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Original Article

In Vitro Evaluation of Antioxidant Activity of Methanol Extract from *Madu* Mango (*Mangifera indica* L. "Madu") Leaves and Fruit Peel

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Abstract

Mango (*Mangifera indica* L.) holds promise as a natural source of bioactive compounds with pharmaceutical potential, owing to its active constituents that exhibit antioxidant properties. Both the leaves and peels of the mango fruit contain secondary metabolites, including alkaloids, phenolics, flavonoids, and steroids. Among these, phenolics and flavonoids are particularly significant for their role in antioxidant activity. This study aimed to quantify the total phenolic and flavonoid contents and to evaluate the antioxidant activity of methanolic extracts obtained from mango leaves and peels. Antioxidant activity was assessed using the ferric reducing antioxidant power (FRAP), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. The results demonstrated that the leaf extract contained considerably higher levels of total phenolics (255.627 mg GAE/g extract) and total flavonoids (43.326 mg QE/g extract) compared to the peel extract, which had 25.777 mg GAE/g extract and 15.632 mg QE/g extract, respectively. Correspondingly, the leaf extract exhibited stronger antioxidant activity, with IC_{50} values of 0.457 ppm for DPPH and 5.430 ppm for ABTS, and a FRAP value of 410.356 µmol Fe^{2+}/g extract. These findings indicate that mango leaves, in particular, have significant potential as a source of natural antioxidants and supporting their use in herbal medicine aimed at preventing degenerative diseases.

Keywords

Antioxidant, extract, free radicals, mango, phytochemical.

1. Introduction

Oxidative stress, a condition resulting from an imbalance between pro-oxidants and antioxidants in the body, represents a serious health issue (Agus et al., 2022). The utilization of plants as a source of antioxidants aligns with the global trend of using herbal medicine. The World Health Organization (WHO) reports that approximately 80% of the world's population relies on plant-based medicines (Purba, 2022; Septriani & Purmini, 2022). Indonesia, with its high biodiversity, possesses more than 300 species of plants with the potential to be used as raw materials for jamu and traditional medicine (Hadi et al., 2024). The WHO also emphasizes the importance of standardization in the processing of herbal raw materials to ensure product quality and safety (World Health Organization, 2023).

One of the plants that has high antioxidant content is *Mangifera indica* L. or mango plant (Herwin & Meilani, 2016). Almost all parts of the *M. indica*, including the leaves, fruit seeds, and fruit peel, contain flavonoid and phenolic compounds that play a role in neutralizing free radicals and preventing degenerative diseases (Herwin & Meilani, 2016). *M. indica* plants have benefits in treating diabetes, hypertension, asthma, and other diseases (Mutiara et al., 2024;

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Paknahad et al., 2020). *M. indica* fruit peel, which is often considered waste, has great potential to be repurposed as a functional ingredient and natural preservative (Aditiyarini et al., 2025). Aditiyarini et al., 2025; Indriyanti et al., (2024) reported that mango peel contains various antioxidant compounds, such as fiber, vitamin C, tocopherols, phenolic compounds, and carotenoids.

Antioxidant activity is determined not only by differences in cultivar but also by the different parts of the plant. This is due to the varying metabolism in producing secondary metabolites in each part of the plant. This is important because the variation in mango cultivars can affect the content of active compounds and antioxidant activity. Antioxidant activity is influenced by differences in plant varieties. In Indonesia, there are several mango varieties, including *Gedong Gincu, Manalagi, Alpukat, Apel, Golek, Malibu, Madu, Kweni, and Cengkir* (Cempaka et al., 2021). One of the mango cultivars, known as *M. indica* L. "Madu" had good antioxidant activity. The ethanol extract of the leaves showed the inhibition of 90.241%, which is classified as high (Mutiara et al., 2024). Ifmaily et al. (2024) reported the antioxidant activity of the *Arumanis* mango peel extract with an IC_{50} value of 18.294 ppm, which is classified as very strong. Winata & Ameliana (2024) reported that the antioxidant activity of the *Manalagi* mango leaf extract has an IC_{50} value of 10.27 ppm. Previous researchers have shown that mango fruit peel extract has strong antioxidant activity with varying IC_{50} values depending on the extraction method and the type of solvent used (Aditiyarini et al., 2025; Indriyanti et al., 2024). The hydroalcoholic extract of *madu* mango fruit peel produced an antioxidant activity of 77.32% (Javid et al., 2024). On the other hand, the ethanol extract of *madu* mango leaves has the highest antioxidant value of 90.241% (Aditiyarini et al., 2025), while another report noted that the *madu* mango leaf extract has an antioxidant activity of 257.917 ppm (Andini & Priyanti., 2022). These findings indicate that both the peel and leaves of the *madu* mango have antioxidant potential, although the level of effectiveness is influenced by the part of the plant used.

