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FORMULATION AND EVALUATION OF NOVEL ANTIAGING CREAM CONTAINING RAMBUTAN FRUITS EXTRACT

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ABSTRACT: Skin aging is a complex process induced by constant exposure to ultraviolet (UV) irradiation and damages human skin. Rambutan (*Nephelium lappaceum*), a delicious tropical fruit that grow in Malaysia and spread widely in most Southeast Asian countries, have strong antioxidants properties due to the presence of ellagic acid, corilagin, geraniin, β-carotene and vitamin C. These compounds scavenge the free radical and thereby protect the skin against oxidative damage. The aim of the present study is to formulate and evaluate an antiaging cream containing Rambutan fruits extract. The SMEP, SMEF, CMEP and CMEF produced significant antioxidant activities and tyrosinase inhibition with low IC_{50} values. All the four extracts were formulated into an antiaging cream and evaluated. The results showed that the formulated antiaging creams and its ingredients were consistent in quality and can be easily used. From the above results, it is concluded that the formulation containing SMEF and CMEF are safe and usable for the skin. The present results demonstrate that the Rambutan fruits extracts has a good potential for cosmetic product development.

INTRODUCTION: Skin aging is influenced by many factors including ultraviolet radiation (UV), excess alcohol consumption, tobacco abuse and environmental pollution. Combined, these factors lead to cumulative deterioration in skin appearance and function [1, 2]. According to Soyun et al., 2009 [3], aging of the skin is characterized by irregular pigmentation, increased wrinkling, loss of elasticity, dryness and roughness. The use of natural compounds in skin protection especially topical application of antioxidants indicates their popularity in decreasing the effect of aging on the skin [4].

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Rambutan (*Nephelium lappaceum*), a delicious tropical fruit that grow in Malaysia and spread widely in most Southeast Asian countries like Indonesia, Philippines, Vietnam, Sri Lanka, Cambodia and Thailand. Rambutan is also called "hairy fruit" due to its red spiny or hairy appearance. Rambutan fruits (pulp, seeds and peels) have strong antioxidants properties such as ellagic acid, corilagin, geraniin, β-carotene and Vitamin C. These compounds scavenge the free radical and thereby protect the skin against oxidative damage. Previous studies have shown N. lappaceum rind extract to exhibit high anti-oxidant, antibacterial, anti-hyperglycemic and anti-Herpes Simplex virus type 1 activities [5-8].

Rambutan fruits are well known for its antioxidant properties. However, so far the Rambutan fruits have not been explored as an antiaging formulation.

Therefore the present study intends to formulate Rambutan extract into a skincare formulation (for example, as cream) that may scavenge free radicals and protect the skin against oxidative damage. This is supported by Nair et al. in 2012 [9], who concluded that it is possible to develop creams containing herbal extracts having antioxidant property and they can be used as the provision of a barrier to protect skin.

For this study, 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 stabilise the final formulation as antioxidants are very unstable and can easily oxidize, becoming inactive before reaching its site of action. Recent research have reported that different combinations of antioxidants appear to have synergistic effects and, therefore, better efficiency when compared to an isolated antioxidant. Hence, in the present study we are interested to evaluate the formulated cream along with its effects on different parameters related to skin aging.

MATERIALS AND METHODS:

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

Collection and Identification: 12 kg of Rambutan (*Nephelium lappaceum*) fruits was purchased from the local market and identified. (**Fig. 1)**

FIG. 1: *NEPHELIUM LAPPACEUM* LINN. (RAMBUTAN)

Extraction: The flesh, peels and seeds of Rambutan fruits were separated, dried in hot air oven at 35-40°C and grinded to coarse powder using blender. The dried powder of flesh and peels of Rambutan (500 g each) were successively extracted with petroleum ether, ethyl acetate, chloroform and methanol (1.5 l each) using Soxhlet apparatus separately for 18-20 hours.

The dried powder of flesh, peels and seeds were also extracted separately using methanol by Soxhlet extraction method. All the extracts were concentrated to dryness under reduced pressure and controlled temperature using rotary evaporator. The percentage yield of all the extracts were calculated. All the extracts were stored in air-tight containers in a refrigerator at 4 °C until further use.

Qualitative Phytochemical Analysis: All the extracts obtained as above were tested for the following qualitative chemical tests for the identification of various phytoconstituents [10].

