

EXTRACTION OF FLAVONOID FROM MANGOSTEEN PEEL (*GARCINIA MANGOSTANA*) USING ULTRASOUND-ASSISTED (UAE) EXTRACTION AND ITS SPF VALUE

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Abstract

*In this study, the peel of mangosteen (*Garcinia mangostana*) will be extracted to develop a natural sunscreen formulation. Mangosteen peel is rich in natural antioxidants, including flavonoids, tannins, triterpenoids, and phenolic compounds. The primary objectives are to determine the yield, Total Flavonoid Content (TFC), and to evaluate the Sun Protection Factor (SPF) of the ethanolic extract obtained from the mangosteen peel. Ultrasound-Assisted Extraction (UAE) will be employed, with a fixed extraction time of 45 minutes. Two concentrations of ethanol are used, 96% and 70%, respectively. The ratio of the material to the solvent used are 1:15, 1:25, and 1:35 gr/mL. This study reports the highest SPF value was obtained from the 70% ethanol extract, using a material-to-solvent ratio of 1:35 g/mL, which yielded 15.111% and exhibited an SPF of 16.009 (unitless). In contrast, the highest TFC was observed in the 96% ethanol extract, with a concentration of 2,549 mg quercetin equivalents (QE) per gram of extract.*

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Mangosteen Peel Ultrasound-Assisted Extraction; Total Flavonoid Content; Sun Protecting Factor

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1. Introduction

The ozone layer which is located in the stratosphere, contains ozone molecules that are crucial for Earth's protection. It forms a protective shield around the Earth to filter out the harmful sun's ultraviolet (UV-B) radiation [1]. The ozone layer is naturally created through a photochemical process with 90% and 10% of the concentration found in the stratosphere and the troposphere, respectively [1]. Every year, the ozone layer is shrunk by chemicals, such as Chlorofluorocarbons (CFCs), which are carried by the winds into the stratosphere after being emitted from the surface. Chlorofluorocarbons (CFCs) are gaseous substances that play a role in the depletion of the ozone layer [2]. When CFC substances are released into the atmosphere, they are broken down by ultraviolet (UV) radiation from sunlight, releasing chlorine atoms that catalyze the destruction of ozone molecules. A single CFC molecule can ultimately destroy up to 100,000 ozone molecules [3].

The mangosteen fruit offers a variety of benefits including antioxidant and antibacterial properties [4]. Notably, the peel—often considered a byproduct also harbors bioactive compounds, among which flavonoids are particularly abundant and contribute significantly to its biological effects. Catechin, a subclass of flavonoids, is among the bioactive compounds identified in mangosteen (*Garcinia mangostana* L.) peel. Flavonoids are known for their strong ultraviolet (UV)-absorbing and antioxidant properties, which make them promising natural candidates for use in sunscreen formulations as contributors to sun protection factor (SPF). In mangosteen peel, the TFC has been reported to reach up to 4.08 mg per 100 grams of fresh peel, highlighting its potential as a

sustainable source of photoprotective agents [5]. Flavonoid compounds can be efficiently extracted using Ultrasound-Assisted Extraction (UAE), a method that enhances mass transfer and cell wall disruption through acoustic cavitation, thereby improving yield and reducing extraction time [4], [6–8]. Despite its advantages in terms of time efficiency and reduced solvent consumption, UAE remains underutilized in Indonesia for the extraction of flavonoids.

2. Material and Method

A. Materials

The materials used in this study include mangosteen peel (*Garcinia mangostana L.*), analytical-grade ethanol, distilled water (aquadest), magnesium powder, 1 M potassium acetate, 2 M hydrochloric acid (HCl), quercetin (as a flavonoid standard), and aluminum chloride (AlCl_3).

B. Mangosteen Peel Preparation.

The mangosteen peel was first washed thoroughly under running water and air-dried at ambient temperature for 24 hours. Subsequently, it was further dried in a drying oven at 45 °C for 2 hours. The dried peel was then ground into a fine powder and sieved through an 850- μm mesh sieve to obtain a uniform particle size for subsequent analysis.

