

Determination of Sun Protection Factor and Total Phenolic Content of Extract and Fraction of Matoa Leaves (*Pometia pinnata*)

[Uji *Sun Protection Factor* dan Penentuan Kadar Fenolik Total dari Ekstrak dan Fraksi Daun Matoa (*Pometia pinnata*)]

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ABSTRACT

Matoa leaves (*Pometia pinnata* J.R. & G. Forst) are known to contain phenolic compounds with potential as natural sunscreen agents due to their ultraviolet (UV) absorption capabilities. The presence of conjugated double bonds or aromatic rings in these compounds allows absorption in the 200–400 nm wavelength range, making them suitable candidates for photoprotection. This study aimed to evaluate the sun protection factor (SPF)—a measure of a substance's ability to protect the skin against UVB radiation—and total phenolic content of the extract and fraction derived from matoa leaves. Extraction was performed using Soxhlet apparatus with 70% ethanol, followed by liquid–liquid partitioning to obtain fractions. Total phenolic content was determined using the Folin–Ciocalteu method, while SPF was evaluated using a UV–Vis spectrophotometer in the 290–320 nm range at 5 nm intervals. The total phenolic contents of the ethanolic extract and ethyl acetate fraction were 50.51 ± 0.18 mg GAE/g and 39.27 ± 0.09 mg GAE/g, respectively. The SPF values were 19.60 ± 0.11 for the ethanolic extract and 16.37 ± 0.80 for the ethyl acetate fraction. These findings suggest that the extract and fraction of matoa leaves exhibit SPF values within the ultra-protection category, indicating their potential as natural sunscreen agents.

ABSTRAK

Daun matoa (Pometia pinnata J.R. & G. Forst) diketahui mengandung senyawa fenolik yang berpotensi sebagai agen tabir surya alami karena kemampuannya menyerap sinar ultraviolet (UV). Keberadaan ikatan rangkap terkonjugasi atau cincin aromatik dalam struktur senyawa ini memberikan kemampuan penyerapan pada panjang gelombang 200–400 nm, menjadikannya kandidat yang sesuai untuk perlindungan terhadap sinar UV. Penelitian ini bertujuan untuk mengevaluasi nilai sun protection factor (SPF)—yaitu ukuran kemampuan suatu zat untuk melindungi kulit terhadap radiasi UVB—dan kadar fenolik total dari ekstrak dan fraksi daun matoa. Ekstraksi dilakukan menggunakan metode sokletasi dengan etanol 70%, dilanjutkan dengan fraksinasi menggunakan metode ekstraksi cair-cair. Penetapan kadar fenolik total dilakukan menggunakan metode Folin–Ciocalteu, sedangkan pengujian SPF menggunakan spektrofotometer UV-Vis pada panjang gelombang 290-320 nm dengan interval 5 nm. Hasil penetapan kadar fenolik total menunjukkan nilai sebesar $50,51 \pm 0,18$ mg GAE/g untuk ekstrak etanol dan 39,27 ± 0,09 mg GAE/g untuk fraksi etil asetat. Nilai SPF yang diperoleh adalah 19,60 \pm 0,11 untuk ekstrak etanol dan 16,37 \pm 0,80 untuk fraksi etil asetat. Berdasarkan hasil tersebut, ekstrak dan fraksi daun matoa memiliki nilai SPF dalam kategori perlindungan ultra, sehingga berpotensi dimanfaatkan sebagai agen tabir surya alami.

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1. INTRODUCTION

Indonesia is located on the equator and has a tropical climate. This geographical position exposes the country to consistently highintensity sunlight throughout the year. Prolonged sun exposure may lead to skin damage primarily caused by ultraviolet (UV) radiation (Rahmawati et al., 2018). Excessive and sustained exposure to UV radiation (UVR) can damage the epidermal layers of the skin and lead to adverse effects such as erythema and sunburn (Umborowati & Rahmadewi, 2014). Therefore, the use of sunscreen is essential as a protective agent against UVR-induced skin damage (Dewi et al., 2019). The effectiveness of sunscreen ingredients in protecting the skin against UVB rays is quantified using the Sun Protection Factor (SPF). The SPF value is defined as the ratio of the amount of solar energy required to produce minimal erythema on protected skin to that required on unprotected skin. A higher SPF value indicates stronger UVblocking activity. SPF assessment can be conducted in vitro using UV-Visible spectrophotometry (Adawiyah, 2019).

