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Development Sunscreen Microemulsion Gel Containing n-Hexane Fraction of Mangosteen Pericarp (*Garcinia mangostana* Linn.).

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ABSTRACT

Sunscreen can protect skin from ultraviolet (UV) radiation, through absorbing, scattering or reflecting radiation. Mangosteen pericarp contain active compounds which can absorb UV rays. The objectives of this research were to determine photoprotective activity of mangosteen pericarp fractions, formulated selected fraction into microemulsion gel dosage forms, and evaluate the physical characteristic and sunscreen activity of the preparation. Extraction was conducted by maceration method using ethanol 96%. Fractionation performed by liquid-liquid extraction using n-hexane, ethyl acetate and water as solvents. Sun protecting factor (SPF) value was determined by Mansur method using Spectrophotometer UV/Vis. The result show that n-hexane fraction had highest value of SPF compared with water and ethyl acetate fractions. Microemulsion gel preparation containing 0.1 % mangosteen n-hexane fractions were stable based on organoleptic, centrifugation, and freeze thaw tests. The SPF value of microemulsion gel as much as 4.01±0.31.

Keywords: Mangosteen , sunscreen, microemulsion gel, n-hexane fraction

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INTRODUCTION

UV radiation (UVR) is composed of UVA (320-400 nm), UVB (290-320 nm), and UVC (100-290 nm). Ambient sunlight is composed primarily of UVA (90–95%) and UVB (5–10%) energy, with most solar UVC absorbed by the ozone layer. UVB is largely responsible for erythema of sunburn and suntan of the skin [1]. Naturally occurring UV radiation is the environmental mutagen responsible for skin pathologies, including erythema and inflammation, degenerative aging changes, and cancer [2]. UVR induced reactive oxygen species (ROS) production and generating photobiologycal reactions on the skin [3].

Sunscreen can protect skin from ultraviolet (UV) radiation, through absorbing, scattering or reflecting radiation. The main goals of sunscreens are to protect against UVB radiation and long wavelength UVA radiation, scavenge ROS, activate cellular repair systems, including DNA repair. The efficacy of sunscreen products has been usually measured in form of sun protection factor (SPF) [4][5].

Mangosteen (*Garcinia mangostana* Linn.) has been long known as the "Queen of Fruits", is in the family of Guttifarae. The fruit is approximately 3.5- 7 cm in diameter with about 1 cm thick dark purplish brown shell and 4-8 white petals inside. Mangosteen pericarp was known have various pharmacological properties including, antioxidant, antitumoral, antiallergic, anti-inflammatory, antibacterial, and antiviral activities [6]. Another health benefit of mangosteen is sun protecting activity. Mangosteen known containing α-mangostin compound considered as the main contributor for the sunscreen activity [7]. The antioxidant effect of mangosteen can increase the value of mangosteen sunscreen formulation. Antioxidants are commonly added in commercial sunscreen preparations in order to reduce the photo-oxidative damage that results from UV-induced ROS production. [8]

Most common types of sunscreens presently in use are the topical preparations. One kind of topical preparation that appropriate to be used as a sunscreen is microemulsion [9]. Microemulsion are optically transparent, thermodynamically stable dispersions of oil and water stabilized by surfactant, usually in combination with a cosurfactant [10]. Microemulsions provide several advantages over conventional topical formulations such as their spontaneous formation as they can be prepared easily by simple mixing of particular components in suitable ratios at ambient temperature [11]. For topical delivery, microemulsion is incorporated in polymer gel base to prolong the local contact to the skin, that called microemulsion gel [12].

There are some in vitro assays methods to determine sun protecting factor (SPF) of the samples, one of them is with measurement of the absorption characteristics of the sunscreens product on the basis of spectrophotometric analysis of dilute solutions. UV spectrophotometric method for SPF determination is easy, rapid, cost effective, and can be used for in vitro determination of SPF value in many cosmetic formulations [13]. The objective of this research was to determine SPF value of mangosteen pericarp fractions and formulate the selected fraction into microemulsion gel preparation. The ultimate goal of this research is to develop an effective herbal sunscreen microemulsion gel.

MATERIALS AND METHODS

Sample collection

Pericarp of mangosteen were collected from Subang, West Java, Indonesia, and determinated at herbarium Bandung Institute of Technology (ITB), Indonesia. The pericarp were dried and powdered to get a coarse powder.

Phytochemical screening

Preliminary screening of secondary metabolites such as alkaloids, flavonoids, saponins, quinons, polyphenol, tannin, terpenoids and steroid were carried out according to the common phytochemical methods [14].

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Extraction and fractionation

The dried powder of mangosteen pericarp was extracted by maceration with ethanol (EtOH) for 24 hours at room temperature for three times, and evaporated using a vacuum rotary evaporator. The extract EtOH was fractionated with n-hexane, ethyl acetate , and water. The fractions were also evaporated using vacuum rotary evaporator.