- **Tests for alkaloids:** Dragendorff's test, Wagner's test, Mayer's test and Hager's test.
- **Tests for carbohydrates:** Molisch test, Fehling's test and Benedict's test.
- **Tests for Proteins:** Biuret test, Xanthoproteic test and Lead acetate test.
- **Tests for steroids and sterols:** Libermann-Burchard test and Salkowaski test.
- **Tests for glycosides:** Legal test, Baljet test, Borntrager's test and Keller-Kiliani test.
- **Test for flavonoids:** Shinoda test.
- **Tests for tannins:** Lead acetate test and gelatin test

- **Test for fixed oils:** Spot test and saponification test.

***In vitro* Antioxidant Activity:** The *in vitro* methods are based on 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. Methods vary greatly as to the generated radical, the reproducibility of the generation process, and the endpoint that is used for the determination.

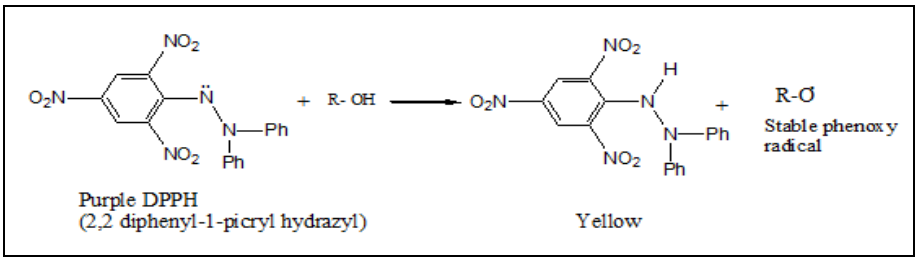
Even though *in vitro* methods provide a useful indication of antioxidant activities, data obtained from *in vitro* methods are difficult to apply to biological systems and do not necessarily predict a similar *in-vivo* antioxidant activity. A number of different methods may be necessary to adequately assess *in vitro* antioxidant activity of a specific compound or antioxidant capacity of a biological fluid. All the extracts were tested for *in vitro* antioxidant activity using several standard methods. The final concentration of the extract and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25 and 15.625, 7.812 µg/ml. The absorbance was measured spectrophotometrically against the corresponding blank solution.

The percentage inhibition was calculated by using the following formula.

$$\text{Radical scavenging activity (\%)} = \frac{\text{OD control - OD sample}}{\text{OD control}} \times 100$$

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

DPPH Assay: [11] The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donor's changes to yellow in colour. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution in ethanol or methanol and the decrease in absorbance was measured at 490 nm.



Reagents: 2, 2-Diphenly 1-picryl hydrazyl solution (DPPH, 100 µM): Accurately weighed 22 mg of DPPH and dissolved 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 of the extracts and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 21 mg/ml concentration. These solutions were serially diluted separately to obtain the lower concentrations.

Preparation of Standard Solutions: Accurately weighed 10 mg each of ascorbic acid and rutin and dissolved in 0.95 ml of freshly distilled DMSO to get 10.5 mg/ml concentration. These solutions were serially diluted with DMSO to get the lower concentrations.

Procedure: To 2 ml of DPPH solution, 100 µl of each of the extract or standard solution was added separately. The solutions were incubated at 37 ^{o}C for 30 min and the absorbance of each solution was measured at 490 nm (Parasuraman et al., 2010) using UV spectrophotometer.

Tyrosinase Inhibition Activity: [12] Tyrosinase, a copper-containing monooxy-genase, is a key enzyme that catalyses melanin synthesis in melanocytes. It catalyzes the conversion of tyrosine to dopa, dopaquinone, and subsequent autopolymerization to melanin. Tyrosinase inhibitor has been used as a whitening agent or antihyperpigment agent because of its ability to suppress dermal-melanin production. Many scientists are working to isolate tyrosinase inhibitors from natural products.

Since the accumulation of excessive epidermal pigmentation leads to various dermatological disorders, such as melasma associated with age, freckling, age spots, and sites of actinic damage, tyrosinase inhibitors have become increasingly important in medication and in cosmetics to prevent hyperpigmentation through the inhibition of enzymatic oxidation.