In recent years, studies have shown that synthetic UVR filters, such as oxybenzone, can cause environmental harm, particularly coral reef bleaching and marine ecotoxicity. Compounds like benzophenone-3, avobenzone, octocrylene, and octinoxate have also been found to be genotoxic to aquatic organisms, including fish, mammals, and corals. Consequently, sunscreen formulations containing oxybenzone, benzophenone-3, and octinoxate have been prohibited in several marine-sensitive regions such as Hawaii, Key West, the U.S. Virgin Islands, Palau, parts of Mexico, and the Caribbean Islands (Carvalho et al., 2023; Resende et al., 2022). Apart from environmental risks, synthetic UV filters such as benzophenone, camphor, and cinnamate derivatives may also disrupt the endocrine system and exhibit hormonal activity and toxicity in in vivo models, including rats (Apel et al., 2018). As a result, the search for bioactive compounds from natural sources with potential UVR-filtering properties has become increasingly important. Natural sunscreens are gaining popularity due to their broad-spectrum UV absorption, lower environmental impact, and perceived safety by consumers (Soleimani et al., 2023).

One promising natural source of sunscreen agents is matoa (Pometia pinnata J.R. & G. Forst) leaves, which are known to contain phenolic and flavonoid compounds. These compounds possess chromophore groups in their molecular structure, enabling them to absorb UV radiation (Ebrahimzadeh et al., 2014). Based on research by Martiningsih et al. (2016), the ethanolic extract of matoa leaves obtained via maceration with absolute ethanol exhibited strong antioxidant activity with an IC₅₀ value of 45.78 µg/mL, attributed to the presence of flavonoids and tannins. Another study reported that ethanolic extracts from maceration using 96% ethanol showed even higher antioxidant activity with an IC₅₀ of 1.403 μ g/mL, likely due to secondary metabolites such as steroids, tannins, and saponins (Islami et al., 2021). Phenolicrich compounds, particularly flavonoids and tannins, are known for their potential as natural sunscreen ingredients (Almeida et al., 2019; Ashari et al., 2020). Testing of the SPF value of matoa leaf extract prepared using 70% ethanol via maceration yielded an SPF of 24.45 ± 0.2 , placing it in the ultra-protection category (Kurnianto & Rahman, 2021).

The current study was designed to determine the total phenolic content and SPF of extracts obtained using the Soxhlet extraction method, as well as fractions from matoa leaves. The Soxhlet method was selected due to its ability to utilize the solvent's boiling point to reduce surface tension and viscosity, thereby enhancing solvent penetration into the plant matrix and improving the solubility of target compounds. Fractionation was employed to achieve better separation of phenolic and flavonoid groups, facilitating the identification of bioactive constituents (Yuliani et al., 2022).

Based on the previous studies on matoa leaf extracts, this research aims to assess the Sun Protection Factor and total phenolic content of extracts and fractions derived from matoa leaves collected in the Muaro Jambi region.

2. METHODS

2.1. Tools and Materials

This study used matoa leaves (*Pometia pinnata* J.R. & G. Forst) collected from Sengeti Village, Muaro Jambi Regency, Jambi Province, and taxonomically verified at the Herbarium Jatinangor, Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University (UNPAD).

The tools used in this study included a blender (Miyako), 60 mesh sieve, a Soxhlet extraction apparatus (Pyrex), rotary vacuum evaporator (Biobase), water bath (B-One), vortex mixer (Biobase), UV–Vis spectrophotometer (BEL Photonics), digital balance (TL-series), 100–1000 μ L micropipette (Nesco Dragon Lab), volumetric flasks (Pyrex), and other standard laboratory glassware.

The materials used included matoa leaves, distilled water (Merck), 70% ethanol (PT. Indo Classica), n-hexane p.a (Merck), ethyl acetate p.a (Merck), methanol p.a (Merck), 96% ethanol (Merck), gallic acid (Merck), Folin reagent (Merck), sodium carbonate p.a (Merck), mercury(II) chloride, potassium iodide, magnesium powder, hydrochloric acid, anhydrous acetic acid, sulfuric acid, iron(III) chloride (Xilong Scientific), and gelatin (Eurotrade World Commerce, S.L.).

2.2. Plant Preparation

A total of 5 kg of matoa leaves were wet-sorted, washed under running water, and air-dried under sunlight while covered with black cloth. After drying, the material was dry-sorted, ground using a blender, and passed through a 60-mesh sieve to obtain uniform powder (Nopiyanti & Aisiyah, 2020).