Several studies have discussed the antioxidant potential in various parts of the mango plant, but none have specifically compared the antioxidant activity of methanol extracts from the leaves and the peels of the *madu* mango fruit. By understanding the antioxidant potential of these two parts of the plant, it is expected to provide a scientific basis for further utilization in the pharmaceutical and food industries. The results of this study are anticipated to produce reliable data for the development of safer, more effective, and sustainable pharmaceutical and food products.

2. Methods

2.1. Materials

The samples in this study were the leaves and fruit peels of *Mangifera indica* L. "Madu" obtained from the city of Denpasar. The mango leaves used were fresh, dark green, free from pests or diseases, and were picked from healthy mango trees. The mango fruit peels used came from ripe mangoes, characterized by a golden-green color, a sweet aroma, and showing no signs of physical damage such as rot or black spots. Plant identification was carried out at the Pharmacognosy Laboratory, Faculty of Pharmacy, Universitas Gadjah Mada. The chemicals used included: methanol, folin ciocalteau, Na-acetate, NaOH, $AlCl_3$, chloroform, acetic acid anhydrous, HCl, H_2SO_4, $FeCl_3$, gallic acid, quercetin, DPPH, ascorbic acid, $K_2S_2O_8$, ABTS, Trolox, $K_2S_2O_8$, acetic buffer, and $FeSO_4$. All reagents were analytical grade and purchased from Merck, Germany.

2.2. Extract preparation

The drained leaves and fruit peels of mango were oven-dried at 50°C for 72 hours. After oven-drying, the samples were ground using a blender and then sieved. The simplicia used was 100 g, and 1 L of methanol solvent was employed for each maceration. The powder of the samples was macerated with methanol solvent for 24 hours and then filtered. Maceration was performed three times. The filtrate was collected and evaporated using a rotary evaporator until a thick extract was obtained. The extract was then placed into a dark glass bottle and stored at 4°C for use in the subsequent method stage.

2.3. Determination of total phenolic content

A volume of 1 ml was taken from the methanol extract stock solution of mango leaves and fruit peels, and this stock solution was diluted 10-fold. Then, 5 ml of 7.5% Folin-Ciocalteu reagent was added, the mixture was vortexed, and allowed to stand for 8 minutes. Subsequently, 4 ml of 1% NaOH solution was added, vortexed until homogeneous, and left to stand for 30 minutes. The absorbance of the solution was measured at 739 nm. The total phenolic content value is expressed as mg GAE/g extract (Putra et al., 2021).

2.4. Determination of total flavonoid content

A total of 0.5 ml of the extract solution was placed into a test tube, followed by the addition of 1.5 ml of methanol, 0.1 ml of 10% $AlCl_3$, 0.1 ml of sodium acetate, and 2.8 ml of distilled water. The mixture was vortexed and allowed to stand for 30 minutes, after which its absorbance was measured using a UV-Vis spectrophotometer at 430 nm. The total flavonoid content value is expressed as mg QE/g extract (Putra et al., 2021).

2.5. The FRAP assay

The ferric reducing antioxidant power (FRAP) assay is used to evaluate the antioxidant capacity of a sample. It specifically measures the sample's ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). To test the extract solution, 1 ml of the extract test solution was placed into a test tube. Then, 2 ml of FRAP reagent was added to the test tube and vortexed until homogeneous. The mixture was incubated in a dark room. The absorbance of the solution was measured at a wavelength of 597 nm using a UV-Vis spectrophotometer.