C. Extraction of Flavonoid Content in Mangosteen Peel

The previously prepared mangosteen peel powder was accurately weighed and transferred into Erlenmeyer flasks containing ethanol–water mixtures as extraction solvents. Three solid-to-solvent ratios were evaluated: 1:15, 1:25, and 1:35 (w/v, g/mL). In addition to the solid-to-solvent ratio, the ethanol concentration was varied at 96% and 70% (v/v), prepared by diluting analytical-grade ethanol with distilled water. These two factors solid to solvent ratio and ethanol concentration were systematically investigated to optimize flavonoid extraction efficiency.

Extraction was performed using an ultrasonic water bath at 45 °C and atmospheric pressure for 45 minutes. The resulting extract was immediately filtered under vacuum using a Büchner funnel and filter paper. The filtrate was then concentrated to dryness using a vacuum oven at 40–50 °C and 0.2 bar pressure for approximately 40 hours, then yielding a dry extract for further analysis.

The extraction yield was calculated by comparing the mass of the dried extract obtained after evaporation with the initial mass of the dried mangosteen peel used. The yield (%) was determined according to Equation 1.

$$\text{Yield}(\%) = \frac{\text{Evaporated extract mass (g)}}{\text{Mangosteen peel mass (g)}} \times 100\% \quad (1)$$

D. Determination of Total Flavonoid Content (TFC) using AlCl_3 Method - Quercetin Standard Curve Creation

A stock solution of quercetin (1000 $\mu\text{g/mL}$) was prepared by accurately weighing 25.0 mg of quercetin standard and dissolving it in ethanol to a final volume of 25 mL in a volumetric flask. A series of working standard solutions with concentrations of 40, 60, 80, 100, and 120 $\mu\text{g/mL}$ were subsequently prepared by appropriate dilution of the stock solution with ethanol. For the calibration assay, 0.5 mL of each standard solution was transferred into separate test tubes and mixed with 1.5 mL of 96% (v/v) ethanol, 0.1 mL of 10% (w/v) aluminum chloride (AlCl_3), 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The reaction mixtures were incubated at 25 °C for 30 minutes to allow complex formation. Absorbance was then measured at 438 nm using a UV-Vis spectrophotometer. A calibration curve was constructed by plotting absorbance (y-axis) against quercetin concentration (x-axis, $\mu\text{g/mL}$), and the resulting linear regression equation was used for quantification of flavonoids in the samples.

E. Determination of Total Flavonoid Content (TFC) using $AlCl_3$ Method

A blank solution was prepared following the same procedure as the standard solutions, except that 0.5 mL of ethanol was substituted for the quercetin standard solution. The blank mixture consisted of 0.5 mL of ethanol, 1.5 mL of 96% (v/v) ethanol, 0.1 mL of 10% (w/v) aluminum chloride ($AlCl_3$), 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The solution was incubated at 25 °C for 30 minutes under the same conditions as the standards. Absorbance measurements for all samples and standards were recorded against this blank using a UV-Vis spectrophotometer at 438 nm.

The test solution was prepared by mixing 0.5 mL of the mangosteen peel ethanol extract (for each extraction condition) with 1.5 mL of 96% (v/v) ethanol, 0.1 mL of 10% (w/v) aluminum chloride ($AlCl_3$), 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water. The mixture was incubated at 25 °C for 30 minutes to allow flavonoid- $AlCl_3$ complex formation. Absorbance was measured at 438 nm using a UV-Vis spectrophotometer, with the blank solution used as the reference. All analyses were performed in duplicate.

The TFC was calculated based on the quercetin calibration curve and expressed as milligrams of quercetin equivalents per gram of dry extract (mg QE/g), using equation 2:

$$F = \frac{c \times V \times f \times 10^{-3}}{m} \quad (2)$$

Where F is the number of flavonoids content using the $AlCl_3$ method, c is quercetin equivalence ($\mu\text{gQE/mL}$), V is total extract volume (mL), f is dilution factor (1) and m is sample weight (g).

F. Calculating SPF Value

The collected data is absorbance data which is measured to determine the potential of sunscreen. The extract was scaled and then the solvent was added to it and put in a measuring cup to obtain a concentration of 300 ppm. The absorbance and absorption values were measured at a wavelength of 290-400 nm using a UV-Vis spectrophotometer. The area under the absorption curve or AUC is obtained from the absorbance value at a wavelength of 290 – 400 nm with an interval of 5 nm which is calculated using equation 3 [6].

$$AUC = \frac{Aa + Ab}{2} \times dp(a - b) \quad (3)$$

Where Aa and Ab represent the absorbance values at wavelengths and (nm), respectively, $dp(a - b)$ denotes the difference between the two wavelengths (a and b).