2.3. Extraction and Fractionation of Matoa Leaves

Twenty-five grams of matoa leaf powder were extracted using the Soxhlet method with 312 mL of 70% ethanol at 78°C, until the siphonate became nearly colorless. The extract was concentrated using a rotary vacuum evaporator and further reduced using a water bath to obtain a thick extract. The extraction was repeated until the required extract amount was achieved.

The resulting extract was fractionated via liquid–liquid extraction. Two hundred and fifty milligrams of the thick extract were dissolved in 250 mL of distilled water, followed by partitioning with 250 mL of n-hexane. After phase separation, the aqueous layer was re-extracted with 250 mL of ethyl acetate. The process continued until the organic layers became nearly colorless. Each organic layer was evaporated using a water bath to yield a dry fraction (Nopiyanti & Aisiyah, 2020).

2.4. Phytochemical Screening of Matoa Leaves Extracts

a. Alkaloid Test

Fifty milligrams of matoa extract were dissolved in 70% ethanol. The solution was treated separately with 5 drops of Mayer's reagent and Wagner's reagent. A white precipitate indicated the presence of alkaloids with Mayer's reagent, while a brown precipitate indicated a positive result with Wagner's reagent (Rossalinda et al., 2021).

b. Flavonoid Test

Fifty milligrams of extract were dissolved in 70% ethanol, and the filtrate was treated with magnesium powder, followed by 2 drops of HCl. A color change from yellow to red indicated the presence of flavonoids (Rossalinda et al., 2021).

c. Saponin Test

Fifty milligrams of extract were mixed with 1 mL of hot distilled water and shaken for 10 minutes until froth persisted (1–10 cm). If the foam remained after adding 2 drops of HCl, it confirmed the presence of saponins (Rossalinda et al., 2021).

d. Triterpene and Steroid Test

Fifty milligrams of extract were dissolved in 0.5 mL of chloroform, then mixed with 0.5 mL of anhydrous acetic acid and 2 mL of sulfuric acid. A brownish or violet color indicated triterpenes; a blue-green color indicated steroids (Rossalinda et al., 2021).

e. Phenol and Tannin Test

For phenols, 50 mg of extract were dissolved in 70% ethanol and treated with 2 drops of 1% $FeCl_3$. A blackish green color indicated phenolic presence (Rossalinda et al., 2021). For tannins, 50 mg of extract were dissolved in 15 mL of hot distilled water, cooled, and filtered. The filtrate was mixed with 3 mL of 10% gelatin solution. A white precipitate indicated tannins. A separate portion was treated with 3 mL of NaCl–gelatin solution to confirm the result (Rossalinda et al., 2021).

2.5. Determination of Total Phenolic Content by Folin-Ciocalteu Method

a. Wavelength Optimization

A 25 μ g/mL gallic acid solution was scanned at 400–800 nm using a UV–Vis spectrophotometer, revealing a maximum absorbance at 755 nm.

b. Preparation of Standard Solution

Gallic acid stock (500 μ g/mL) was prepared by dissolving 50 mg in 100 mL methanol. Dilutions of 15–45 μ g/mL were used to construct the standard curve, following modified guidelines from the Indonesian Ministry of Health (2017).

c. Preparation of Sample Solution

Fifty milligrams of extract or fraction were dissolved in 10 mL methanol, stirred for 30 minutes, filtered into a 10 mL flask, and topped up with methanol to obtain a 5000 μ g/mL stock. This was diluted to 50 μ g/mL for testing.

d. Test Procedure

The Folin–Ciocalteu method (Martínez et al., 2022) was modified for wavelength. Aliquots of 0.5 mL were mixed with 2.5 mL of 1:10 Folin reagent and 2 mL of 7.5% Na_2CO_3 . Incubation occurred at 45°C for 15 min in a water bath, followed by 30 min at room temperature. Absorbance was read at 755 nm. Analyses were performed in triplicate. Results were expressed as mg gallic acid equivalents (GAE) per 100 g of extract or fraction.

2.6. Determination of SPF Value by UV-Vis Spectrophotometric Method

Approximately 0.25 g of extract or fraction were dissolved in 100 mL of 96% ethanol to yield a 2500 μ g/mL solution. Ethanol was used as blank. Absorbance was measured at 290–320 nm in 5 nm intervals using a UV–Vis spectrophotometer. All analyses were performed in triplicate. SPF was calculated using Mansur's equation (Nopiyanti & Aisiyah, 2020).

