

DETERMINATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT MATOA LEAVES (*Pometia pinnata*) BY SOXHLET EXTRACTION

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Abstract. The matoa plant (*Pometia pinnata*) is one of the medicinal plants frequently used for traditional medicine by the Indonesian community. This research aims to determine the total phenolic content and antioxidant activity of matoa leaf extract obtained through the soxhlet extraction method using 70% ethanol as a solvent. The total phenolic content of the matoa leaf ethanol extract was analyzed using the Folin-Ciocalteu method, and the antioxidant activity was tested using the DPPH method with gallic acid as a comparison compound, measured using a UV-Vis Spectrophotometer. The total phenolic content of the ethanol extract from the soxhlet-extracted matoa leaves at the maximum absorption wavelength of 762 nm was found to be 13.98% b/b. The antioxidant activity test of gallic acid yielded an IC₅₀ of 7.922 µg/mL, and the antioxidant activity of the ethanol extract from the soxhlet-extracted matoa leaves at the maximum absorption wavelength of 521 nm yielded an IC₅₀ of 112.854 µg/mL. Based on the IC₅₀ values, it can be concluded that the antioxidant activity of the ethanol extract from the soxhlet-extracted matoa leaves falls into the moderate category, specifically in the range of 100-150 µg/mL. The equivalence of the antioxidant activity of the matoa leaf ethanol extract to gallic acid is 1:14.2456 mg, meaning that 1 mg of gallic acid is equivalent to 14.2456 mg of matoa leaf ethanol extract.

Key words: Phenolic content, *Pometia pinnata*, Soxhletation, Antioxidants, UV-Vis Spectrophotometer

INTRODUCTION

The Papuan community traditionally uses matoa leaves as medicine. The chemical compounds found in matoa leaves are alkaloids, tannins, saponins, terpenoids, coumarins, and flavonoids [1]. Flavonoids are compounds that contain phenolates. Phenolic compounds are phenol derivatives that have antioxidant activity. Antioxidants are compounds or molecules that can prevent the process of oxidation. Various scientific evidence shows that antioxidant compounds can reduce the risk of various chronic diseases such as cancer and coronary heart disease (Kuntorini and Astuti, 2010). Antioxidants can be obtained both naturally and synthetically. Synthetic antioxidants such as Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), and Propyl gallate (PG) are indeed very effective, but their use is toxic to the human body. Therefore, it is necessary to find a source of natural antioxidants that are safer than synthetic antioxidants to be developed, such as plant-based antioxidants rich in flavonoids and tocopherols [2]. Several studies related to the matoa plant have been conducted, including screening and testing the antioxidant activity of ethanol extracts from matoa leaves (*Pometia pinnata*) using the DPPH method. Based on the research results, the ethanol extract of matoa leaves has an IC₅₀ value of 45.78 ppm [3], antioxidant activity of matoa leaves (*Pometia pinnata*) with variations in drying temperatures,

yielding results at 30 °C (IC₅₀ 64.8404), 60 °C (IC₅₀ 49.3608), and 90 °C (IC₅₀ 68.2175) [4], and the determination of total phenolics and flavonoids in ethanol extracts of matoa bark (*Pometia pinnata* J.R & G.Forst) using spectrophotometry, yielding phenolic and flavonoid content of 201.450 ± 0.017 mg GAE/g and 3.092 ± 0.005 mg QE/g extract, respectively [5].

From the review of previous literature, studies using different solvents, extraction methods, and antioxidant activity test methods have yielded varying results in terms of metabolite compounds and antioxidant activity. This variation can occur due to several factors such as light, pH, temperature, and the altitude of the plant's growing location, which can cause differences in the phytochemical content of the plants, even though they are of the same species [6] Based on the above description, a study was conducted to determine the total phenolic content and antioxidant activity of the ethanol extract of matoa leaves (*Pometia pinnata*) obtained through soxhlet extraction. The analysis of phenolic content was conducted using the Folin-Ciocalteu method, and antioxidant activity was measured using the DPPH method with a UV-Vis spectrophotometer.