After determining the area under the curve (AUC) at each wavelength across the UV range, the individual AUC values were summed to obtain the total AUC. The sun protection factor (SPF) was then calculated using the following equation:

$$\text{Log SPF} = \frac{[AUC]}{\lambda_n - \lambda_1} \quad (4)$$

Where λ_n is the largest wavelength (400 nm), and λ_1 is the smallest wavelength (290 nm).

3. Result and Discussion

A. Yield of Mangosteen Peel Extract

In this study, both qualitative and quantitative analyses were conducted, including extraction yield, phytochemical screening, total flavonoid content (TFC), sun protection factor (SPF) assessment, and Fourier-

transform infrared (FTIR) spectroscopy. The extraction yield was evaluated to determine the influence of solvent concentration and solid to solvent ratio on extract production. Two ethanol concentrations (96% and 70%, v/v) were tested, each combined with three solvent volumes 15 mL, 25 mL, and 35 mL corresponding to solid to solvent ratios of 1:15, 1:25, and 1:35 (w/v), based on a fixed mass of dried mangosteen peel.

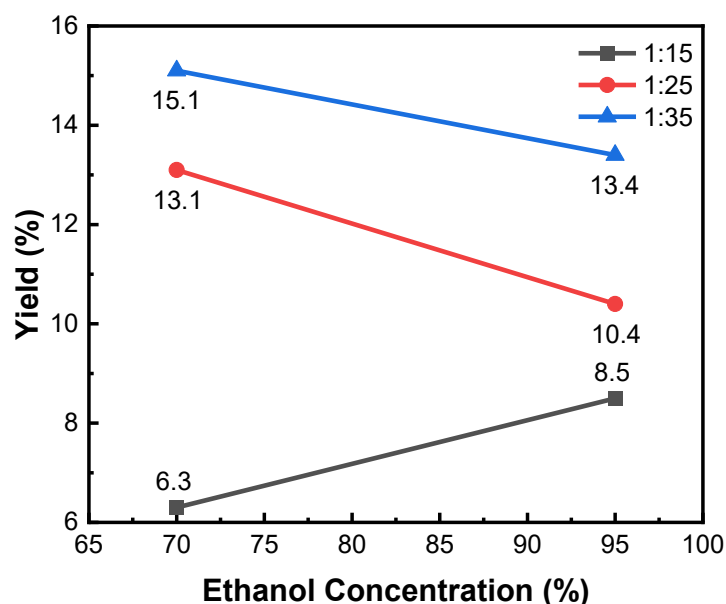


Figure 1. Effect of concentration and ratio of ingredients/solvents on yield.

As demonstrated in Figure 1, increasing the ratio of raw material to solvent significantly improved the overall extraction yield. Mechanistically, a higher solvent volume relative to the solute promotes superior solvent penetration (diffusivity) into the cellular structures, thereby accelerating the dissolution kinetics and subsequent mass transfer of the desired compound [9]. Means, the larger the volume of solvent, the faster the diffusion process and facilitates the mass transfer process [10]. Furthermore, the increased material-to-solvent ratio synergistically enhanced the effects of ultrasonic treatment. A larger solvent volume relative to the material intensified the ultrasonic cavitation, leading to greater fragmentation, erosion, and pore formation within the plant matrix. This physical disruption, combined with the resultant increase in the material-solvent contact area, significantly improved mass transfer rates and consequently boosted the final extraction yield [10]. Conversely, higher ethanol concentrations resulted in lower extraction yields, suggesting that a greater proportion of water in the hydroethanolic solvent enhances the extraction efficiency. This observation is consistent with literature findings [11]. The increased yield is likely due to the co-extraction of highly polar, non-phenolic compounds, such as proteins and carbohydrates, which exhibit significantly greater solubility in water than in ethanol [12].

B. Phytochemical of Mangosteen Peel Extract

To assess the extract's capacity to support antioxidant activity, a qualitative phytochemical screening was performed to determine the content of major secondary metabolites, shows in the Table 1. The procedure involved the addition of class-specific chemical reagents to a small sample of the extract to identify the presence of phenols, flavonoids, tannins, alkaloids, and triterpenoids [6-7].

