Comparative Analysis of Antioxidant Activities of Matoa *(Pometia pinnata)* Peel Extracts Using DPPH, ABTS, and FRAP Assays

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Abstract. The fruit peel of matoa *(Pometia pinnata)*, commonly discarded as waste, is known to contain bioactive compounds with high antioxidant potential. This study aims to compare the antioxidant activity of *P. pinnata* fruit peel extracts using solvents of varying polarity (ethanol, n-butanol, and ethyl acetate) and assays (DPPH, ABTS, and FRAP). IC_{50} values were calculated using a four-parameter logistic model, and FRAP values were reported as mg GAE/g of extract. Phytochemical screening revealed that the ethanolic extract (ETM) contained the most diverse secondary metabolites, including phenolics, flavonoids, tannins, terpenoids, and triterpenoids. The DPPH assay showed the highest antioxidant capacity in ETM (IC_{50} = 44.90 ppm), followed by ethyl acetate (EAM, 95.66 ppm) and n-butanol (NBM, 397.80 ppm). In the ABTS assay, ETM and EAM demonstrated weaker activities (IC_{50} = 3424 ppm and 13,485 ppm, respectively), while NBM showed no significant activity. The FRAP results indicated that ETM exhibited the strongest reducing power (6.26 ± 0.08 mg GAE/g), followed by EAM (5.44 ± 0.07 mg GAE/g) and NBM (0.77 ± 0.03 mg GAE/g). Solvent polarity influences antioxidant activity and bioactive composition, and ethanol is the most effective solvent for extracting antioxidant compounds from matoa peel.

Keywords: Matoa *(Pometia pinnata)*, antioxidant activity, extract, ABTS, DPPH, FRAP

1. Introduction

Reactive oxygen species (ROS) are free radicals produced naturally during cellular metabolism or in response to environmental stress. Excessive levels of ROS can lead to oxidative stress, causing damage to biomolecules such as lipids, proteins, and DNA. They are also involved in the pathogenesis of various degenerative diseases, including cancer, diabetes, neurodegenerative, and cardiovascular disorders [1]. Antioxidants thus serve an essential function in counteracting free radicals and mitigating oxidative damage. However,

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the prolonged use of synthetic antioxidants, such as tocopherol and ascorbic acid, raises concerns about their stability and potential toxicity [2]. Consequently, the search for natural antioxidant sources derived from biological materials has become a key focus of current research in the fields of functional food and biomedicine [3].

Plants are natural sources rich in antioxidant compounds. The bioactive constituents in plants, particularly phenolics and flavonoids, possess the ability to donate hydrogen atoms and electrons in the process of free radical scavenging [3]. One tropical plant with potential as a natural antioxidant source is *Pometia pinnata* J.R. Forst. & G. Forst. (matoa). This species is widely distributed across Malesia, Southeast Asia, and Papua. While the fruit pulp is commonly consumed, the peel is often discarded and underutilized, resulting in waste. However, the peel of *P. pinnata* contains high levels of phenolic and flavonoid compounds [4], presenting an opportunity for its utilization as a natural antioxidant source and for adding value to this agricultural by-product.

The antioxidant potential of plant extracts is significantly influenced by the presence of bioactive constituents and the type of solvent used during the extraction process. Solvent polarity determines the range of bioactive compounds that can be efficiently extracted [5]. Typically, ethanol is used to extract polar compounds, n-butanol for semi-polar compounds, and ethyl acetate for less polar compounds. Using solvents of varying polarities aims to obtain a broader spectrum of active compounds [5,6]. Furthermore, a comprehensive evaluation of antioxidant activity requires multiple in vitro assays, as each method reflects a distinct mechanism. The DPPH assay measures hydrogen-donating ability, ABTS assesses radical scavenging in both hydrophilic and lipophilic environments, and FRAP evaluates electron-donating capacity, indicating reducing power [7].

Previous studies on *Pometia pinnata* have reported antioxidant activity primarily from leaf, bark, and seed extracts, with most using only a single extraction solvent and one antioxidant assay. For example, ethanolic leaf extracts have demonstrated strong DPPH scavenging activity [8], while bark extracts exhibit moderate radical-scavenging effects [9]. However, research specifically focused on the fruit peel remains limited. Existing studies on peel extracts have generally used ethanol as the sole extraction solvent and evaluated antioxidant capacity solely through the DPPH method [10]. Although these findings confirm the peel as a promising source of antioxidants, they do not investigate how solvent polarity affects the phytochemical profile or antioxidant mechanisms of the extract.