MATERIALS AND METHODS

Preparation Of Matoa Leaf Ethanol Extract

Matoa leaf extract was prepared by soxhletation. 50 g of powdered matoa leaf simplicia was wrapped in filter paper, placed into a soxhlet apparatus, and 70% ethanol was added. Extraction was performed until the dripping cycle was colorless. The obtained liquid extract was then concentrated using a rotary evaporator at 50 °c and further concentrated with a water bath until a viscous extract was obtained [7].

Phytochemical Screening Of Matoa Leaf Extract

0.5 g of matoa leaf ethanol extract was weighed and placed into a test tube. Then, 5 ml each of chloroform and water (1:1) were added, shaken vigorously, and allowed to stand until two layers, water and chloroform, were formed.

Flavonoid test

1-2 drops of the aqueous layer were placed on a spot plate, a small amount of mg metal powder and a few drops of concentrated hcl were added. The appearance of an orange-red color indicates the presence of flavonoids.

Phenolic test

1-2 drops of the aqueous layer were added to a spot plate, then 1-2 drops of FeCl₃ reagent were added, which gave a blue color indicating the presence of phenolics.

Saponin test

The aqueous layer was put into a test tube and stirred. If permanent foam (± 15 minutes) was formed, it indicated the presence of saponins.

Terpenoid and steroid test

The chloroform layer was filtered with activated charcoal. 2-3 drops of the filtrate were pipetted and allowed to dry on a spot plate. After drying, 2 drops of acetic anhydride and 1 drop of concentrated H₂SO₄ (lieberman-bouchard reagent) were added. If a red color appeared, it indicated the presence of terpenoids, and if a blue or green color appeared, it indicated the presence of steroids.

Alkaloid test

A small amount of the chloroform layer was taken, 1 ml of 0.05 N Ammonia chloroform was added, stirred gently, then 1 ml of 2N H₂SO₄ was added and stirred gently, then allowed to separate. The acidic layer was taken and 1-2 drops of

mayer's reagent were added. A positive reaction for alkaloids showed a white turbidity to white precipitate [8].

Total phenolic content determination

Preparation of standard concentration series and calibration curve

From the 100 µg/ml gallic acid stock solution, 4, 5, 6, 7, and 8 ml were pipetted, diluted with methanol:distilled water (1:1) in a 10 ml volumetric flask to the mark, yielding gallic acid concentrations of 40, 50, 60, 70, and 80 µg/ml. 0.5 ml of each concentration solution was pipetted, then mixed with 5 ml of diluted folin-ciocalteu reagent, and 4 ml of 1M sodium carbonate solution was added. The mixture was allowed to stand for 15 minutes, and the absorbance was measured with a uv-vis spectrophotometer at the maximum absorption wavelength of gallic acid-folin, which would give a blue color complex [9].

Determination of total phenolic content in matoa leaf extract (*pometia pinnata*)

0.0252 g of sample was weighed, then dissolved with 0.5 ml of methanol and placed into a 50 ml volumetric flask. Distilled water was then added to the mark to obtain a test solution of 500 µg/ml. 0.2 ml of the extract test solution was pipetted into a vial, then 5 ml of folin-ciocalteu reagent was added, followed by 4 ml of 1M sodium carbonate. The mixture was shaken until homogeneous. It was allowed to stand for 15 minutes to form a blue color complex. The absorbance was measured at the maximum absorption wavelength of gallic acid-folin using a uv-vis spectrophotometer [10].

Antioxidant activity test as free radical scavenger using DPPH method with uv-vis spectrophotometer

Determination of maximum absorption wavelength (λ) of DPPH

4 ml of the freshly prepared 35 µg/ml DPPH solution was pipetted into a vial, then 2 ml of ethanol and distilled water mixture (1:1) was added. the vial was capped and allowed to stand for 30 minutes in a dark place. subsequently, its absorbance was measured at a maximum wavelength of 400-800 nm using a UV-Vis spectrophotometer.

Determination of antioxidant activity of gallic acid comparison

From the 100 µg/ml gallic acid stock solution, (0.2; 0.4; 0.6; 0.8; 1) ml were pipetted respectively and placed into 10 ml volumetric flasks. A mixture of ethanol and distilled water (1:1) was added to the mark to obtain concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm. 2 ml of each solution was pipetted into a vial, then 4 ml of 35 µg/ml dpph solution was added. The mixture was allowed to stand for 30 minutes in a dark place. The absorbance of the solution was

measured using a uv-vis spectrophotometer at the maximum absorption wavelength of 35 µg/ml DPPH.

