Determination Of Antioxidant Activity and SPF Value Of Purified And Crude Extracts Of Mango Leaves (*Mangifera Indica* L.)

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Abstract

This research aimed to examine the differences in antioxidant activity and *Sun Protection Factor* (SPF) between crude and purified extracts of mango leaves (*Mangifera indica* L.), and to evaluate the role of purification in enhancing their effectiveness. The background of the study is related to the increasing demand for natural compounds with dual potential as antioxidants and UV protectors, considering that free radicals and ultraviolet exposure are the major causes of oxidative stress and skin damage. The research process included several stages, namely plant determination, simplicia preparation, maceration extraction using 70% ethanol, purification with n-hexane, phytochemical screening, quality evaluation, antioxidant assay using the DPPH method, and SPF determination with spectrophotometry. The results showed that the purified extract had a higher quality profile with a yield of 50%, water content of 5.78% ± 0.29, and ash content of 0.22%. Phytochemical tests indicated the presence of flavonoids, alkaloids, tannins, phenolics, and saponins, with flavonoids more concentrated in the purified extract. Antioxidant assays revealed IC_{50} values of 24.64 ± 0.52 µg/mL for the purified extract and 48.28 ± 0.61 µg/mL for the crude extract, both classified as very strong. SPF results showed a value of 44.89 at 12,000 ppm for the purified extract and 30.23 for the crude extract. ANOVA analysis confirmed significant differences ($p < 0.05$). These findings highlight the promising potential of purified mango leaf extract as an herbal sunscreen ingredient.

Keywords: *Mangifera indica* L., extract purification, extract, antioxidant, SPF

1. INTRODUCTION

Global climate change and increased industrial activity have significantly reduced environmental quality, particularly in areas like Cikarang. Higher temperatures, fluctuating humidity, and intense UV radiation during the dry season are worsened by air pollution from vehicles and manufacturing. These conditions accelerate free radical formation, which can cause health problems, including skin tissue damage (Ainurrohmah *et al.*, 2022; Wiyono *et al.*, 2023; Fadhilla *et al.*, 2025). Free radicals are unstable atoms or molecules with unpaired electrons, making them highly reactive and capable of damaging lipids, proteins, and DNA.

Ultraviolet B (UVB) radiation is a major trigger of free radical formation in the skin, leading to oxidative stress. This condition can cause premature ageing, hyperpigmentation, inflammation, and skin cancer, as well as contribute to degenerative diseases such as cancer, cardiovascular disorders, diabetes, and neurological conditions (Hindrianingtyas *et al.*, 2023). Prevention can be achieved by using antioxidants and external protection such as *Sun Protection Factor* (SPF). Antioxidants neutralize free radicals, while SPF limits UV penetration by absorbing, reflecting, or scattering ultraviolet rays (Asrifaturofingah *et al.*, 2024; Hajar *et al.*, 2024). The synergy between antioxidants and sun protection is considered the most effective strategy to prevent oxidative stress–related skin damage (Susanti *et al.*, 2020).

The mango tree (*Mangifera indica* L.) is a widely distributed tropical species in Indonesia and contains abundant bioactive compounds such as flavonoids, alkaloids, and tannins, which exhibit strong antioxidant activity (Mahdiyah *et al.*, 2020). A 24-hour maceration process was found to produce a considerable amount of flavonoids, reaching 49.07%, which indicates the effectiveness of this extraction method (Afifah *et al.*, 2023). Numerous studies have confirmed the antioxidant potential of mango leaves (Anggraheni *et al.*, 2019) reported an IC_{50} of 13.54 ppm, indicating very strong activity. The DPPH assay is commonly used because it is simple, reliable, and allows comparison of extract effectiveness across methods or plant varieties, where lower IC_{50} values indicate stronger antioxidants. In addition to these properties, mango leaf extract has also been identified as a promising natural sunscreen. A study conducted by (Cahyani *et al.*, 2024) reported SPF values of mango leaf extract ranging from 3.57 to 37.10, with the highest at 10,000 ppm, classified as high protection. This confirms the dual role of mango leaves as antioxidants and UV protectants. However, comparisons between crude and purified extracts remain limited. Purification can increase active compound concentration, remove impurities, and improve stability and pharmacological activity (Wahyuningsih *et al.*, 2024). Thus, this study examines how purification influences the antioxidant activity and SPF of mango leaf extracts. This study compares the antioxidant activity and SPF of crude and purified mango leaf extracts. The direct comparison of both extract types is rarely reported. The results are expected to guide the development of effective natural products with antioxidant and photoprotective properties.

