
Identification Of Flavonoid Compounds and Antioxidant Activity Test of Ethyl Acetate Extract of Matoa Leaves (*Pometia Pinnata*) Using the Dpph Method

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Abstract

*This research aimed to identify the flavonoid compounds and test the antioxidant activity of the ethyl acetate extract of matoa leaves (*Pometia pinnata*). The extraction process was carried out using the maceration method with ethyl acetate as the solvent. Phytochemical screening was conducted to determine the groups of secondary metabolites, while specific identification of flavonoids was performed using Thin Layer Chromatography (TLC). The antioxidant activity was analyzed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging method and measured with a UV-Vis spectrophotometer to determine the Inhibitory Concentration 50 (IC₅₀) value. The phytochemical screening results showed that the extract positively contained alkaloids, flavonoids, tannins, saponins, and triterpenoids. The TLC test further confirmed the presence of flavonoid compounds, suggesting that matoa leaves are rich in bioactive components. The antioxidant test results revealed that the ethyl acetate extract of matoa leaves exhibits strong antioxidant activity with an IC₅₀ value of 72.32 µg/mL. This value indicates a high potential of *Pometia pinnata* as a natural antioxidant source that could be utilized for the development of herbal-based pharmaceutical products, health supplements, or functional foods. Therefore, further research on the isolation and structural characterization of flavonoid compounds is recommended to explore their specific roles and mechanisms in antioxidant activity.*

Keywords: Antioxidant, DPPH, Flavonoid, IC₅₀, Matoa Leaves, *Pometia pinnata*

INTRODUCTION

Indonesia is known for its abundant biodiversity, including various types of plants that have long been used by people to maintain health and treat illnesses. One plant that has attracted attention is the matoa (*Pometia pinnata*), which has been used traditionally in medicine, for example, by using its bark to treat burns and smallpox, and a mixture of leaves and bark to treat mouth infections, diarrhea, and diabetes (Małecka et al., 2021).

Matoa leaves are known to contain secondary metabolites with potential antioxidant properties, such as flavonoids, tannins, triterpenoids, glycosides, and saponins. Flavonoids, in particular, play a crucial role in counteracting free radicals—unstable molecules that can damage body cells and trigger oxidative stress. This antioxidant ability has been proven through several studies, where daxon matoa extract showed significant activity in neutralizing free radicals, even at low concentrations (Ahmad et al., 2022)..

Based on previous research, the ethyl acetate extract of matoa leaves was reported to have an IC₅₀ value of 261.07 µg/mL, while the ethanol extract showed a lower IC₅₀ value of 45.78 ppm. For comparison, vitamin C, as a standard antioxidant, has an IC₅₀ value of 42.0 µg/mL. These findings indicate that matoa leaves have the potential to be developed as an effective source of natural antioxidants (Islami et al., 2021).

Therefore, this study was conducted to identify the presence of flavonoid compounds in ethyl acetate extract of matoa leaves and to test its antioxidant activity using the DPPH method. The research results are expected to contribute to the development of Indonesian natural materials, particularly the use of matoa leaves as a source of bioactive compounds that are beneficial for health.

RESEARCH METHODS

This experimental research was conducted at the Pharmaceutical Laboratory of the Deli Husada Deli Tua Health Institute for approximately three months, starting in March 2025. This study aims to identify flavonoid compounds and test the antioxidant activity of ethyl acetate extract of Matoa (*Pometia pinnata*) leaves using the DPPH method, which is a common method for measuring the ability of a compound to neutralize free radicals.

Matoa leaf samples were obtained from Sei Bamban Hamlet, Batang Serangan District, Langkat Regency, North Sumatra, using a purposive sampling technique. The collected leaves are then washed, thinly sliced, and air-dried at room temperature without direct sunlight to preserve the active compounds. The resulting dried herbal medicine is then ground into a powder using a blender for further extraction.

The extraction process was carried out using the maceration method using ethyl acetate as a solvent. A total of 500 grams of powdered herbal medicine was soaked in the solvent for 5 days with periodic stirring. The maceration results were then filtered, and the residue was remacerated. The collected filtrate was concentrated using a rotary evaporator until a thick extract was obtained, which was then stored in a desiccator to maintain its stability.