To date, no study has systematically compared multiple solvents of differing polarity in combination with multiple antioxidant assays (DPPH, ABTS, and FRAP) specifically for *P. pinnata* fruit peel, nor evaluated extracts prepared using ultrasound-assisted extraction. This gap results in an incomplete understanding of how extraction parameters affect the antioxidant performance of the peel. Accordingly, this study investigates the antioxidant activity of *P. pinnata* fruit peel extracts obtained using three solvents of differing polarity and three in vitro methods. The results are expected to provide insights into the effect of solvent polarity on antioxidant capacity and support the potential utilization of *P. pinnata* fruit peel as a natural antioxidant source.

2. Materials and Methods

2.1. Material

Ethanol, n-buthanol, ethyl acetate, ethanol p.a, n-hexane, distilled water, Wagner's reagent and Mayer's reagent were purchased from Brataco Indonesia, ABTS (2,2'-azino-bis-(3-ethyl benzothiazoline-6-ammonium sulfonate), TPTZ [2,4,6-Tris(2- pyridyl)-striazine], DPPH (2,2-diphenyl-1- picrylhydrazyl) (Sigma-Aldrich), DMSO, gallic acid, HCL, H_2SO_4, $FeCl_3$, sodium acetate, ascorbic acid, Glacial Acetic Acid, and H (Merck).

2.2. Plant Material and Extraction

Pometia pinnata fruit peel was obtained from the Laboratory of UPT Materia Medika Batu, Jl. Lahor 87, Pasanggrahan, Batu, East Java, Indonesia (coordinates: -7.86754, 112.51924), with identification number 074/631/102.20-A/2022. Ultrasound-assisted extraction (UAE) of *P.pinnata* fruit peel powder was conducted at 40 kHz for 20 minutes at 30 °C. Three solvents—ethanol, n-butanol, and ethyl acetate—were selected for use in the extraction process. Solvent separation from the extracts was carried out using a rotary evaporator at 40°C. Phytochemical screening for saponins, alkaloids, flavonoids, phenolics, tannins, terpenoids, steroids, and triterpenoids was performed following standard procedures [11].

2.3. Antioxidant Assay

2.3.1 DPPH Assay

The DPPH assay was conducted with modifications based on the methods described by Tomala et al.[12]. A total of 80 µL of sample or standard solution (ascorbic acid) was added to a 96-well microplate in decreasing concentrations. Each concentration was prepared in triplicate. Then, 80 µL of 0.1 mM DPPH solution was added to each well containing the sample or standard. The blank contained 80 µL of solvent mixed with 80 µL of 0.1 mM DPPH solution. The mixtures were homogenized for 30 seconds and incubated for 25 minutes at 25°C in the dark. Absorbance was then measured using a microplate reader at 517 nm. The percentage of inhibition was calculated using Equation [1]:

$$\% \text{ Inhibition} = (\text{Abs blank} - \text{Abs sample}) / \text{Abs blank} \times 100 \quad [1]$$

Antioxidant capacity was expressed as the IC_{50} value, representing the extract concentration needed to inhibit 50% of DPPH radical activity.

2.3.2 ABTS Assay

The ABTS assay was carried out with minor modifications to the procedures described by Wu *et al*. [13]. The $ABTS^+$ radical cation stock solution was prepared by mixing 7 mM ABTS with 140 mM potassium persulfate and incubating the mixture in the dark at room

temperature for 16 hours. The ABTS stock solution was diluted with absolute ethanol to obtain a working solution with an absorbance of 0.70 ± 0.02 at 734 nm. A total of 290 µL of the ABTS working solution was mixed with 10 µL of the sample or standard solution (gallic acid), homogenized, and incubated in the dark at room temperature for 6–10 minutes. Absorbance was subsequently measured at 734 nm, and the percentage of radical scavenging activity was calculated using the same formula applied in the DPPH assay (Equation 1). The half-maximal inhibitory concentration (IC_{50}) was used to evaluate antioxidant activity, defined as the concentration required to achieve 50% inhibition of free radical activity.