2. METHOD

Tools

Aluminium foil, an analytical balance, a beaker, a blender, a bottle, a closed scale, a cup, an electric furnace, an erlenmeyer flask, filter paper, a funnel, a glass jar, a hotplate, a knife, a measuring flask, a micropipette, an oven, a pipette, a rotary evaporator, a spatula, the SPSS software application, a stirring rod, an ultrasonicator (Laboratory Ultrasonic Cleaner), a UV-Vis spectrophotometer (Computer-Integrated Uv-Vis Spectrophotometer System), a watch glass, and a 40 mesh sieve.

Materials

Ammonia (Merck, USA), amyl alcohol (Sigma-Aldrich, USA), aquadest (Brataco, Indonesia), anhydride acetate (Merck, Germany), chloroform (Merck, Germany), Dragendorff reagent (Brataco, Indonesia), ethanol 70% (Brataco, Indonesia), ethylhexyl methoxycinnamate (Sigma-Aldrich, USA), $FeCl_3$ 1% (Merck, Germany), H_2SO_4 2N (Merck, Germany), Mayer reagent (Brataco, Indonesia), magnesium powder (Mg) (Merck, Germany), methanol p.a. (Merck, Germany), n-hexane (Merck, Germany), purified mango leaf extract (*Mangifera indica* L.), crude mango leaf extract (*Mangifera indica* L.), silica gel (Merck, Germany), blank solution, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich, USA), vitamin C (Brataco, Indonesia), dan Wagner reagent (Brataco, Indonesia).

Plant Determination

The identification of mango plants (*Mangifera indica* L.) was carried out at BRIN to ensure accurate species verification so that the test material used met the required standards.

Preparation of Simplicia

Approximately 2 kg of mango leaves were cleaned, cut, and separated into midribs and lamina. The lamina was dried in an oven at 40–60 °C, ground into powder, and sieved through a 40-mesh filter to obtain fine simplicia (Kunti Mulangsri *et al.*, 2020).

Preparation of Crude Extract

The extraction process employed the maceration method, where simplicia was immersed in 70% ethanol solvent with a ratio of 1:10 (w/v). After the soaking process, the extract solution was filtered and concentrated using a rotary evaporator until a thick extract was obtained (Cahyani *et al.*, 2024).

Preparation of Purified Extract

A total of 20 g of crude extract was dissolved in 250 mL of 70% ethanol, followed by the addition of 250 mL of n-hexane. The mixture was shaken and left until two distinct layers

formed. The ethanol fraction was separated and repeatedly extracted with n-hexane until clear, then evaporated to yield a concentrated purified extract (Kunti Mulangsri *et al.*, 2020).

Phytochemical Screening

Phytochemical analysis of mango leaf extract revealed the presence of flavonoids, indicated by color changes with HCl, amyl alcohol, and magnesium; alkaloids, confirmed by precipitate formation with Dragendorff, Mayer, and Wagner reagents; tannins and phenolics, detected through $FeCl_3$-induced color changes; and saponins, identified by the formation of stable foam lasting over 10 minutes. (Aristyawan *et al.*, 2024; Sukmanastiti *et al.*, 2024).