Table 1. Phytochemical test result on mangosteen peel extract.

Test Parameters	Observation Result	Test Results	Compound Examples
Flavonoid	Formed orange color	+	Quercetin, catechin, gallocatechin [13]
Triterpenoids	No red color formed	-	Lupenone [14]
Tannins	Formed blackish green color	+	Tannin [14]
Phenol	Formed black color	+	Chrysin, caffeine acid, and cinnamic acid [15]
Alkaloids	An orange precipitate is formed	+	Phenylalanine [16]

C. Total Flavonoid Content (TFC) of Mangosteen Peel Extract

The quantification of total flavonoids relies on the formation of a stable acid complex between the target compounds and $AlCl_3$. This complexation specifically occurs through chelation between the Al^{3+} ion and the keto group at the (C-4) position alongside a hydroxyl group at either the (C-3) or (C-5) position found on flavones and flavonols [17]. Quercetin, a representative flavonol possessing both the (C-4) keto and hydroxyl groups at (C-3) and (C-5) (Figure 2), was therefore chosen as the reference standard for the calibration curve, making it an ideal candidate for total flavonoid determination [17].

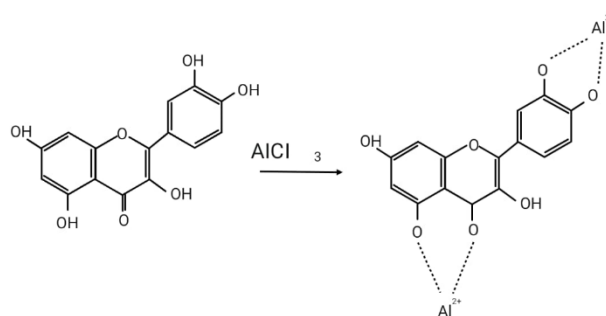


Figure 2. Chemical Reaction of Quercetin and $AlCl_3$ [18].

The TFC was quantified following the addition of 1M potassium acetate, a step specifically included to confirm the presence of flavonoids bearing a 7-hydroxyl group. A 30-minute incubation period was applied to ensure the reaction reached completion, thereby guaranteeing maximum color intensity and accurate quantification [19]. The TFC value can be calculated using a standard Quercetin curve and the results are expressed in mg QE (Quercetin Equivalent)/gr mangosteen peel. The standard curves were made by 96%, and 70% ethanol concentrations, respectively, with the absorbance measurement wavelength used, which was 438 nm [20]. The following equations for each standard curve are:

$$\text{Etanol 96\%} \rightarrow y = 0.0033x + 0.0181 ; R^2 = 0.9852 \quad (5)$$

$$\text{Etanol 70\%} \rightarrow y = 0.0035x + 0.0242 ; R^2 = 0.985 \quad (6)$$

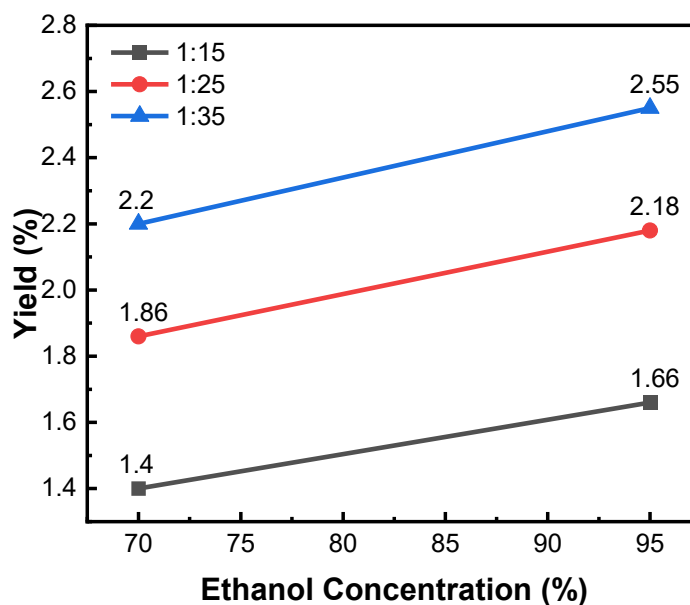


Figure 3. The concentration and solvent ratio affect the TFC of Mangosteen Peel Extract.