2.7. Data Analysis

The SPF value was calculated using the Mansur equation (Kera et al., 2024),

SPF = CF x $\sum_{290}^{320} EE(\lambda)$ x I (λ) x Abs (λ)

Where CF = correction factor (10); EE(λ) = erythemal effect spectrum; I(λ) = solar intensity spectrum; Abs(λ) = absorbance of sample at wavelength λ . Each absorbance value was multiplied by the corresponding EE × I value from **Table 1**. The sum was multiplied by the correction factor (CF = 10) to obtain the SPF. Sunscreen categories were defined as follows: SPF < 15 (low), SPF 15–30 (moderate), SPF 30–50 (high), and SPF > 50 (very high) (Kera et al., 2024).

Wavelength (nm)	EE x I	
290	0.0150	
295	0.0817	
300	0.2874	
305	0.3278	
310	0.1864	
315	0.0839	
320	0.0180	
Total	1	
		1

3. RESULTS AND DISCUSSION

3.1. Extraction Yield of Matoa Leaves Extract and Fraction

The parameter to determine the effectiveness of the extraction process used is the yield value. Extract yield is the ratio between the weight of the extract obtained and the weight of the initial plant material. The extract yield obtained was 17.59% (w/w). The fraction yield is the ratio between the weight of the fraction obtained and the weight of the extract. The fraction yield obtained was 24.82% (w/w). This value is higher than the extract yield. This occurs because the fractionation, carried out using the liquid–liquid partition method on the ethanolic extract of matoa leaves, is designed to separate compounds based on their solubility in solvents of different polarities (Abubakar & Haque, 2020).

The fractionation process begins with n-hexane as a non-polar solvent, followed by the addition of water, which acts as a polar solvent. The next step uses ethyl acetate, a semi-polar solvent. This stepwise use of solvents with increasing polarity enables gradual extraction of compounds with different affinities (Yuliani et al., 2022). The yield is expressed as a percentage (% w/w), where a higher yield reflects the greater extraction of secondary metabolite compounds by the solvent (Alviola Bani et al., 2023). The yield results for matoa leaves extract and its fractions are presented in **Table 2**.

Table 2. Extract and fraction yields of Matoa eaves

Sample	Yield (%, w/w)
Ethanolic extract	17.59
Ethyl acetate fraction	24.82

3.2. Phytochemical Screening of Matoa Leaves Extract

The results of phytochemical screening of matoa leaves extract are shown in **Table 3**. Matoa leaf extract was found to contain alkaloids, flavonoids, saponins, phenols, and tannins. According to Islami et al. (2021), a 96% ethanolic extract of matoa leaves contains steroids, tannins, and saponins. These differences are likely due to variations in solvent concentration.

In this study, 70% ethanol was used as the extraction solvent. Because it contains a higher proportion of water, it has higher polarity compared to 96% ethanol. This increased polarity enhances the ability of the solvent to extract a broader range of polar compounds (Hikmawanti et al., 2021). Solvent polarity plays a critical role in increasing the solubility of phenolic compounds (Boeing et al., 2014). The ability of 70% ethanol to extract phenolic compounds is due to hydrogen bonding interactions between the polar part of the compound and the solvent (Mohammed et al., 2022).

3.3. Total Phenolic Content of Matoa Leaves Extract and Fraction

In this total phenolic content determination, the standard used was gallic acid, a stable and pure compound. In addition, gallic acid is a naturally occurring phenolic compound that is relatively inexpensive compared to other phenolic standards. Gallic acid is derived from hydroxybenzoic acid and classified as a simple phenolic acid. When gallic acid reacts with Folin–Ciocalteu reagent, it produces a yellow color, indicating the presence of phenolic compounds. The reaction mixture is then adjusted to alkaline conditions by the addition of sodium carbonate (Na₂CO₃). Under these conditions, the solution changes to blue due to the formation of phosphomolybdate–phosphotungstate–phenol complex (Hatami et al., 2014; Roskiana Ahmad et al., 2015).

In this study, the ethanolic extract exhibited the highest total phenolic content ($50.51 \pm 0.18 \text{ mg GAE/g extract}$), followed by the ethyl acetate fraction ($39.27 \pm 0.09 \text{ mg GAE/g fraction}$). The detailed results are presented in **Table 4**. The high phenolic content of the ethanolic extract is attributed to the ability of polar solvents to extract polyphenolic compounds more efficiently (Hydroethanolic > ethanol > ethyl acetate) (Hatami et al., 2014; Sridhar et al., 2021). However, it was expected that the ethyl acetate fraction might yield a higher phenolic content due to its selectivity based on solvent polarity, especially when targeting phenolic and flavonoid subgroups (Yuliani et al., 2022).