2.3.3 Frap ABTS

The FRAP assay was assessed based on the method described by Donoso-Bustamante et al.[14]. The FRAP reagent consisted of a mixture of 25 mL of 0.3 mM acetate buffer (pH 3.6), 2.5 mL of 10 mM 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution prepared in 40 mM HCl, and 2.5 mL of 20 mM ferric chloride ($FeCl_3 \cdot 6H_2O$) solution. A total of 20 µL of sample or standard (gallic acid) solution was added to a 96-well microplate in decreasing concentrations, with each concentration analyzed in triplicate. Then, 280 µL of FRAP reagent was added to each well, and the plate was incubated in the dark at room temperature for 10 minutes before measuring the absorbance at 595 nm using a microplate reader. FRAP values were calculated based on a standard curve generated from serial dilutions of gallic acid, using the linear correlation between concentration and absorbance. Results were expressed as mg GAE/g using the linear regression equation obtained from the gallic acid standard curve.

2.4. Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Data analysis was conducted using GraphPad Prism version 10.6.1 (GraphPad Software, San Diego, CA, USA). For the DPPH and ABTS assays, the IC_{50} values of ascorbic acid and *P.pinnata* fruit peel extracts were determined by nonlinear regression analysis using a four-parameter logistic (4PL) sigmoidal model. The Hill slope, coefficient of determination (R^2), and 95% confidence interval (95% CI) were used to evaluate model fit and data variability. For the FRAP assay, a standard curve of gallic acid was generated using linear regression, and the results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g). Differences among extract groups were analyzed using one-way ANOVA followed by Tukey's post hoc test, with a significance level of $p < 0.05$.

3. Results and discussion

3.1. Phytochemical screening

The results of qualitative phytochemical screening of the three *P.pinnata* fruit peel extracts are presented in Table 1. The analysis revealed that all three extracts contained various classes of secondary metabolites, each with a distinct phytochemical profile.

Table 1. Phytochemical constituents of *P. pinnata* fruit peel extracts.

Extracts	Phytochemical constituent							
	Alkaloids	**Phenolics**	**Flavonoids**	**Saponins**	**Tannins**	**Steroids**	**Terpenoids**	**Triterpenoids**
ETM	+	+	+	+	+	–	+	+
NBM	+	–	–	–	–	+	+	–
EAM	–	+	+	–	–	+	–	–

(+) = present; (−) = absent.
ETM = ethanolic extract; NBM = n-butanol extract; EAM = ethyl acetate extract.

The ethanol extract (ETM) exhibited the most comprehensive profile of bioactive compounds compared to the n-butanol (NBM) and ethyl acetate (EAM) extracts. ETM contained alkaloids, phenolics, flavonoids, saponins, tannins, terpenoids, and triterpenoids, but no steroids. NBM showed the presence of steroids, terpenoids and alkaloids, while saponins, phenolics, and flavonoids were not detected. Meanwhile, EAM contained phenolics, flavonoids, and steroids. These differences in the phytochemical content among the three extracts reflect the varying polarity of the solvents used, which influenced the types of bioactive compounds extracted.

3.2. Antioxidant Activities

The antioxidant activity of *P. pinnata* fruit peel extracts was assessed using three different methods: DPPH, ABTS, and FRAP, to better understand their underlying mechanisms of action. In general, antioxidant mechanisms are classified into two major types: Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) [7,15]. The DPPH and ABTS assays involve a combination of both HAT and SET mechanisms, while the FRAP assay specifically represents the SET mechanism through the reduction of Fe^{3+} to Fe^{2+} ions. In the HAT mechanism, antioxidant activity occurs through the donation of a hydrogen atom to neutralize free radicals. In contrast, the SET mechanism involves electron transfer to reduce oxidants to a more stable form [15].

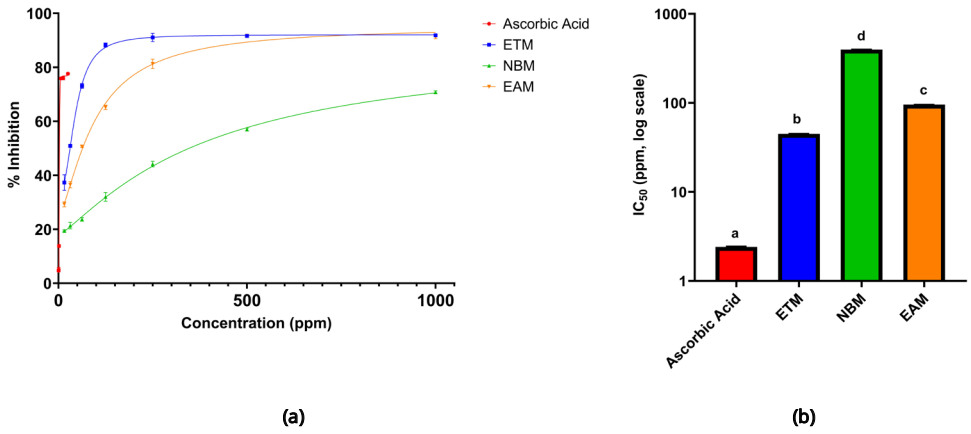

Fig. 1. **(a)** Dose–response curve of DPPH radical scavenging activity of *P. pinnata* fruit peel extracts; **(b)** IC_{50} values of ascorbic acid and *P. pinnata* peel extracts.