Figure 3 shows that the (TFC) value increases with the addition of more solvent, which corresponds to a decreased solute-to-solvent ratio at the same solvent concentration. The observed effect is due to the solvent's enhanced diffusivity into the cells, which facilitates the dissolution of the flavonoids [9]. During increasing solute/solvent ratio, the viscosity and concentration of the extraction medium will decrease, thereby increasing the cavitation effect in the UAE method and maximizing the extraction process [10]. Meanwhile, if the material/solvent ratio is too small, it can lead to not optimal extraction process.

The solvent volume also influences the contact area between the material and the solvent, specifically, increasing the solvent volume widens the contact area and improves solvent distribution [21]. According to Mas'ud, an even distribution of the solvent, coupled with an increased solvent volume, enhances the extraction yield by reducing solvent saturation, thereby facilitating the complete extraction of target components [21]. Furthermore, increasing the solvent volume enhances the solvent's extractive capacity and prolongs the effective contact time between the material and the solvent [22]. Moreover, because UAE relies on the cavitation principle for mass transfer, an increase in solvent volume will enhance the extraction efficiency [23]. Consequently, the highest TFC of mangosteen peel extract in this study was achieved using the 96% ethanol concentration with a 1:35 g/mL material-to-solvent ratio. This optimum result aligns with the "like-dissolves-like" principle, indicating that the polarity of 96% ethanol is highly effective for dissolving the dominant flavonoids in the peel.

D. SPF of Mangosteen Peel Extract

In this study, a Sun Protecting Factor (SPF) test was conducted to determine the potential of mangosteen peel extract as a basic ingredient for making sunscreen. Mangosteen peel extract with a ratio of 1:35 which has been separated from the solvent (after using a vacuum oven) was scaled and dissolved with each solvent to obtain a concentration of 300 ppm. The mangosteen peel extract absorbance was measured across the 290-400 nm range. The Area Under the Curve (AUC) value was subsequently obtained, and the Total AUC can be used to determine the Sun Protection Factor (SPF) value. The SPF results obtained are different from those for the TFC. Similar results were also obtained from a study by Ebrahimzadeha, where the SPF value correlated in parallel to the Total Phenolic Content (TPC) in *Crataegus pentagonal* extract, using methanol as a solvent via the UAE method [24].

Although flavonoids are widely studied and generally considered superior in UV radiation protection, phenolic compounds as a whole also demonstrate photoprotective potential [25-26].

The low TFC obtained in this study meant that the resulting Sun Protection Factor (SPF) value, which was primarily driven by the high Total Phenolic Content (TPC), was not quantitatively tested. The purity of the ethanol solvent had contrasting effects on the compounds extracted: higher ethanol purity led to a higher TFC value, but lower TPC values. Specifically, the maximum TPC was achieved with 50% ethanol [9]. This means that the total phenolic compounds in the mangosteen peel extract have a higher polarity than the TFCs so in 70% ethanol, there are more phenolic compounds, and in 96% ethanol, there are more flavonoid compounds. The presence of these compounds in the extract is influenced by the solubility of each compound in the solvent used for the extraction process. The polarity of the solvent has an important role in increasing the solubility of each of these compounds [11]. The results was ethanol with a concentration of 96% and 70% has an SPF value of 14.074 and 16.009, respectively. From the SPF value, it can be concluded that the potential of sunscreen for ethanol concentrations of 96% and 70% can be categorized as maximum protection.

E. Fourier transform infrared (FTIR)

The results of mangosteen peel extraction using solvents with ethanol concentrations of 96% and 70% were tested using FTIR to identify the compounds contained in the chemical groups of these compounds, the tests were carried out on variations in the ratio 1:35 gr/mL.