Characterization of the simple drug includes determining the water content, water-soluble and ethanol content, and total ash and acid-insoluble ash content, which is carried out according to the Indonesian Herbal Pharmacopoeia (FHI) standards. Next, phytochemical screening was performed to detect the presence of secondary metabolites such as alkaloids, flavonoids, saponins, and tannins. Further identification of flavonoids was performed using Thin Layer Chromatography (TLC) to separate and identify compounds based on their R_f values (Ii, 2017).

Antioxidant activity was tested using the DPPH method. A DPPH solution with a concentration of 40 µg/ml was prepared, and its maximum absorption wavelength was determined at 516 nm. The operating time was determined by measuring the absorbance every minute until stable. The extract and reference (vitamin C) were tested at various concentrations, and the percentage of free radical scavenging was calculated. The IC₅₀ value, which indicates the sample concentration required to scavenge 50% of DPPH radicals, was analyzed using a linear regression equation from the relationship curve between concentration and percentage inhibition (Syarifuddin, 2023).

RESULTS AND DISCUSSION

RESULTS

Table 1. Extract yield results

Leaf	Powder	Extraction Results	Yield%	Yield Requirements (FHI, 2017)
Matoa leaves	500 gr	45gr	9%	≤8,8%

Table 2. Results of the test of the characteristics of the simple matoa leaf (*Pometia Pinnata*)

No	Parameter	Results (%)	Statement In Indonesian Medical Material (MMI) 6th ed. Page. 116 – 119
1	Water content	6,16%	≤ 10%
2	Water soluble fiber content	12,33%	≤ 15%
3	Ethanol soluble content	8,66%	≤ 13%
4	Total ash content	2,45%	≤ 7,6%
5	Acid insoluble ash content	0,65%	≤ 1%

Table 3. Results of phytochemical screening of Matoa Leaf simplicia

Compound groups	Reagent	Results	Conclusion
alkaloid	Mayer's reagent (yellow/white precipitate)	White precipitate forms	(+) alkaloid
	Dragendorff's reagent (brick red precipitate)	A reddish brown color is formed	
	Bouchardat reagent (brown-black precipitate)	A thick reddish brown precipitate forms.	
Tannin	Add 2 ml of 96% ethanol, 1-2 drops of iron (III) chloride	A blackish green color is formed	(+) Tannin
Flavonoid	Mixture of 0.1 gr mg powder, 1 ml HCl (p), 2 ml amyl alcohol	A yellow color forms on the amyl alcohol layer	(+) flavonoid
Saponin	Hot distilled water and shake vigorously	Foam forms	(+) saponin
	By adding 1-2 drops of 2N HCl	Foam/foam doesn't disappear	
Triterpenoid	Add 2 ml of 96% ethanol, 1 ml of chloroform, 1 ml of acetic anhydride, HSO ₄ (p)	A reddish color forms	(+) Triterpenoid

Results of Thin Layer Chromatography

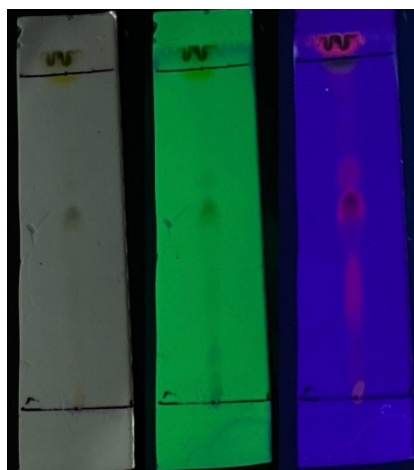


Figure 1. Results of thin layer chromatography

$$\text{RF Value} = \frac{\text{Distance traveled by the substance being analyzed}}{\text{Distance traveled by the solvent}}$$

❖ Matoa Leaf Extract Sample

$$\text{Rf Value} = \frac{5,6}{8} = 0,7$$

The Rf results meet the requirements, with an Rf value of 0.2-0.8.

The maximum wavelength absorption curve for DPPH is 40 µg/ml.