Quality Characterization Tests

- Organoleptic Evaluation: Included assessment of color, form, and aroma of the extract using sensory observation (Mangalu *et al.*, 2022).
- Identity Test: Included information on the extract's name, scientific plant name, plant part used, and its common Indonesian (RI, 2000).
- Ash Content Test: A 2 g sample was ignited in an electric furnace at 550 ± 10 °C until ash was obtained (Tuapattinaya *et al.*, 2021).
- Water content test

Moisture content was determined using the gravimetric method, in which a 2 g sample was heated

Determination of Antioxidant activity

Antioxidant activity was evaluated by the DPPH method using a UV-Vis spectrophotometer. A 100 µg/mL DPPH solution in methanol p.a. was prepared, with 500 µg/mL vitamin C as the positive control. Purified and crude extracts (3000 ppm; 5–30 µL) were mixed with 1 mL DPPH, diluted to 5 mL, incubated for 30 minutes at 37 °C, and measured at 516 nm. Activity was expressed as percentage inhibition and determined through IC_{50} values based on the decrease in DPPH absorbance. using the following formula:

$$Inhibisi\ presentase = \frac{Abs.Blanco - Abs.Sample}{Abs.Blanco}\ x\ 100\%$$

Table 1. Classification of Antioxidant Activity Values $\boldsymbol{IC_{50}}$

(Rusydi *et al.*, 2022)

Antioxidant Activity	Velue IC_{50}
Very Strong	<50Ppm
Strong	50-100Ppm
Moderate	100-150Ppm
Weak	>150Ppm

SPF Velue Determination

The SPF test was performed by dissolving 1800 mg of purified and crude extracts in 150 mL methanol p.a., ultrasonicated for five minutes, and filtered. Dilutions of 9000, 6000, and 3000 ppm were prepared to 100 mL. Absorbance was measured using a UV-Vis spectrophotometer at 290–320 nm (5 nm intervals) with methanol p.a. as the blank. SPF values were calculated using Mansur's method (1986), with low (2–11), medium (12–29), and high (30–50) categories. Ethylhexyl Methoxycinnamate was used as the control at 12,000–3000 ppm (Rusydi *et al.*, 2023).

$$SPf = CF\ x \sum_{290}^{320} \text{EE}\ (\lambda)\ x\ I\ (\lambda)\ x\ \text{abs}\ (\lambda)$$

CF = Correction Factor (10)

EE = Erythema Effect Spectrum

I = Light Intensity Spectrum

Abs = Sample Absorbance At Wavelength Λ

Table 2. Normalized Product Function

NO	Wavelength	EE x I
1	290	0.0150
2	295	0.0817
3	300	0.2874
4	305	0.3278
5	310	0.1864
6	315	0.0839
7	320	0.0180

Table 3. Classification of Sun Protection Factor (SPF) Values

(Rusydi *et al.*, 2023)

Level	Nilai SPF
low	2 - 11
Medium	12 - 29
High	30 – 50

Data Analysis

Data analysis in this study was performed using IBM SPSS Statistics 20, involving data processing in Microsoft Excel, followed by normality testing, homogeneity testing, and One-Way ANOVA.

3. RESULTS AND DISCUSSION

Determination

According to the Herbal Pharmacopoeia of Indonesia (2017), plant identification verifies specimen authenticity. At BRIN, the mango leaf (*Mangifera indica* L.) was confirmed as Anacardiaceae, ID B-1427/II.6.2/IR.01.02/3/2025, ensuring taxonomic accuracy for the study.

Preparation of Simplicia

The results of the preparation of mango leaf (*Mangifera indica* L.) simplicia are presented in Table 4

Table 4 Drying Results

Initial Weight (g)	Final Weight (g)	Drying Loss (%)
2000,01	962,02	51,89

The drying of mango leaf (*Mangifera indica* L.) simplicia decreased its weight from 2000.01 g to 962.02 g, corresponding to a 51.89% loss, caused by water evaporation. Such a reduction is typical for leaves with high moisture content and confirms that the drying process effectively produced stable simplicia suitable for subsequent extraction and phytochemical studies.