Fourier-transform infrared (FTIR) spectroscopy (Figure 2) revealed that the functional groups present in both extracts across all tested concentrations were nearly identical to those of the quercetin standard. Key functional groups characteristic of flavonoids were identified, including hydroxyl (O-H), carbonyl (C=O), aromatic (C=C), and aromatic (C-H) stretching vibrations (Table 2) [27]. The peaks observed in the Fourier-transform infrared (FTIR) spectra were consistent with the characteristic wavenumber ranges of flavonoid compounds, confirming the presence of key functional groups including hydroxyl (O-H), (C-H), carbonyl (C=O), aromatic (C=C), (C-O), and the fingerprint region. Interestingly, a specific functional group (C-H) appear in a small intensity in 96% concentration of the FTIR results. Meanwhile, the aliphatic (C-H) stretching vibration was higher intensity present, being identified in 70% concentration of the spectra with a peak centered at 2974.41 cm^{-1} .

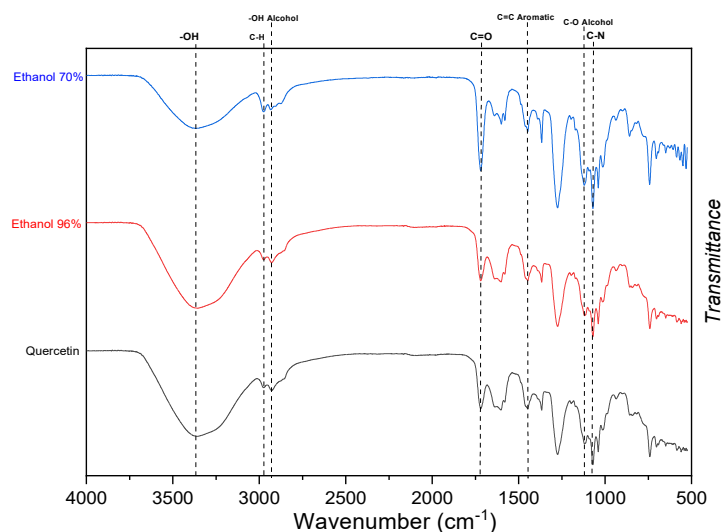


Figure 4. FTIR of Mangosteen peel extraction using solvents with ethanol concentrations of 96% and 70%.

Table 2. FTIR references to identify the functional groups compound in Mangosteen Peel Extraction.

Functional groups	Wavenumber Frequency (cm ⁻¹)	Mangosteen Peel Extract Wave Number (cm ⁻¹)		References	Identification Results
		96%	70%		
-OH	3200-3500	3356,09	3366,30	[28-32]	Phenol, Flavonoid, Tannin, Alkaloid, Saponin
C-H	2700-3000	-	2974,41		Ethanol, Tannin, Saponin
-OH alkohol	2700-3000	2928,76	2933,43		Ethanol
C=O	1650-1900	1719,89	1719,64		Flavonoid, Tannin, Phenol, Saponin
N-H	1680-1550	1601,90	1600,52		Alkaloid
C=C aromatic	1475-1500	1447,51	1447,48		Flavonoid, Tannin, Phenol, Alkaloid, Saponin
C-O eter	1085-1150	1014,46	1013,23		Flavonoid, Tannin, Phenol
C-O alcohol	1000-1260	1119,31	1120,75		Alcohol, Flavonoid, Tannin, Alkaloid, Saponin
C-N	1020-1250	1070,93	1070,95		Alkaloid
Fingerprint region of flavonoid	900-1300	1039,68	1039,55		Flavonoids

4. Conclusion

In conclusion, this research shows that mangosteen peel can be one of the potential raw materials for sunscreen production due to its unique components, including the SPF value and the flavonoid content. The study indicates that the increasing amount of solvent can lead to the inclining trend of yield and Total Flavonoid Content (TFC). The highest yield and TFC were discovered at the ratio of material to solvent 1/35 gr/mL. Furthermore, the solvent concentration showed an effect on the yield result. It shows that the smaller amount of the solvent concentration leads to the greater the yield, namely at 70% ethanol concentration. In contrast, the effect of concentration on TFC showed a declining trend. The smaller yield is observed at the smaller concentration of TFC produced. The largest yield of TFC is at an ethanol concentration of 96% and the lowest is at 70% ethanol concentration. The highest flavonoid content in mangosteen peel was obtained at a concentration of 96% with an ingredient/solvent ratio of 1/35 gr/mL, which is 2,549 mg QE/gr. The highest Sun Protecting Factor (SPF) value of mangosteen peel extract was obtained at 70% ethanol concentration, which was 16.009 in the ultra-protection category.

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