The extracts that showed significant antioxidant activity in DPPH method were selected for tyrosinase inhibition assay. The potent extracts were assayed for tyrosinase inhibition by measuring their effect on tyrosinase activity in a 96-well reader. 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 ^{o}C (sometimes1.5 mM l-tyrosine and 100 U/mL mushroom tyrosinase). The reaction mixture was pre-incubated for 10 min before adding substrate. The change of the absorbance at 475 nm (sometimes 490 nm) was measured. The percent inhibition of tyrosinase was calculated as follows:

$$\text{Inhibition (\%)} = [(A - B)/A] \times 100$$

Where A is absorbance without plant extract and B is the change in absorbance with Rambutan extract. Extracts that showed significant tyrosinase inhibition were selected for formulation of antiaging cream.

Formulation of Antiaging Cream: SMEF (Successive methanol extract (Flesh)), CMEF (Crude methanol extract (Flesh)), SMEP (Successive methanol extract (Peels)) and CMEP (Crude methanol extract (Peels)) were chosen to prepare antiaging cream. The composition of the antiaging cream were shown in **Table 1**.

TABLE 1: COMPOSITION OF ANTIAGING CREAM

Components	Amount (%w/w)
Active Ingredient	
Rambutan fruits extract (SMEF/ CMEF/SMEP/CMEP)	3%
Oily Phase	
Stearic acid	10%
Cetyl alcohol	6%
Liquid paraffin	6.6%
Aqueous Phase	
Glycerin	5%
Methyl paraben	0.05%
Propylene glycol	30%
Deionised water q.s.	100%

Heated oily phase and aqueous phase, both up to 70°C were mixed using homogenizer by addition of methyl paraben, extract and fragrance. With constant mixing, the remaining distilled water is added and continuously stirred until the mixture cools. Cream was formed when the consistency of the mixture was viscous and opaque. Base is prepared without extract. The same method will be adopted to prepare the base without extract. The formulated creams are shown in **Fig. 2.**

FIG. 2: FORMULATED ANTIAGING CREAMS (CMEF, SMEF, CMEP AND SMEP)

Evaluation of Antiaging Cream: [13, 14] 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 occurs 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 were checked.

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

Acid Value: 10 g of the cream was dissolved in 50 ml mixture of equal volume of alcohol and solvent ether in a flask. The flask is connected to a reflux condenser and slowly heated, until the sample dissolve completely, to this 1 ml of phenolphthalein was added and titrated with 0.1N NaOH, until faintly pink color appears after shaking for 30 sec.

$$\text{Acid value} = n*5.61/w$$

n = the number of ml of NaOH required; w = the weight of cream

Saponification Value: 2 g of the cream was refluxed with 25 ml of 0.5 N alcoholic KOH for 30 min, to this 1 ml of phenolphthalein is added and titrated immediately, with 0.5 N HCL.

$$\text{Saponification value} = (b-a)*28.05/w$$

a = the volume in ml of titrant; b = the volume in ml of titrate, w = the weight of cream

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 hrs and reported.

Microbial Limit Test: Petri dishes of 9-10 cm in diameter are used. Soybean-casein digest agar medium was used for bacteria detection and Sabouraud glucose agar was used for fungi detection. 20 ml of sterilized agar medium, previously melted and kept below 45 °C is added and mixed evenly.

10 g of cream was dissolved in phosphate buffer (7.2) and made up to 100 ml. The microbial limit test was performed using spread plate method. 100 µl of the dissolved cream was instilled on the solidified and dried surface of the agar medium and spreaded uniformly using a spreader. After the agar solidifies, it was incubated for 5 days at 30—35 °C for bacteria detection and at 20—25 °C for fungi detection. The number of colonies developed per plate was calculated and recorded.

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.

Evaluation of Antiaging Cream on Skin: A total of 10 healthy volunteers in both genders, aged from 35 to 65 years were selected in the study after securing their informed consent. Persons with known hypersensitivity to any of the ingredients of the formulation, any facial wound or abrasion, and who were not willing to give informed consent were excluded from the study. Prior to the tests, the volunteers were examined by a cosmetology expert for any serious skin disease or damage especially on cheeks and forearms. All the skin tests were performed at 21 ± 1 °C and 40 ± 2 % relative humidity.

Patch Test (Burchard test): [15, 16] The experiment was carried out on the forearms of volunteers as forearms are exposed to UV radiations. On the first day, the patch test (Burchard test) was performed on the forearms of each volunteer to determine any possible reactions to the emulsions. In this test, 5 x 4 cm region was marked on the forearms. The patch (bandage disc) for the right forearm was saturated with 1 g of base while the patch for left forearm was saturated with 1 g of the Rambutan extract formulation. Each was applied to the marked region separately on each forearm and covered with surgical dressing after application. The patches were removed after 48 hrs, the forearms washed with physiological saline and scores were recorded for the presence of erythema (skin redness) using a scale of 4 points, ranging from 0 to 3; where 0 stands for absence of erythema, 1 for mild erythema, 2 for moderate erythema and 3 for severe erythema.