Preparation of Extract and Purified Extract

The results of the preparation of mango leaf (*Mangifera indica* L.) extracts and purified extracts are presented in Table 5.

Table 5. The yield results of mango leaf (*Mangifera indica* L.) extract and purified extract

Sample	Weight Before Extraction (g)	Extract Weight (g)	Yield (%)
Extract	962,02	85,29	8,96
Purified Extract	65, 08	32,04	50

The extraction was carried out by maceration using 70% ethanol (1:10) to preserve heat-sensitive bioactive compounds. The purified extract produced 32.04 g (50%), whereas the crude extract gave 85.29 g (8.96%), showing that purification enhances compound concentration even though it reduces total yield. Variations in solvent, temperature, and purification technique may account for these differences.

Phytochemical Screening

The phytochemical screening results of mango leaves (*Mangifera indica* L.) are presented in Table 6.

Tabel 6 Phytochemical Screening Results of Crude and Purified Extracts

Secondary Metabolite	Observation	Requirement (RI, 2000)	Note	
			Extract	Purified Extract
Flavonoids	Formation of red solution	Formation of yellow, blue, orange, or red color	+	+
Alkaloids				
• Dragendorff	Formation of red precipitate	Formation of red precipitate	+	+
• Mayer	Formation of white precipitate	Formation of white precipitate	+	+
• Wagner	Formation of brown precipitate	Formation of brown precipitate	+	+
Tanins	Formation of greenish-black solution	Formation of greenish-black solution	+	+
Phenolics	Formation of greenish-black solution	Formation of greenish-black solution	+	+
Saponins	Formation of stable foam	Formation of stable foam	+	+

Note: (+) indicates the presence of compound groups;
(–) indicates the absence of compound groups.

Phytochemical screening confirmed that both the crude and purified mango leaf extracts contain secondary metabolites such as flavonoids, alkaloids, tannins, phenolics, and saponins. The flavonoid test, in particular, showed a stronger response in the purified extract, indicating that purification increased the concentration of bioactive compounds. Flavonoids and phenolics are known for their strong antioxidant activity and UV-absorbing properties, while saponins and alkaloids provide additional biological benefits. These results suggest that the purification process does not remove active constituents but rather enriches key metabolites that enhance the extract's overall biological effectiveness.

Quality Characteristic Test

The results of the quality characteristic tests for mango leaf extract (*Mangifera indica* L.) Are presented in Table 7

- **Organoleptic test**

Table 7. Organoleptic Test Results

Sample	Foam	Color	Odor
Extract	Thick	Dark Brown	Characteristic of mango leaves
Purified Extract		Jet Black	

Based on Table 7, the organoleptic test results show differences in the physical properties between the standard mango (*Mangifera indica* L.) leaf extract and the purified extract. In terms of consistency, the two samples exhibited different viscosities: the standard extract was thicker, whereas the purified extract was more concentrated. This difference is likely due to the purification process, which removes some of the solvent and inactive fillers, thereby increasing the concentration of dissolved solids in the extract.

- **Ash Content Test**

The results of the ash content analysis of the mango (*Mangifera indica* L.) leaf extract and purified extract showed significant differences (Table 8)

Table 8. Non-Specific Parameter Results of Crude and Purified Mango Leaf (*Mangifera indica* L.) Extracts

Sample	Ash content (%) ± SD	Requirement	Note
Extract	2,31% ± 0,1%	No more than 4% (Kemenkes, 2017)	Meets requirement
Purified Extract	0,22% ± 0,2%		

The standard extract had a water content of 2.31% ± 0.1%, while the purified extract had a lower water content of 0.22% ± 0.2%. Both values are still below the maximum limit set by the Ministry of Health (2017), which is no more than 4%, thus meeting the requirements. This difference in water content is likely due to the purification process, which removes some solvents and insoluble materials, resulting in the purified extract having a lower water content. The low water content of the purified extract also indicates better physical stability and a lower microbiological risk, making it more resistant to microbial growth and more suitable for long-term storage.