Figure 1a presents the DPPH radical scavenging activity of *P. pinnata* fruit peel extracts. All samples exhibited a characteristic sigmoidal dose–response curve, as modeled using the four-parameter logistic (4PL) regression. A comparison of IC_{50} values among the samples is presented in Figure 1b. Significant differences among the samples were determined using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$). Ascorbic acid, used as a positive control, exhibited the highest antioxidant activity with an IC_{50} value of 2.41 ppm. Among the three extracts, the ethanol extract (ETM) demonstrated the strongest radical scavenging ability (IC_{50} = 44.90 ppm), followed by the ethyl acetate extract (EAM, 95.66 ppm) and the n-butanol extract (NBM, 397.8 ppm). The DPPH assay utilizes a neutral, lipophilic radical (DPPH•) in an organic solvent, such as ethanol or methanol, which facilitates more efficient interaction with phenolic compounds, flavonoids, and terpenoids identified in ETM and EAM [15]. These compounds are known to have high hydrogen and electron-donating capabilities, which contribute to the relatively low IC_{50} values observed in the DPPH assay.

The ABTS scavenging profiles of *P. pinnata* fruit peel extracts are illustrated in Figure 2a, using gallic acid as the reference antioxidant. The concentration–response relationships followed a typical sigmoidal trend consistent with the four-parameter logistic (4PL) model. The corresponding IC_{50} values for each sample are summarized in Figure 2b. Statistical evaluation through the extra sum-of-squares F-test confirmed that the variation in IC_{50} values among the samples was highly significant ($p < 0.0001$).

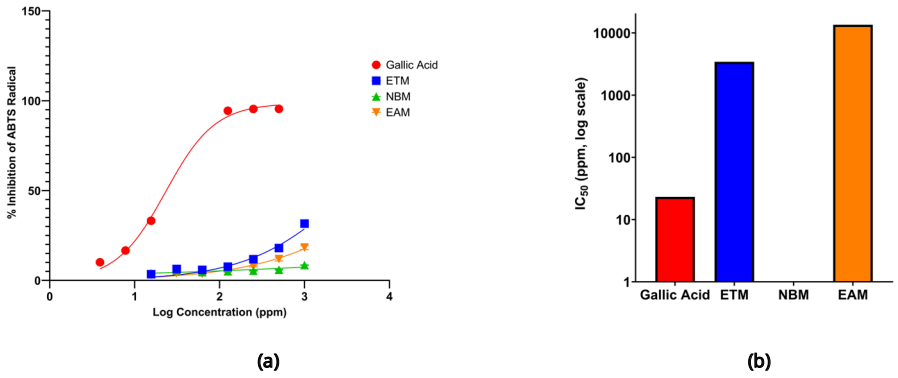

Fig. 2. (a) Dose–Response Curves of Gallic Acid and *P.pinnata* fruit peel extracts in the ABTS Radical Scavenging Assay; **(b)** Comparison of IC_{50} values of Gallic Acid and *P.pinnata* Fruit peel Extracts

Gallic acid exhibited the most potent antioxidant activity, with an IC_{50} value of 23.14 ppm (R^2 = 0.996), indicating a very strong effect. Among the extracts, the ETM displayed the highest activity, with an IC_{50} value of 3424 ppm (R^2 = 0.948), followed by the ethyl acetate extract (EAM) at 13,485 ppm (R^2 = 0.960). The NBM did not reach 50% inhibition within the tested concentration range (R^2 = 0.645) and was therefore considered insignificant. These results indicate that the polar extract (ETM) has a greater electron-donating capacity compared to the semi-polar and non-polar extracts.