Each volunteer were asked to note the degree of irritation/itching and assign a score from the same scale. The mean scores were calculated.

RESULTS:

Extraction and Qualitative Phytochemical Studies: The nature and yields of the extracts were given in **Table 2**. The qualitative phytochemical analysis showed the presence of carbohydrates, fixed oils, and absence of proteins in all the extracts. Alkaloids were present in all the crude methanol extracts.

***In vitro* Antioxidant Activity:** The DPPH radical scavenging activities of all the extracts were assessed, the SMEP, SMEF, CMEP and CMEF showed potent antioxidant activity with IC_{50} values of 38.88µg/ml, 93.85µg/ml, 103.84µg/ml and 98.71µg/ml respectively. The extracts of SCEP, SEEP, SCEF, SEEF and CMES showed moderate activity with IC_{50} values of 101.07µg/ml, 350.29µg/ml, 403.45µg/ml, 393.44µg/ml and 687.22µg/ml, respectively. The extracts SPEP and SPEF showed weak activity with IC_{50} values of 747.04 µg/ml and >1000 µg/ml, respectively. However, all the extracts were found to be less active compared to the standards used. The standard ascorbic acid and rutin potent antioxidant activity with IC_{50} values of 11.50 and 0.60 µg/ml, respectively. (**Table 3** and **Fig. 3)**

Tyrosinase Inhibition Activity: Based on the *in-vitro* antioxidant results, SMEP, SMEF, CMEP and CMEF were selected for tyrosinase inhibition study. The CMEP and CMEF showed potent tyrosinase inhibition activity with IC_{50} values of 38.88 µg/ml and 43.80µg/ml respectively. The SMEP and SMEF showed moderate tyrosinase inhibition activity with IC_{50} values of 51.44 µg/ml and 358.47 µg/ml, respectively. However, all the extracts were found to be less active compared to the standard gallic acid with IC_{50} value of 22.50 µg/ml. Based on tyrosinase inhibition activity, SMEP, SMEF, CMEP and CMEF were selected to formulate antaging cream. (**Table 4** and **Fig. 4)**

TABLE 2: NATURE, PERCENTAGE YIELD AND QUALITATIVE PHYTOCHEMICAL ANALYSIS OF THE EXTRACTS

Extract	Nature	% Yield	Phytoconstituents								
			Alkaloids	Carbohydrates	Proteins	Amino acids	Steroids	Glycosides	Flavonoids	Tannins	Fixed oils
Successive petroleum ether extract (Peels)	Yellowish semisolid	1.31	A	P	A	A	A	P	A	A	P
Successive chloroform extract (Peels)	Dark reddish brown semisolid	1.09	A	P	A	A	P	A	A	A	P
Successive ethyl acetate extract (Peels)	Dark brown semisolid with residues	1.87	P	P	A	A	P	A	P	P	P
Successive methanol extract (Peels)	Dark brown semisolid	21.36	P	P	A	A	A	A	P	P	P
Successive petroleum ether extract (Flesh)	White semisolid	1.07	A	P	A	A	P	P	P	A	P
Successive chloroform extract (Flesh)	Dark brown semisolid with residues	1.18	A	P	A	A	A	A	P	A	P
Successive ethyl acetate extract (Flesh)	Dark brown semisolid with residues	1.36	P	P	A	A	P	A	P	P	P
Successive methanol extract (Flesh)	Dark brown semisolid	52.38	A	P	A	A	A	A	P	A	P
Crude methanol extract (Peels)	Dark brown semisolid	29.84	P	P	A	A	A	A	P	P	P
Crude methanolextract (Flesh)	Dark brown semisolid	50.24	P	P	A	A	A	A	P	A	P
Crude methanol extract (Seeds)	Golden yellow semisolid	4.48	P	P	A	P	P	P	A	P	P