The FRAP value was expressed as µmol Fe^{2+}/g (Putra & Suarjana, 2025).

2.6. The DPPH assay

Into a cuvette, 2 mL of the extract solution at various concentrations was placed and then mixed with 2 mL of DPPH. Subsequently, it was incubated for 15 minutes in a dark room. The absorbance was measured at 516 nm. The same procedure was also performed on the quercetin as a positive control. The DPPH radical scavenging activity was expressed as the IC_{50} value (Putra et al., 2022).

2.7. The ABTS assay

Into a cuvette, 1 mL of the extract solution at various concentrations was placed and mixed with 1 mL of $ABTS^+$ solution. Subsequently, it was incubated for 6 minutes in a dark room. The absorbance was measured at a wavelength of 745 nm. The same procedure was also performed on quercetin as a positive control. The $ABTS^+$ radical scavenging activity was expressed as the IC_{50} value (Putra et al., 2025).

2.8. Data analysis

Statistical analysis was performed using an independent t-test to examine the differences between samples in the determination of total phenolic content, total flavonoids, and FRAP. Meanwhile, DPPH and $ABTS^+$ radical scavenging activities were analyzed using a one-way ANOVA test, followed by Tukey's test to identify significant differences among the sample groups tested.

3. Results and Discussion

Extraction yield was calculated based on the ratio of the final weight (extract obtained) to the initial weight (simplicia weight) multiplied by 100%. The yield values for the extracts of mango leaves and fruit peels can be seen in Table 1. Based on the table, it is observed that the extraction yields for the mango leaves and fruit peel were 17.17% and 15.50%, respectively.

Table 1. Extraction yield

Extract	Simplicia weight (g)	Extract weight (g)	Yield (%)
Leaf	100	17.17	17.17
Fruit peel	100	15.50	15.50

Yield is the ratio between the weight of the extract obtained and the weight of the simplicia as the raw material. The extract yield indicates the amount of secondary metabolites that can be extracted by the solvent. The higher the yield, the greater the amount of secondary metabolites obtained (Nahor et al., 2020.). The results of this study indicate that the yield of the methanol extract of *madu* mango leaves was higher than that of the methanol extract of *madu* mango fruit skin. The yield obtained for the methanol extract of mango leaves in this study was 17.17%, and for the methanol extract of mango fruit skin was 15.5%. Factors influencing the amount of extract or yield in the extraction process include the nature and polarity of the solvent, the number of active compound components present in the sample, and the extraction method used. The maceration method is widely chosen in research because the process is easy to perform and the resulting extract yield is quite good. Additionally, active compounds are not damaged because no heating is used. In terms of its polarity level, methanol is a universal solvent capable of extracting polar, semi-polar, and non-polar compounds. Pandey et al. (2017) showed that the methanol extract of a simplicia or plant exhibits the best antioxidant activity.

The results of the total phenolic content analysis (Table 2) show that the *madu* mango leaf extract contains a much higher total phenolic content (255.627 ± 3.889 mg GAE/g extract) compared to the methanol extract of *madu* mango fruit skin (25.777 ± 0.117 mg GAE/g extract), with a p-value < 0.05. Based on Table 2, the total flavonoid content of the methanol extract of mango leaves was 43.326 ± 1.549 mg QE/g extract, and that of the methanol extract of *madu* mango fruit skin was 15.632 ± 0.262 mg QE/g extract. Phenolics are secondary metabolites involved in plant defense against free radicals. Leaves play a primary role in photosynthesis and are susceptible to oxidative damage due to exposure to UV light and oxygen, thus requiring higher phenolic content as antioxidants and protective defense mechanisms. Fruit skin also contains phenolics, but in lower concentrations because its main function is as a physical barrier and structural defense mechanism (Lin et al., 2016). The results of this study are supported by Aji et al. (2024), who showed similar findings: the total phenolic content test results for the extract of *Arumanis* mango leaves were 246.94 mg GAE/g, and for Kweni mango leaves were 176.11 mg GAE/g. The measurement results for total phenolic content of the ethanol extract of Arumanis mango skin were 53.4182 mg GAE/g, and for the ethanol extract of *Manalagi* mango skin were 102.4281 mg GAE/g (Safitri et al., 2023).