Sun protection value determination test of the fractions

The test was done in vitro using spectrophotometric analysis (UV-Visible spectrophotometer/Shimadzu type UV mini-1240, Japan). Sample as much as 10.0 mg was weighed individually, transferred to 100 ml volumetric flask and finally diluted to volume with ethanol (100 ppm). Then, the solution was diluted to get sample concentration 50 ppm. The absorbance of samples in solution was measured in the wavelength range of 290 to 320 nm with every 5 nm interval. Each measurement was performed in triplicates. SPF value was calculated according to the Mansur equation.

$$SPF_{spectrophootometric} = CF \times \sum_{290}^{230} EE(\lambda) \times 1(\lambda) \times Abs(\lambda)$$

Where EE (λ) - erythemal effect spectrum; I (I) - solar intensity spectrum; Abs-Absorbance of sunscreen product ; CF-correction factor (=10). The value of EE x I are constant and predetermined. (EE: Erythemal effect spectrum; I: solar intensity spectrum)

Table 1: **The value of EE x I**

Wave leght (λ/nm)	EE x I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

Formulation mangosteen fraction microemulsion gel

The composition of microemulsion gel was shown on table 2.

Table 2: Microemulsion gel formulation

Ingredients	Microemulsion gel (%)
Mangosteen Fraction	0.1
Olive oil	6
Tween 80	35
Glycerin	20
Propylene Glycol	10
Methyl paraben	0.18
Propyl paraben	0.02
Alpha-tocopherol	0.03
Gel carbopol 1 %	10
Purified water until	100

First, the stock gel was prepared by dispersing Carbopol 934 in heated purified water (80 °C) and the gel pH was adjusted by Triethanolamine (TEA), and the dispersion was cooled and left overnight. Fraction, methyl paraben, propyl paraben and alpha-tocopherol was added to the mixtures of oil, surfactant, and

cosurfactant, then an appropriate amount of water was added to the mixture by stirring the mixtures at the temperature 40-50^{0}C. Carbopol gel was slowly mixed with microemulsion under stirring.

Physical stability evaluation of microemulsion gel

Physical appearance

The microemulsion gel was inspected visually for their color, transparency, homogenecity and consistency.

Centrifugal test

Centrifugal test was performed at room temperature and at 3750 rpm for 5 hours by placing the 10 g of sample in centrifugal tubes.

Freeze thawing test

At one cycle of this test, microemulsion gel was stored at the refreegerator (4^{0}C) for 48 hours (freezing) and then stored at the oven (40^{0}C) for 48 hours. The test was conducted in 5 cycle. Observation about oraganoleptic and phase separation of the preparationl was done at the end of each cycle.

Rheological Study

The viscosity of th microemulsion gel formulations was determined by using viscometer brookfield RV (DV-I Prime).

SPF determination of microemulsion gel

Determination SPF value of the cinnamon bark microemulasion gel and the base, were done use the same method with the extract, using spectrophotometer. The sample was preparated by ethanol dilution (1:500) and mix vigorously until homogeny. The absorbance of samples in solution was measured in the wavelength range of 290 to 320 nm with every 5 nm interval. Each measurement was performed in triplicates. SPF value was calculated according to the Mansur equation. [15]

RESULTS AND DISCUSSION

Coarse powder of mangosteen pericarp was extracted by maceration process using universal solvent (ethanol). From 1800 g cinnamon powder, it was yield 208.338 g ethanol extract (11.57%). The phytochemical analysis of the coarse powder and ethanol extract of the mangosteen pericarp showed the presence of different groups of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, quinons, terpenoids, and steroid which are medicinally importance.

Table 3: **Phytochemical screening on the plant and extract**

Class of compounds	Coarse Powder	Ethanol extract
Alkaloids	+	+
Flavonoids	+	+
Saponins	-	+
Tannins	+	+
Quinons	+	+
Polyphenol	+	+
Steroids & Triterpenoids	+	+
Monoterpenes & sesquiterpenes	-	-

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The extract was fractionated by liquid-liquid extraction using three kinds of solvents. The fractionation process resulting 62.34% water fraction, 7.99% ethyl acetate fraction, and 0.13% n-hexane fraction. SPF value determination were done to all of the mangosteen fractions. The result show at table 4.