A= absent, P= present

TABLE 3: *IN VITRO* ANTIOXIDANT ACTIVITY OF THE EXTRACTS BY DPPH METHOD

Extract	Concentration (µg/ml)							Extracts Abbreviation
	1000	500	250	125	62.5	31.25	IC_{50}	
	% Inhibition							
Successive petroleum ether extract (Peels)	52.32	46.43	45.20	21.36	-	-	747.04	SPEP
Successive chloroform extract (Peels)	78.70	76.13	71.99	50.06	40.43	2.37	101.07	SCEP
Successive ethyl acetate extract (Peels)	73.64	56.06	49.09	29.70	3.64	-	350.29	SEEP

Successive methanol extract (Peels)	85.40	83.17	82.22	78.41	69.52	47.62	38.88	SMEP
Successive petroleum ether extract (Flesh)	27.81	16.58	-	-	-	-	>1000	SPEF
Successive chloroform extract (Flesh)	61.61	58.33	33.04	-	-	-	403.45	SCEF
Successive ethyl acetate extract (Flesh)	76.19	53.97	38.62	29.84	10.05	-	393.44	SEEF
Successive methanol extract (Flesh)	72.50	67.94	63.57	57.92	39.16	33.70	93.85	SMEF
Crude methanol extract (Peels)	85.89	83.54	81.97	60.82	29.78	18.65	103.84	CMEP
Crude methanol extract (Flesh)	>100	>100	87.80	56.85	35.71	27.68	98.71	CMEF
Crude methanol extract (Seeds)	61.45	44.58	27.41	-	-	-	687.22	CMES
Ascorbic acid								**11.50**
Rutin								**0.60**

- No inhibition

TABLE 4: TYROSINASE INHIBITION OF THE POTENT EXTRACTS

Extract	Concentration (µg/ml)							
	1000	500	250	125	62.5	31.25	IC_{50}	
	% Inhibition							
Successive methanol extract (Peels)	-	-	77.69	67.77	53.72	34.95	51.44	SMEP
Successive methanol extract (Flesh)	-	59.68	41.94	32.26	26.61	-	358.47	SMEF
Crude methanolic extract (Peels)	85.40	83.17	82.22	78.41	69.52	47.62	38.88	CMEP
Crude methanolic extract (Flesh)	-	79.03	75.00	61.29	59.68	44.35	43.80	CMEF
Gallic acid								**22.50**

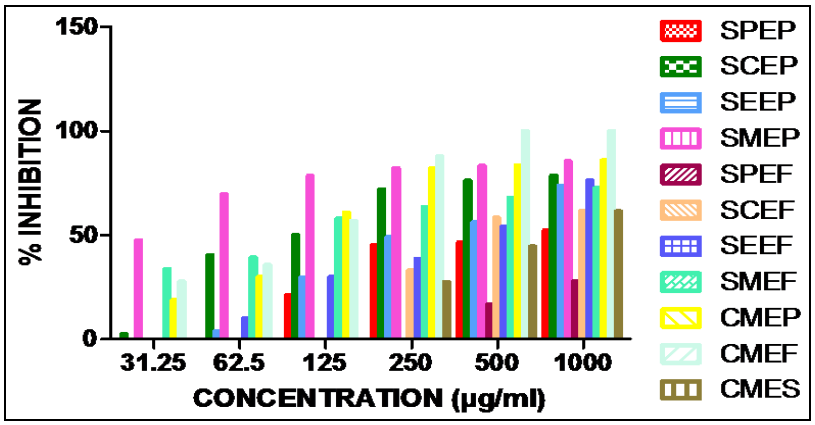


FIG. 3: *IN VITRO* ANTIOXIDANT ACTIVITY OF THE EXTRACTS BY DPPH METHOD

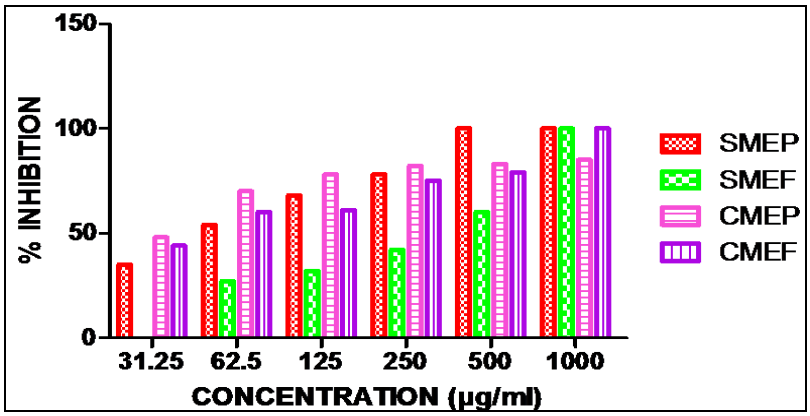


FIG. 4: TYROSINASE INHIBITION OF THE POTENT EXTRACTS

Evaluation of formulated antiaging cream:

The dye test was confirms that all the formulations were o/w type of emulsion cream. The pH of the formulated cream was found to be in range of 4.30 to 5.20 which is good and recommended pH for the skin. The acid value and saponification value of all the formulations were presented in **Table 5**, and showed satisfactorily values.

