's test and Legal test.

Test for fixed oils: Spot test.

Test for tannins and phenolic compounds: Lead acetate test and gelatin test.

Test for flavonoids: Shinoda's test, test with Sodium hydroxide solution and test with sulphuric acid.

Test for steroids: Libermann- Burchard test.

Table 1: Composition of antioxidant cream.

Components	Amount (% w/w)	
Active Ingredient	Formulation 1 (F1)	Formulation 2 (F2)
D.longan Peel extract	2.5 %	2.5 %
Oily Phase		
Stearic acid	7.00 %	7.00 %
Cetyl alcohol	2.00 %	2.00 %
Mineral oil	20.00 %	20.00 %
Aqueous Phase		
Glycerin	10.00 %	10.00 %
Methyl paraben	0.05%	0.05%
Tween 80	2.00 %	-
Triethanolamine (TEA)	-	2.00 %
Deionised water q.s	100 %	100 %

In vitro Antioxidant activity

The in vitro method is based on the inhibition. Samples are added to a free radical – generating system, inhibition of the free radical action is measured and this inhibition is related to antioxidant activity of the sample. Method vary greatly as to the generated radical, the reproducibility of the generation process, and the endpoint that is used for determination. Both the extracts were tested for in vitro antioxidant activity. The final concentration of the extract and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25, 15.625 and 7.812 µg/ml. the absorbance was measured spectrophotometrically against the corresponding blank solution.

The percentage inhibition was calculated by using the following formula.

$$\% \text{ Inhibition} = \frac{\text{OD control} - \text{OD Sample} \times 100}{\text{OD control}}$$

IC_{50}, which is the concentration of the sample required to scavenge 50% of free radicals was calculated.

DPPH Assay

The present study on estimation of free radical scavenging activity of peels and seeds of *D. longan* on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical was determined[8].

Reagents

2, 2-Diphenyl-1-picryl hydrazyl solution (DPPH, 100 µM): Accurately weighed 22 mg of DPPH in 100 ml of methanol. From this stock solution, 18 ml was diluted to 100 ml with methanol to obtain 100 µM DPPH solution.

Preparation of Extract Solutions

Accurately weighed 21 mg of each extracts and dissolved in 1 ml of freshly distilled DMSO to obtain solutions of 21 mg/ml concentration. These solutions were serially diluted separately to obtain the lower concentrations.

Table 2: Nature, Percentage Yield of the Extracts.

Extract	Nature	Percentage Yield
Crude methanol extract (Seeds)	Light Yellow semisolid	5.26
Crude methanol extract (Peels)	Dark brown semisolid	30.10

Table 3: Phytochemical analysis of the extracts.

Phytoconstituents	Seed extract	Peel extract
Alkaloids	A	A
Proteins	P	P
Carbohydrates	P	P
Glycosides	P	P
Fixed oils	P	P
Tannins and Phenolic compounds	P	P
Flavonoids	P	P
Steroids	P	P

A = Absent, P = Present

Preparation of Standard Solutions

Accurately weighed 10 mg of ascorbic acid and dissolved in 0.95 ml of freshly distilled DMSO to get 10.5 mg/ml concentration. These solutions were serially diluted separately to obtain the lower concentrations.

Procedure

To 2 ml of DPPH solution, 100 µl of each of the extract or standard solution was added seperately. The solution were incubated at 37 ℃ for 30 min and the absorbance of each solution was measured at 490 nm using UV spectrophotometer[9].

Preparation of Formulation

D. longan methanolic peels extract is used to prepare the antioxidant cream. For testing the maximum stability between the formulations F1 and F2, two types of stabilizers were chosen such as tween 80 and triethanolamine. The composition of the cream were shown in Table 1. The formulation F1 and F2 adopts the same method to prepare the cream. The oily phase and aqueous phase components were heated separately up to 70 ℃ and were mixed using homogenizer by addition of methyl paraben, extract and perfume. Care was taken for constant and even mixing, the remaining deionised water is added with continuous stirring until the mixture cools and formed as cream. Base cream is prepared in the same method as formulation without extract. The formulated creams were shown in Figure 2.

Evaluation of Antioxidant Cream

The following parameters were used to evaluate the antioxidant cream. The standard procedure was followed to evaluate all the parameters[10].

Physical Properties

The cream was observed for colour, odour and appearance.

Determination of pH

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.