Table 4: SPF value of extract and fractions of mangosteen pericarp (50 ppm)

Samples	SPF Value	Protection Category (refer to FDA)
Water Fraction	0.89 ± 0,14	-
Ethyl Acetate Fraction	3.63 ± 0.25	Minimum
N-hexane Fraction	7.15 ± 1.56 *	Extra
Extract	1.31 ± 0.019	-

*statistically different (P<0.05) with other samples

The result shows that n-hexane fraction has the highest SPF value compare with extract and the other fractions. Of the result, that known that the major active compound of mangosteen pericarp with give photo protection activity is non polar compound. Alfa-mangosteen was known as major compound of n-hexane fraction that has photo protection activity [7]. Based on FDA regulations that categorize the effectiveness of sunscreen, the n-hexane fraction has extra protection activity (SPF value 6-8) [16].

Table 5: SPF calculation of n-hexane fraction

Wave lenght(nm)	EE x I	Abs (Avarage)	(EE x I) x abs x CF
290	0.0150	0.475	0.071
295	0.0817	0.554	0.452
300	0.2874	0.625	1.796
305	0.3278	0.725	2.376
310	0.1864	0.831	1.549
315	0.0839	0.896	0.751
320	0.0180	0.875	0.157
SPF Value			**7.152**

N-hexane fraction of mangosteen was formulated to microemulsion gel preparation. Microemulsions have the ability to deliver larger amounts of topically applied agents into the skin than other traditional vehicles such as lotions or creams because they act as a better reservoir for a poorly soluble drug through their capacity for enhanced solubilization. Olive oil was used as oil phase in the formulation. Oily vehicles are more effective for producing a uniform and long-lasting film of sunscreen on the skin, and their emollient properties protect the skin against the drying effects of exposure to wind and sun. Olive oil has photo protective activity and give the best SPF values compared with another herbal oil including coconut oil, almond oil, castor oil, and sesame oil [17].

Microemulsion gel formulation was found to be homogenous of liquid-semisolid consistency, colored yellow transparent, having the little odor of mangosteen. The average pH values of all prepared formulation was 6.62. Centrifugation and freeze thaw tests were done to evaluate the physical stability. No phase separation after centrifugation and freeze thaw tests was seen in mangosteen microemulsion gel. Centrifugation and freeze thaw tests have been widely used as an accelerated path to judge emulsion stability.

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Figure 1: Microemulsion gel of n-hexane mangosteen pericarp fraction

Knowledge of rheological properties of the formulation may lead to optimize topical drug delivery from topical formulations. The resulting rheogram shows the presence of a pseudoplastic-thixotropic behavior (Fig 2). This is a desirable behavior for topical drug delivery systems, since the formulation could remain stable following the preparation and packaging within the container, and then could break down as a result of shearing stresses applied during exit from the container and spreading on the skin surface. [18]

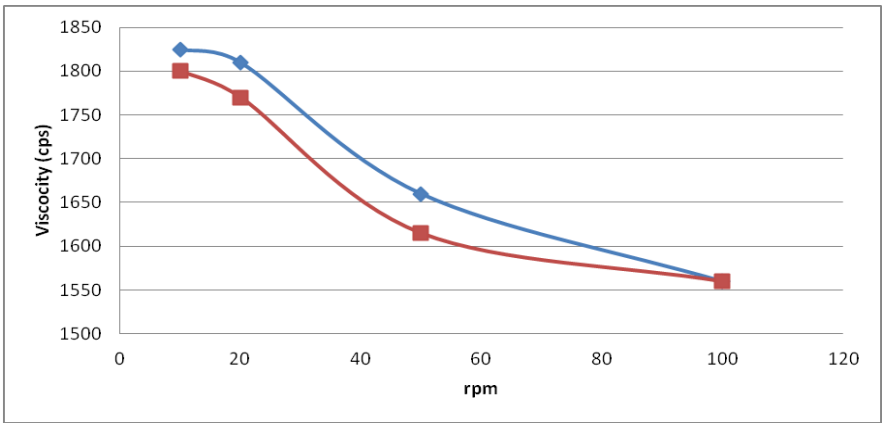

Figure 2. Rheogram of microemulsion gel (upward/backward curve)

SPF value determination was performed to mangosteen microemulsion gel and also the microemulsion gel base b(without fraction) using spectrophotometer method (Table 6). The result shows that mangosteen microemulsion gel having an SPF value higher than the base. The result informed that n-hexane mangosteen fraction give significant effect for sunscreen activity of the formulated microemulsion gel. The preparation has moderate protection activity since the SPF value 4-6. The concentration of n-hexane fraction must be increased (>0.1%) in microemulsion gel preparation to increase the photo protective activity.

Table 6: SPF value of microemulsion gel

Samples	SPF value
Mangosteen microemulsion gel	4.010 ± 0.310
Microemulsion gel base	0.720 ± 0.072

CONCLUSION

N-hexane fraction had highest value of SPF compared with extract and other fractions. Microemulsion gel preparation containing 0.1 % mangosteen n-hexane fractions were stable based on organoleptic, centrifugation, and freeze thaw tests. The SPF value of microemulsion gel was 4.01±0.31.

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