- **Moisture Content Test**

Moisture content analysis showed that the crude mango leaf extract (8.57% ± 0.72%) contained more moisture than the purified extract (5.78% ± 0.29%), with both values within the acceptable limit set by the Ministry of Health (≤10.4%) (table 9).

Table 9. Non-Specific Parameter Results of Crude and Purified Mango Leaf (*Mangifera indica* L.) Extracts

Sample	Moisture Content (%) ± SD	Requirement	Note
Extract	8,57% ± 0,72%	No more than 10.4% (Kemenkes, 2017)	Meets requirement
Purified extract	5,78% ± 0,29%		

The lower water content in the purified extract is due to the purification process, which removes part of the solvent and insoluble materials, resulting in a more concentrated product with reduced moisture. This is beneficial because it improves stability, lowers the risk of microbial growth, extends shelf life, and ensures consistent quality for use in pharmaceutical and herbal preparations.

Determination of Antioxidant Activity

The results of the antioxidant activity assessment of mango leaf extract are presented in Figure 1 and statistical analysis table 10

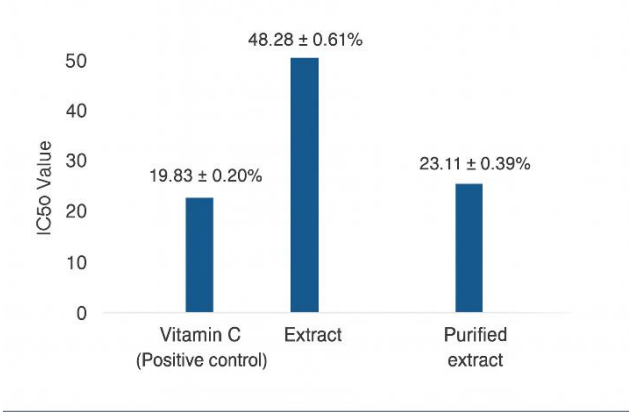


Figure 1. IC50 Values of Crude Extract, Purified Mango Leaf (Mangifera indica L.) Extract, and Vitamin C Control

Table 10. Results of Normality Test, Homogeneity Test, and One-Way ANOVA

Sample	Sig	Requirement	Note
Normality test • Extract • Purified Extract	 • 0.852 • 0.652	Sig (2-tailed) >0.05 (Zulkifli *et al.*, 2025)	Data are normallly distributed
Homogenity test	0.721	Sig (2-tailed) >0,05 (Zulkifli *et al.*, 2025)	Data are homogeneous
One Way Anova	0.044	Sig < 0.05 (Arif *et al.*, 2023)	H1 accepted; significant differences exist

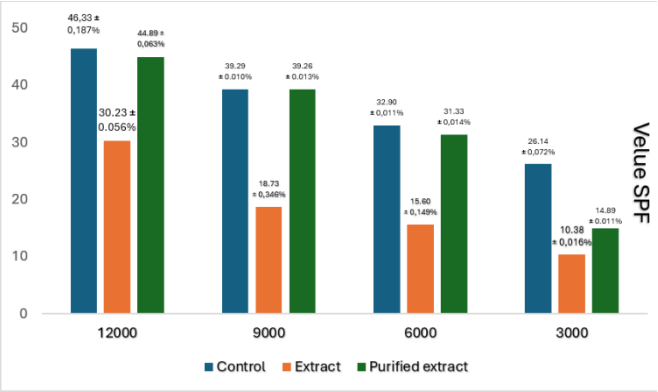
The antioxidant activity assay showed distinct IC_{50} values for the three tested samples: vitamin C (positive control), crude mango leaf extract, and purified mango leaf extract. As illustrated in Figure 1, vitamin C had the lowest IC_{50} (19.83 ± 0.20 µg/mL), indicating the strongest antioxidant capacity. The purified extract recorded an IC_{50} of 23.11 ± 0.39 µg/mL, while the crude extract had the highest IC_{50} at 48.28 ± 0.61 µg/mL. Since lower IC_{50} values reflect higher free radical scavenging activity, the antioxidant potency ranks as vitamin C > purified extract > crude extract. Both mango leaf extracts fall into the "very strong" antioxidant category (<50 µg/mL) according to Molyneux (2004). The results emphasize that purification

enhances antioxidant performance by concentrating bioactive compounds, particularly flavonoids and polyphenols, while removing inactive substances, making the purified extract more effective at neutralizing free radicals than the crude extract.