The ABTS assay is conducted in an aqueous medium and involves a polar radical cation ($ABTS^{\bullet+}$), making it more selective for hydrophilic antioxidants [7, 13]. The bioactive components identified in ETM, EAM, and NBM—such as phenolics, flavonoids, steroids, and terpenoids—are predominantly semi-polar to non-polar, which limits their solubility in the aqueous ABTS system. As a result, the interaction between $ABTS^{\bullet+}$ and antioxidant molecules is less efficient [7,15], leading to higher IC_{50} values: 3,424 ppm for ETM and 13,485 ppm for EAM.

The ability of *P.pinnata* fruit peel extracts to reduce Fe^{3+} to Fe^{2+} was evaluated using the FRAP assay. The standard calibration curve using gallic acid is shown in Figure 3a, demonstrating a linear relationship between concentration (0–60 ppm) and absorbance at 595 nm. The regression equation ($Y = 0.0316X$, $R^2 = 0.999$) demonstrated a strong linear relationship between concentration and absorbance and was used to calculate the FRAP values of the extracts. A comparison of FRAP values among the extracts is presented in Figure 3b. One-way ANOVA followed by Tukey's post hoc test ($p < 0.05$) was performed to evaluate differences among the samples.

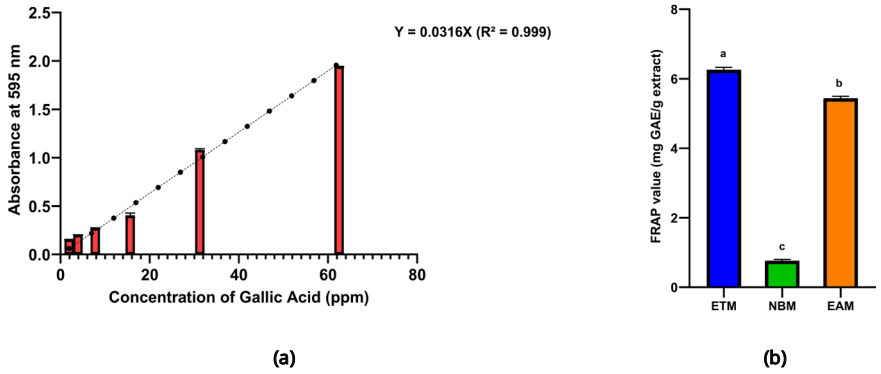

Fig. 3. (a) Standard Calibration Curve of Gallic Acid for the FRAP Assay; (b) Comparison of ferric reducing antioxidant power (FRAP) of *P.pinnata* peel extracts.

In the FRAP assay, ETM demonstrated the highest electron-donating capacity (6.26 ± 0.08 mg GAE/g), followed by EAM (5.44 ± 0.07 mg GAE/g) and NBM (0.77 ± 0.03 mg GAE/g). FRAP values reflect the extract's ability to reduce Fe^{3+} to Fe^{2+} ions, serving as an important indicator of a compound's reducing strength [14,15]. The high linear correlation ($R^2 = 0.999$) between standard concentration and absorbance confirms the reliability of this method for determining antioxidant capacity. The elevated FRAP value in ETM is closely associated with its high polyphenol content [3]. The hydroxyl groups on polyphenolic aromatic rings play a crucial role in electron transfer and the formation of stable complexes with transition metal ions [3,15].

Solvent polarity strongly influences the composition of extracted bioactive compounds and the resulting antioxidant potential of the extract [5,6]. Phytochemical screening results showed that ETM contained a broader range of secondary metabolites compared to EAM and NBM. Ethanol, being more polar than the other two solvents, was able to extract higher amounts of phenolics, flavonoids, saponins, and tannins. Polar solvents, such as ethanol, are

more effective at dissolving polar compounds that contribute significantly to antioxidant activity, particularly phenolics and flavonoids, which are known for their hydrogen- and electron-donating properties [6]. This aligns well with the antioxidant assay results, where the ethanol extract consistently demonstrated superior activity compared to the n-butanol and ethyl acetate extracts.

4. Conclusions

The polarity of the extraction solvent significantly influenced the phytochemical content and antioxidant capacity of *P.pinnata* fruit peel extracts. The ethanol extract (ETM) exhibited the most diverse phytochemical composition and the highest antioxidant activity across the DPPH, ABTS, and FRAP assays. These findings indicate that ethanol is the most effective solvent for extracting bioactive radical-scavenging constituents from *P. pinnata* fruit peel. This study supports the potential application of *P. pinnata* fruit peel as a natural source of antioxidants for the development of functional foods and biomedical products.

The authors declare no conflict of interest.

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