TABLE 5: pH, ACID VALUE AND SAPONIFICATION VALUE OF FORMULATED ANTIAGING CREAM

Formulation	pH	Acid value (mg NaOH/g of cream)	Saponification number (mg KOH/g of cream)
CMEF	5.20	19.07	47.70
CMEP	5.30	20.20	44.90
SMEF	4.30	19.75	46.30
SMEP	4.50	20.42	46.30

Irritancy test was conducted with 5 healthy volunteers to identify the safety, skin irritation and allergic sensitization were scarce or absent. All the formulations showed no redness, edema, inflammation and irritation during irritancy studies. The results indicates that all the formulations were safe to be used on the skin (**Table 6**).

TABLE 6: TYPE OF ADVERSE EFFECT OF FORMULATIONS

Formulation	Irritant	Erythema	Edema
CMEF	NIL	NIL	NIL
CMEP	NIL	NIL	NIL
SMEF	NIL	NIL	NIL
SMEP	NIL	NIL	NIL

The formulated antiaging creams were evaluated for several physicochemical tests and the results were shown in **Table 7**. The type of smear formed on the skin was not greasy after the application of all formulated creams. All the formulated creams when applied on skin were easily removable by washing with water. All the formulations were produce 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.

TABLE 7: PHYSICOCHEMICAL EVALUATION OF THE FORMULATED ANTIAGING CREAM

Parameter	Results			
	CMEF	CMEP	SMEF	SMEP
Homogenity	Good	Good	Good	Good
Appearance	No change in colour	No change in colour	No change in colour	No change in colour
Odour	Good	Good	Good	Good
Spreadability	Good	Good	Good	Good
After feel	Emollient and slipperiness	Emollient and slipperiness	Emollient and slipperiness	Emollient and slipperiness
Type of smear	Non greasy	Non greasy	Non greasy	Non greasy
Removal	Easy	Easy	Easy	Easy
Microbial limit test	<100 colonies			
Stability	Stable for 2 months			

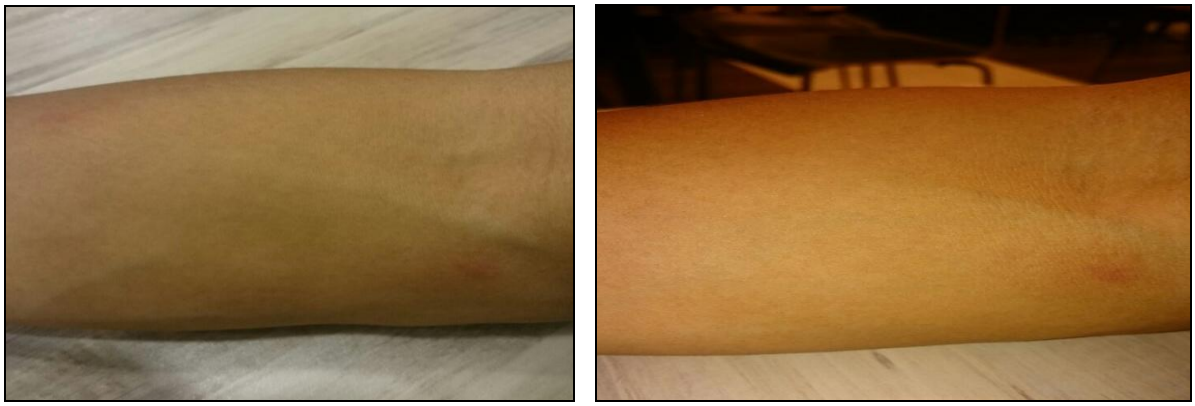
All the physiochemical parameters were maintained during the accelerated stability studies at temperatures 8°C ± 0.1°C in refrigerator and at 25°C ± 1°C, 40°C ± 1°C and 40°C ± 1°C in incubator for 8 weeks. The results of accelerated stability test showed that there were not any particular changes in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

Evaluation of Antiaging Cream on Skin (Patch Test): No severe erythema was occurred in any of the volunteers for formulation containing CMEF and CMEP. Mild erythema was occurred for three volunteers in formulation containing CMEF and four volunteers in CMEP formulation. The results were presented in (**Table 8** and **Fig. 5** and **6)**.