Determination of Emulsion Type (Dye test)

The emulsion type was determined by using dye test. The scarlet red dye is mixed with the cream. Placed a drop of cream on a microscopic slide covers it with a cover slip and examined it under a microscope. If the disperse globules appears colourless the ground is red, the cream is oil in water type. The reverse condition occurs in water in oil type cream. i.e. the disperse globules appear red in the colourless ground.

Homogenecity

The formulations were tested for the homogeneity by visual appearance and by touch.

After Feel Effect

Emolliency, slipperiness and amount of residue left after the application of fixed amount of cream was checked.

Loss on Drying

1 g of cream was taken in china dish and kept in an oven at 105 ℃ for 2 hours.

Rheological Studies

The formulated cream was found to be non-newtonian. Take a fixed quantity 10 g of cream in a 10 ml beaker. Keep it impact for 1 hr. The beaker was inclined to one side see whether consistency has changed or not. The beaker was again tilted and checked for pourability of the cream.

Stability Studies

To assess the formulation stability, stability studies were carried out as per ICH guidelines. The cream filled bottle was kept in humidity chamber maintained 30 $\pm$ 2 ℃ with 65 $\pm$ 5 % RH for two months. At the end of the studies, samples were analysed for the physical properties.

Test for Microbial Growth in Formulated Creams

The formulated creams were inoculated on the plates of Muller Hilton agar media by streak plate method. The plates were placed in the incubator and are incubated at 37 ℃ for 24 hours. After the incubation period, plates were taken out and check the microbial growth by comparing it with the control.

RESULTS AND DISCUSSION

Extraction and Qualitative Phytochemical studies

The percentage yield and nature of the extracts were given in Table 2. The quantitative phytochemical analysis of seeds and peels extracts showed the presence of glycosides, flavonoids, tannins and phenolic compounds, carbohydrates, proteins, fixed oils and steroids as shown in Table 3.

DPPH Radical Scavenging Activity

The antioxidant activity of methanolic extracts of peels and seeds from *D. longan* were assessed using DPPH radical scavenging activity. The results were shown in the Table 4 and 5. The highest radical scavenging activity was recorded in the peels extract. Peels extract had the lowest concentration to exhibit 50 % of the percentage inhibition when compared to that of seeds extract. Moreover, when compared to ascorbic acid, the ascorbic acid had higher antioxidant properties with 11.50 µg/ml compared to peels 23.50 µg/ml and 32.13 µg/ml of seeds as shown in Table 6. However, the extracts were found to be less active compared to the standard ascorbic acid. Thus the result can conclude that the present study proposed that the *D. longan*

Table 4: DPPH radical scavenging activity of seeds extract.

Concentration(µg/ml)	S.D.	Mean of % inhibition	% inhibition ± SEM
7.818	0.162	37.31	37.31 ± 0.162
15.625	1.613	39.87	39.87 ± 1.613
31.25	0.911	48.77	48.77 ±0.911
62.5	0.433	74.05	74.05 ±0.433
125	0.491	79.26	79.26 ±0.491
250	0.583	90.97	90.97 ±0.583
500	0.285	92.05	92.05 ±0.285
1000	0.162	94.98	94.98 ±0.162

Table 5: DPPH radical scavenging activity of peels extract.

Concentration (µg/ml)	S.D.	Mean of % inhibition	% inihibition ± SEM
7.818	1.58	40.91	40.91 ±1.58
15.625	0.783	46.88	46.88 ±0.783
31.25	0.483	53.41	53.41 ±0.483
62.5	0.629	73.58	73.58 ±0.639
125	0.624	94.6	94.6 ±0.624
250	1.206	97.16	97.16 ±1.206
500	0.505	96.59	96.59 ±0.505
1000	0.412	92.61	92.61 ±0.412

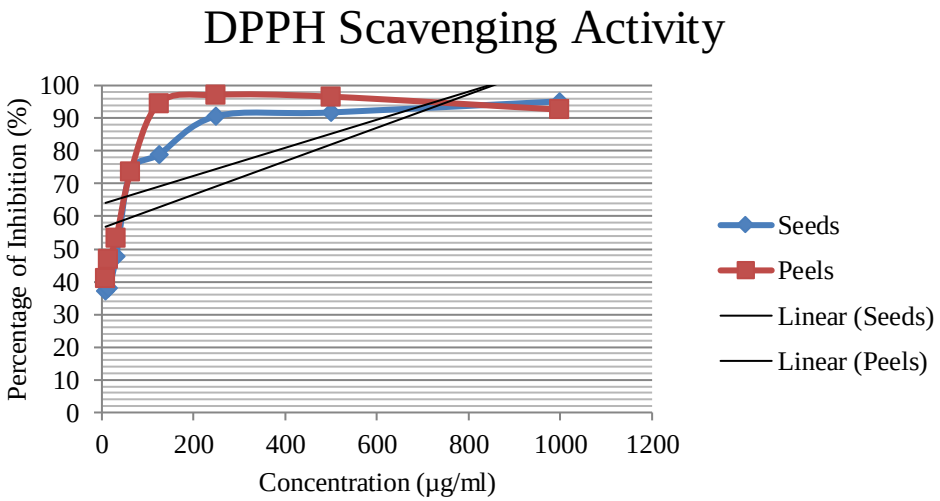

Figure 3: DPPH scavenging activity of peels and seeds.