Table 2. The TPC and TFC of mango leaf and fruit peel extracts

Extract	TPC (mg GAE/g extract)*	TFC (mg QE/g extract)*
Leaf	255.627 ± 3.889	43.326 ± 1.549
Fruit peel	25.777 ± 0.117	15.632 ± 0.262

The experiment was conducted in triplicate. * = significantly different based on independent t-test ($p < 0.05$); TPC = total phenolic content; TFC = total flavonoid content; GAE = gallic acid equivalent; QE = quercetin equivalent.

Flavonoids are the largest group of naturally occurring phenolic compounds. Flavonoids possess pharmacological effects as antioxidants, anti-aging, anti-inflammatory, anti-viral agents, and others. Flavonoids have various subgroups including flavones, flavonols, flavanones, flavanonols, flavanols or catechins, anthocyanins, and chalcones (Putra et al., 2021). Based on the calculation of total flavonoid content, it was found that the leaf sample was much higher compared to the fruit skin sample. This indicates that the highest total flavonoid content was found in the methanol extract of mango leaves. Essentially, fruit skin is presumed to have low flavonoid compounds because the flavonoids present in the skin are bound to glycoside groups (Fauzi et al., 2023), whereas in leaves, flavonoids are produced in large quantities as a response to environmental stress and metabolic needs to protect photosynthetic tissue (Putri & Yawahar, 2023). In previous research, Aji et al., (2024) examined the total flavonoid content of the methanol extract of *Arumanis* mango leaves, finding it to be 129.95 mg QE/g, while for Kweni mango leaves it was 26.50 mg QE/g. The measurement results for the flavonoid content of the ethanol extract of *Arumanis* and *Manalagi* mango skin varieties were 4.4071 mg QE/g and 7.6601 mg QE/g, respectively (Safitri et al., 2023).

Table 3 presents the FRAP values of both extracts. Based on the table, it can be observed that the methanol extract of mango leaves has a higher FRAP value compared to the methanol extract of fruit peels ($p<0.05$). The FRAP assay confirmed the superiority of the leaf extract, with a value of 410.356 ± 17.980 µmol Fe^{2+}/g extract, which was significantly higher ($p < 0.05$) than that of the fruit peel extract (366.910 ± 8.829 µmol Fe^{2+}/g). This value reflects the capacity of the leaf extract as an effective electron donor to reduce Fe^{3+} to Fe^{2+}. Ferjančič et al. (2022) stated that the high FRAP activity in the leaf extract is presumably derived from compounds such as tannins and phenolic acids, which possess strong reducing groups. The *madu mango* leaves show greater potential as a source of reducing antioxidants. This finding aligns with the study by Kumar et al. (2023) regarding the proanthocyanidin content in mango leaves that contributes to reduction activity. Compared to previous studies, the results of this research demonstrate higher values. Işık et al. (2025) reported that FRAP values of 0.118 ± 0.07 (in unit of 0.2 µg/mL) for the ethanol extract of leaves. Bai et al. (2018) in their study on the ethanol extract of mango fruit peel, obtained a value of 4.7 ± 0.5 µM TE/kg. The higher antioxidant activity observed in this study may be influenced by the use of a high-polarity solvent, which enabled more optimal extraction of active compounds. Additionally, variations in fruit elements and environmental growing conditions may also affect the content of secondary metabolites.