This discrepancy may be explained by thermal degradation of phenolic compounds during fractionation. Fractionation typically involves repeated solvent evaporation, which may degrade or evaporate thermolabile or volatile phenolic and flavonoid compounds, thereby reducing their final concentration in the fraction (Sridhar et al., 2021).

Screening	Reagent	Result	
Alkaloid	Mayer	(+) white precipitate	
	Wagner	(+) brown precipitate	
Flavonoid	Mg powder + HCl	(+) orange color	
Saponin	Hot distilled water + HCl	(+) there is foam (2	
		cm high)	
Phenol	FeCl3 1%	(+) green color	
Tanin	10% gelatin solution	(+) white precipitate	
	NaCl-gelatin solution	(+) white precipitate	
Triterpene	Anhydrous acetic acid +	(-)	
and	concentrated sulfuric acid		
Steroid			

Table 3. Phytochemical screening results of matoa leaves extracts

Notes: (+) indicates the presence of the tested compound; (-) indicates absence.

 Table 4. Results of total phenolic content of matoa leaves extract

 and fraction

	Total Phenolic	Total Phenolic Content
Sample	Content	(mg GAE/ g extract)
	(mg GAE/ g extract)	Mean ± SD
Ethanolic	50.60	50.51 ± 0.18
extract	50.63	
	50.30	
Ethyl	39.20	39.27 ± 0.09
acetate	39.23	
fraction	39.37	

3.4. Determination of SPF Value of Matoa Leaves Extract and Fraction

Based on Table 5, the SPF value of the ethanolic extract was higher than that of the ethyl acetate fraction. This result is consistent with the total phenolic content, where the ethanolic extract also exhibited a higher concentration of phenolic compounds compared to the ethyl acetate fraction. Despite the difference in SPF values, both samples fall under the same sunscreen protection category, i.e., medium protection. Polyphenolic compounds are natural bioactive substances known for their photoprotective effects. Phenolic compounds are believed to operate through redox-sensitive signalling pathways that inhibit DNA damage. They also prevent the formation of free radicals induced by ultraviolet (UV) radiation and lipid peroxidation. Furthermore, their antioxidant properties play a crucial role in protecting the skin from UV damage (Ebrahimzadeh et al., 2014; He et al., 2021). This finding is further supported by Almeida et al. (2019), who reported a strong correlation (0.7 \leq r² \leq 1.0) between total phenolic content and antioxidant activity, and a moderate correlation (0.5 \leq r² \leq 0.7) between total phenolic content and SPF activity.

Table 5. SPF values of matoa leaves	extract and	fraction
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Sample	Concentration	SPF	Type of
	(µg/mL)	Value ± SD	Protection
Ethanolic	2500	19.60 ± 0.13	Medium
extract			
Ethyl acetate	2500	16.37 ± 0.97	Medium
fraction			

Phenolic compounds are categorized into phenolic acids, flavonoids, and polyphenols, all of which possess photoprotective activity. Phenolic acids have been shown to protect phospholipid membranes from UVR-induced peroxidation by inhibiting lipid peroxidation chain reactions. These compounds also demonstrate photoprotective effects against oxidative stress, MMP-1 induction, and reactive oxygen species (ROS) formation in human keratinocytes by enhancing antioxidant defense systems, including glutathione (GSH), catalase, and glutathione peroxidase (GPx).

Flavonoid compounds can absorb UV radiation and reduce oxidative damage caused by ROS. The presence of double bonds in the flavonoid structure increases their UV absorption capacity, while hydroxyl groups attached to the aromatic ring contribute to their ROS-scavenging properties. Additionally, flavonoids help inhibit MMP activity in skin cells by directly suppressing MMP expression and enhancing the expression of tissue inhibitors of MMP (Torres-Contreras et al., 2022).

CONCLUSION

The total phenolic contents of the ethanolic extract and ethyl acetate fraction of matoa leaves were 50.51 ± 0.18 mg GAE/g extract and 39.27 ± 0.09 mg GAE/g fraction, respectively. The Sun Protection Factor (SPF) values for the ethanolic extract and ethyl acetate fraction were 19.60 ± 0.13 and 16.37 ± 0.97 ,

respectively, classifying both in the medium protection category. These results indicate that both the extract and fraction of matoa leaves possess photoprotective properties and have potential to be developed as natural sunscreen agents.

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