Table 6: IC_{50} value of standard vs sample extracts.

Extracts	IC_{50} (µg/ml)
Ascorbic acid	11.50
D. longan seeds extract	32.13
D. longan peels extract	23.50

peels methanolic extracts possess high potential of antioxidant properties. The observed antioxidant effects can be attributed majorly to the presence of polyphenolic compounds in the *D. longan*. A comparison graph of DPPH radical scavenging activity of peels and seeds is presented in the Figure 3.

Evaluation of Cream

The methanolic extract of *D. longan* peels were chosen to formulate the cream because of its higher antioxidant activities when compared to seeds. The dye test confirms that the formulated creams were o/w type of emulsion cream. The p^H of the formulated creams was found to be 4.6 to 6.2. The formulated antioxidant cream were evaluated for several physicochemical tests and the results were shown in Table 7. The formulated F1 and F2 creams showed pleasant odour and yellowish and light brownish coloured creams respectively. The formulated cream was not greasy after application to the skin. The formulated creams were easily removable by washing with tap water. The cream showed homogenous distribution of extract in the cream which was confirmed by visual examination. There was no change in colour of formulated cream upon keeping for long time. After feel test showed that the formulated cream were emollient and slipperiness. The loss on drying of the formulated cream was found to be within the limit to standard procedure. All the physicochemical parameters were well maintained during the period of accelerated stability studies at temperatures 8^0 C ± 0.1°C in refrigerator and at 25°C ± 1°C, 40 °C ± 1 °C

Table 7: Physicochemical Evaluation of the formulated cream.

Parameter	F1	F2
Homogeneity	Homogenous	Homogenous
Appearance	Yellowish semisolid cream	Light brown semisolid cream
Odour	Good	Good
Loss on drying	0.25 %	0.11 %
Spreadability	Good	Good
After feel	Emollients and slipperiness	Emollients and slipperiness
Removal	Easily removed	Easily removed
Stability	Unstable	Stable for two months
Microbial limit test	< 100 colonies	< 100 colonies

F1 = Formulation 1, F2= Formulation 2

in incubator for 8 weeks for both the formulations. However, the formulation 1 (F1) showed cracking and the globules of the disperse phase become coalesced and showed separation between oil and water phase. The formulation 2 (F2) showed good stability in colour and consistency until the end of accelerated study period.

CONCLUSION

The *D. longan* is commonly known as longan or dragon eye fruit in Malaysia and widely used for its medicinal value in the traditional system of medicine. The extract of the longan fruits contained three major polyphenolic compounds which are corilagin, gallic acid, and ellagic acid which are responsible for the antioxidant properties[4]. The antioxidant creams are widely used today as it appears to be an interesting way to safeguard the skin against oxidative stress caused by various extrinsic sources. To maintain the effectiveness of antioxidants against free radicals, it is important to stabilise the final formulation on its antioxidant properties. As a part of synergistic effects the current practice moves towards in the formulation of different combinations of antioxidants instead of single antioxidant products. The present study revealed that the peels were having higher radical scavenging activity compared to that of seeds extract in DPPH method. The evaluation test reveals that the formulated cream from peels extract showed that it is safe to be used in the skin to protect from extrinsic oxidation sources. Moreover, our study presented that formulation of F2 is more stable during the shelf storage. The research work suggests that, to ensure the quality and purity of the cream it must have the consistency and uniformity in the ingredients of the herbal antioxidant cream. The trend of using herbal skin cream is becoming in demand since it is proven that topical application of anti-oxidant cream will be effective against UV radiation and protect the skin from major consequence of UV damage. In conclusion, the topical application of the formulated cream from *D. longan* extract will help in reducing oxidative damage and give the antioxidant effect to our skin due to its high antioxidant values. In conclusion, the topical application of the formulated cream from *D. longan* extract will help in reducing oxidative damage and give the antioxidant effect to our skin due to its high antioxidant values.

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