Table 3. The antioxidant activity of mango leaf and fruit peel extracts

Extract	FRAP value (µmol Fe^{2+}/g extract)*	Scavenging activity, IC_{50}		Category
		DPPH (ppm)	ABTS (ppm)	
Leaf	410.356 ± 17.980	0.457 ± 0.098^{a}	5.430 ± 0.280^{b}	Very strong
Fruit peel	366.910 ± 8.829	13.962 ± 0.206^{c}	19.428 ± 0.333^{c}	Very strong
Quercetin	–	1.383 ± 0.016^{b}	1.120 ± 0.013^{a}	Very strong

* = significantly different based on independent t-test ($p < 0.05$); letters a – c indicate significant differences based on the One Way ANOVA test followed by the Tukey post hoc test ($p < 0.0001$).

Based on the DPPH assay, the methanol extract of mango leaves exhibited an IC_{50} value of 0.457 ± 0.98 ppm, which was significantly lower than that of the fruit peel extract (13.962 ± 0.206 ppm) with $p < 0.0001$. This indicates that the methanol extract of the leaves possesses stronger DPPH free radical scavenging activity. Similarly, in the ABTS assay, the methanol extract of *madu* mango leaves showed an IC_{50} value of 5.430 ± 0.280 ppm, while the methanol extract of the fruit peel had an IC_{50} value of 19.428 ± 0.333 ppm ($p < 0.0001$). However, when compared to quercetin, which had an IC_{50} value of 1.120 ± 0.013 ppm, the IC_{50} value of the *madu* mango leaf methanol extract was higher ($p < 0.0001$). This indicates that the leaf extract exhibits stronger $ABTS^{+}$ free radical scavenging activity compared to the mango fruit peel methanol extract but is weaker when compared to quercetin.

The results of this study indicate that the methanol extract of *madu* mango leaves exhibits very strong antioxidant activity in scavenging DPPH and $ABTS^{+}$ free radicals. This suggests that the leaf extract is more effective at neutralizing free radicals. Phytochemical analysis revealed that the methanol extract of *madu* mango leaves contains significantly higher levels of total phenolic and total flavonoid content compared to the mango fruit peel extract. Total phenolic content and total flavonoid content can be used to determine antioxidant activity, as there is a linear correlation between total phenolic content and antioxidant activity (Aryal et al., 2019). Furthermore, a study conducted by Johari & Khong (2019) stated that the higher the total phenolic content, the higher the antioxidant activity. Thus, the leaf extract has the potential to serve as an efficient source of natural antioxidants. This finding is consistent with the study by Lestari et al. (2021), which reported an IC_{50} value of 83.61 ppm for the ethanol extract of kasutri leaves. Research conducted by Bai et al. (2018) showed an IC_{50} value of 92 ± 4.2% for the ethanol extract of *madu* mango fruit peel. Fatmawati & Ersam (2015) demonstrated that the methanol extract of mango leaves had an IC_{50} of 3.18 ppm. Another study by Bai et al. (2018) indicated that the $ABTS^{+}$ radical scavenging rate value for the ethanol extract of *madu* mango fruit peel was 79 ± 2.5%. Differences in IC_{50} values may be attributed to variations in the solvents used and the types of mango plants studied. Compared to these findings, the methanol extract of *madu* mango leaves falls into the very strong category according to Ulfa et al. (2021).

4. Conclusion

Based on the results of this study, it can be concluded that the leaves of the mango possess significantly higher bioactive compound content and superior antioxidant potential compared to the fruit peel. The leaves exhibited substantially greater concentrations of total phenolics and flavonoids, which correlated with their markedly more effective radical scavenging activity, as evidenced by much lower IC_{50} values in both the DPPH and ABTS assays. While the fruit peel also demonstrated notable reducing power, particularly in the FRAP assay, the consistently higher performance of the leaves across all parameters indicates that *madu* mango leaves are a considerably richer source of natural antioxidants and warrant further investigation for potential applications in nutraceuticals or functional foods.

Supplementary Material

No supplementary materials are associated with this manuscript.

Acknowledgments

Not Applicable.

Author Contributions

Ayun: conceptualization, data curation resources, and writing–original draft; **Amelia Christania**: data curation, methodology, validation, and supervision; **I Made Gde sudyadnyana sandhika**: methodology, software, formal analysis, investigation, writing–review & editing, and supervision.

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Conflicts of Interest

The authors declare no conflicts of interest.

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