Figure 2. Maximum wavelength absorption curve of DPPH 40 µg/ml

Table 4. Operating time measurement results

Minute To-	Absorbance
2	0,9182
3	0,8963
4	0,8761
5	0,8612
6	0,8476
7	0,8364
8	0,8259
9	0,8169
10	0,8081
11	0,8003
12	0,7937
13	0,7872
14	0,7811
15	0,7750
16	0,7697
17	0,7647
18	0,7597
19	0,7556
20	0,7507
21	0,7427
22	0,7388
23	0,7352
24	0,7316
25	0,7283
26	0,7254
27	0,7222
28	0,7251

29	0,7190
30	0,7161
31	0,7138
32	0,7120
33	0,7097
34	0,7073
35	0,7046
36	0,7021
37	0,6975
38	0,6965
39	0,6956
40	0,6956
41	0,6945
42	0,6925
43	0,6904
44	0,6885
45	0,6870
46	0,6856
47	0,6848
48	0,6834
49	0,6821
50	0,6802
51	0,6791
52	0,6777
53	0,6769
54	0,6757
55	0,6743
56	0,6727
57	0,6718
58	0,6710
59	0,6707
50	0,6695
61	0,6683
62	0,6679
63	0,6665
64	0,6655
65	0,6646
66	0,6631
67	0,6627
68	0,6613
69	0,6608
70	0,6595
71	0,6592
72	0,6580
73	0,6573
74	0,6569
75	0,6559
76	0,6550
77	0,6541
78	0,6537
79	0,6497
80	0,6479

Table 5. Results of Percentage of DPPH Reduction on EEDM and Vitamin C

Test Solution	Concentration (µg/ml)	% Damping Percentage
Ethyl Acetate Extract of Matoa Leaves	0	0
	25	13,21
	50	40,86
	75	65,08
	100	77,29
	150	83,04
Vitamin C	0	0
	50	78,38
	100	83,66
	200	87,59
	400	88,62