TABLE 8: THE MEAN SCORE OF THE HEALTHY VOLUNTEERS OPINION ON THE PATCH TEST AFTER TREATMENT WITH FORMULATED ANTIAGING CREAM (N=10)

Score	CMEF	CMEP
0	7	6
1	3	4
2	0	0
3	0	0
Mean	0.30	0.40

0 = absence of erythema, 1 = mild erythema, 2 for moderate erythema and 3 for severe erythema.



Before After

FIG. 5: PATCH TEST OF THE FORMULATED ANTIAGING CREAM

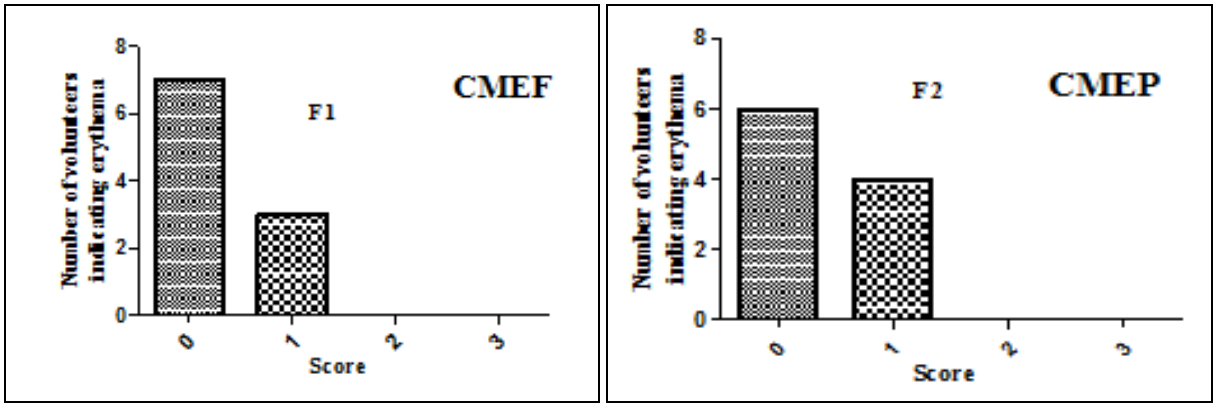


FIG. 6: OPINION ON THE PATCH TEST AFTER TREATMENT WITH CMEF AND CMEP FORMULATION (N=10)

DISCUSSION: *Nephelium lappaceum* is well known for its medicinal value in the traditional system of medicine. Rambutan fruits (pulp, seeds and peels) have strong antioxidants properties such as ellagic acid, corilagin, geraniin, β-carotene and vitamin C. These compounds scavenge the free radical and thereby protect the skin against oxidative damage [7, 8, 17].

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 stabilise the final formulation as antioxidants are very unstable and can easily oxidize, becoming inactive before reaching its site of action. Recent research have reported that different combinations of antioxidants appear to have synergistic effects and, therefore, better efficiency when compared to an isolated antioxidant. The SMEP, SMEF, CMEP and CMEF showed potent antioxidant activity in DPPH method. So, these extracts can be considered as a natural antioxidant. It is well known that natural antioxidants have 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 SMEP, SMEF, CMEP and CMEF.

The formulated cream was o/w type emulsion, hence can easily washed with water and gives better consumer compliance. Our study indicated that, all the formulated creams SMEP, SMEF, CMEP and CMEF 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. All the formulations passed the antimicrobial limit test.

The patch test showed that there no severe erythema was occurred in any of the volunteers for formulation containing CMEF and CMEP. This indicates that the formulated creams were safe for the consumers.

CONCLUSION: The SMEP, SMEF, CMEP and CMEF were produced significant antioxidant activities and tyrosinase inhibition. The results demonstrated that the formulated antiaging creams and its ingredients were consistent in quality and can be easily used. From the above results, it is concluded that the formulation containing SMEF and CMEF are safe and usable for the skin. The present results demonstrate that the Rambutan fruits extracts has good potential for cosmetic product development.

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