Normality testing yielded significance values of 0.852 (crude extract) and 0.652 (purified extract), both above 0.05, confirming normal data distribution. The homogeneity test showed a significance of 0.721 (>0.05), indicating homogeneous variance. These conditions allowed for parametric analysis using One-Way ANOVA. The ANOVA result (sig. = 0.044; <0.05) demonstrated a significant difference among the groups, confirming that the variations in IC_{50} values are real and directly linked to the active compound content of each sample. In conclusion, purification is a crucial step to enhance the antioxidant quality of mango leaf extract. The purified extract, with activity close to vitamin C, highlights the potential of mango leaf secondary metabolites as a valuable natural antioxidant source.

Determination of SPF Value

The results of SPF testing on mango leaf extract are shown in Figure 2 and statistical analysis table 11



Gambar 2. SPF Values of Crude and Purified Mango Leaf Extracts

Tabel 11. Results of Normality Test, Homogeneity Test, and One-Way ANOVA

Sampel	Sig	Syarat	Keterangan
Normality test • **Extract** • **Purified extract**	0.699 0.914	Sig (2-tailed) >0.05 (Zulkifli *et al.*, 2025)	Data are normallly distributed
Homogenity test	0.613	Sig (2-tailed) >0.05 (Zulkifli *et al.*, 2025)	Data are homogeneous
***One Way* Anova**	0.047	Sig < 0.05 (Arif *et al.*, 2023)	H1 accepted; significant differences exist

The SPF evaluation of mango leaf extract and its purified form is shown in Figure 2. In general, SPF values increased with higher sample concentrations, indicating that larger amounts of extract improve UVB absorption and blocking. At the highest concentration (12,000 ppm), the purified extract had a higher SPF than the crude extract, approaching the control's value. This pattern was consistent across most concentrations, with the purified extract consistently providing stronger protection. The enhanced effect is likely due to the purification process, which removes inactive components and enriches bioactive compounds such as flavonoids, tannins, and polyphenols that play a key role in UV absorption. As a result, the purified extract shows greater potential for sun protection.

Normality testing gave significance values of 0.699 for the crude extract and 0.914 for the purified extract, both above 0.05, confirming normal data distribution. The homogeneity test showed a significance of 0.613 (>0.05), indicating homogeneous variance. These results justified the use of parametric analysis via One-Way ANOVA, which yielded a significance of 0.047 (<0.05), demonstrating a statistically significant difference among the groups. Therefore, differences in SPF values between the crude extract, purified extract, and control are real and reflect their active compound content. In conclusion, purified mango leaf extract improves UV protection, and its higher SPF highlights the potential of mango leaf secondary metabolites as natural active ingredients for sunscreen formulations.

4. CONCLUSION

The study indicates that purified mango leaf extract (Mangifera indica L.) has stronger antioxidant and SPF activities than the crude extract. Its lower IC_{50} value points to improved antioxidant capacity, while the SPF test shows notable UVB protection, comparable to some synthetic sunscreens. These results suggest that purification effectively concentrates bioactive compounds, especially flavonoids and phenolics, which play a key role in both antioxidant and photoprotective effects. Overall, purification is an important step to enhance the quality and effectiveness of herbal extracts for use in pharmaceuticals and natural cosmetics. The findings also support the potential of mango leaves as a natural, safer, and environmentally friendly source for herbal sunscreen products.

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