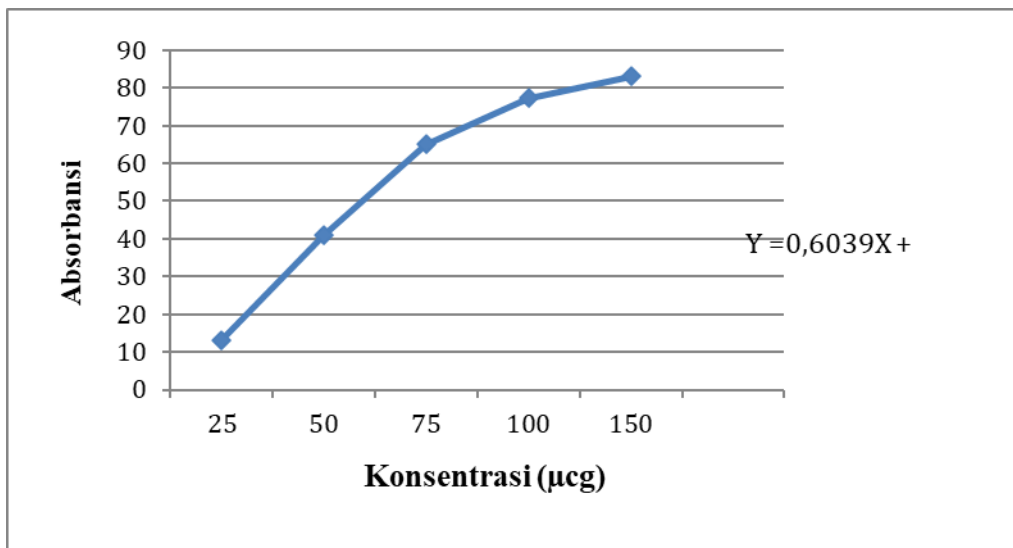


Figure 3. EEDM Antioxidant Activity Test Result Curve

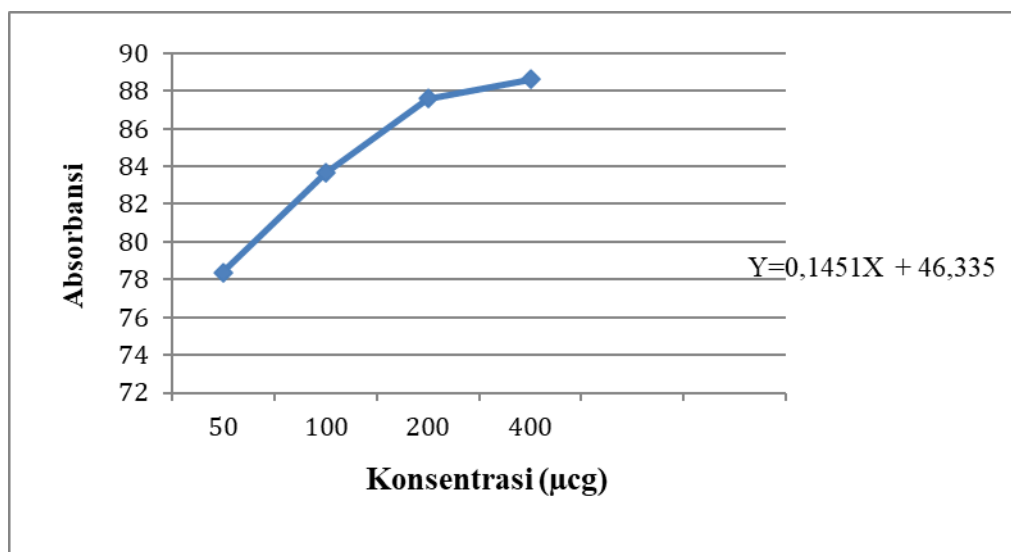


Figure 4. Vitamin C Antioxidant Activity Test Result Curve

DISCUSSION

The results of the identification carried out at the Medanese Herbarium, University of North Sumatra, confirmed that the sample used in this study was Matoa leaves (*Pometia pinnata*) from the Sapindaceae family. The processing of simple herbs begins with washing, slicing, and drying without sunlight to protect bioactive compounds that cannot withstand heat. This drying process aims to reduce the water content, halt enzymatic reactions, and prevent fungal contamination, thus facilitating subsequent extraction. Extraction with ethyl acetate solvent through the maceration method produced a yield of 9%, which meets standard requirements.

Characterization of the simplicia showed that all parameters tested, such as water content (6.16%), water-soluble extract content (12.33%), ethanol-soluble extract content (8.66%), total ash content (2.45%), and acid-insoluble ash content (0.65%), had met the standards set out in the Indonesian Materia Medika (Depkes RI, 1995).

The phytochemical screening test results for the ethyl acetate extract of Matoa leaves showed positive results for several groups of secondary metabolites, namely alkaloids, flavonoids, saponins, tannins, and triterpenoids. The presence of flavonoids was further confirmed through TLC testing, which showed an R_f value of 0.7. This value is within the required range (0.2–0.8), thus strengthening the evidence for the presence of flavonoid compounds in the extract (Niati Ambarsari, 2023).

Antioxidant activity test using DPPH method showed that Matoa leaf ethyl acetate extract (EEDM) was able to reduce free radicals in a concentration-dependent manner. The higher the extract concentration, the greater the percentage of reduction produced, which was indicated by a decrease in the absorbance of the DPPH solution due to a color change from purple to yellow. Linear regression analysis of the data produced the equation $Y = 0.6039X + 6.3241$, which was used to calculate the IC_{50} value. The IC_{50} value obtained for EEDM was 72.32 µg/mL (Leswana & Linden, 2024).

Based on the IC_{50} value of 72.32 µg/mL, the ethyl acetate extract of Matoa leaves is categorized as having strong antioxidant activity, because its value is in the range of 50-100 µg/mL. For comparison, vitamin C used as a positive control has an IC_{50} value of 25.25 µg/mL, which is classified as very strong. These findings prove that the ethyl acetate extract from Matoa

leaves does have the potential as an effective source of natural antioxidants (R. Islamiyati, D.E. Mugitasari, L.N. Nafiah, 2024).

CONCLUSION AND SUGGESTIONS

CONCLUSION

1. Ethyl acetate extract of matoa leaves (*Pometia Pinnata*) contains flavonoid compounds which can be identified by TLC.
2. Ethyl acetate extract of matoa leaves (*Pometia Pinnata*) has antioxidant activity.
3. Matoa leaf extract (*Pometia Pinnata*) has an IC₅₀ antioxidant inhibition value.

SUGGESTIONS

It is recommended that more in-depth research be carried out regarding the identification of flavonoid compounds from other parts of the matoa plant, such as the flowers, fruit, and stems of the plant.

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