Preparation of sample solution and determination of antioxidant activity of sample (*Pometia pinnata*)

25 mg of each matoa leaf ethanol extract (*Pometia pinnata*) was weighed, dissolved with 0.5 ml of ethanol, then ethanol was added into a 25 ml volumetric flask to the mark to obtain a 1000 µg/ml extract stock solution. From the sample solution, (1; 1.2; 1.4; 1.6; 1.8) ml were pipetted. Then, ethanol:distilled water (1:1) was added to a 10 ml volumetric flask to the mark. This yielded samples with concentrations of 100 ppm, 120 ppm, 140 ppm, 160 ppm, and 180 ppm. 2 ml of each sample solution concentration was pipetted using a measuring pipette and placed into a vial, then 4 ml of 35 µg/ml dpph was added. The mixture was homogenized and allowed to stand for 30 minutes in a dark place. The absorbance was measured at the maximum wavelength of 35 µg/ml DPPH using a uv-vis spectrophotometer [11].

Determination of IC₅₀

The IC₅₀ value was calculated using a linear regression equation. IC₅₀ is a value that indicates the concentration of the extract. The extract concentration inhibits 50% of free radical activity. To determine IC₅₀, a standard curve equation is needed with percentage inhibition as the y-axis and antioxidant extract concentration as the x-axis. IC₅₀ is calculated by substituting the value of 50% into the standard curve equation as the y-axis, and then calculating the x-value as the IC₅₀ concentration. A smaller IC₅₀ indicates stronger antioxidant activity.

RESULTS AND DISCUSSION

The collected matoa leaves were thoroughly washed with water, drained, and air-dried for 7 days in a room not exposed to direct sunlight. The drying process aims to reduce the water content of the sample and prevent enzymatic reactions that can lead to the decomposition or damage of compounds present in the sample. Furthermore, the drying process can make the simplicia durable and long-lasting. The matoa leaf simplicia was then powdered to reduce the size of the simplicia and expand the contact surface between the simplicia and the solvent, allowing the solvent to penetrate quickly into the sample and optimize the extraction process.

Soxhlet extraction was performed by setting up the Soxhlet apparatus, then 50 grams of powdered matoa leaves, which had been wrapped in filter paper, were placed into the Soxhlet tube. Then, the solvent (70% ethanol) was added, and heating was started according to the solvent's boiling point. The solvent would evaporate upwards, and when the solvent vapor reached the condenser, it would become liquid and drip onto the powdered biomass below. This process would repeat until the dripping cycle was colorless. The results obtained from Soxhletation were then evaporated using a rotary evaporator to obtain a viscous extract. From

50 g of matoa leaf simplicia, 10.5109 grams of viscous extract were obtained, with a yield of 21.02%.

The matoa leaf extract was found to be a viscous liquid, dark green in color, and with a characteristic odor. Organoleptic examination aims to directly identify the specific characteristics of the extract using the five senses. In addition to organoleptic tests, phytochemical screening was also performed, and the results showed that the ethanol extract of matoa leaves positively contained flavonoids, saponins, phenolics, and steroids.

The drying shrinkage of the extract was 4.56%. Drying shrinkage aims to provide a limit for the amount of compounds lost during the drying process conducted at a temperature of 105 °C, because at this temperature water can evaporate and compounds with boiling points lower than water also evaporate (Lisa, 2016). The obtained drying shrinkage met the standard drying shrinkage of less than 10% (Depkes, 2000). Furthermore, the total ash content of the extract was 1.37%. Ash content measurement aims to provide an overview of the mineral content remaining after the ashing process. The ash content results met the standard ash content, which is not more than 8.4% [12].

The determination of total phenolic content from the Soxhletated matoa leaf extract was performed using the Folin-Ciocalteu method. The principle of the Folin-Ciocalteu method is the oxidation reaction of phenolic compounds under basic conditions by the Folin-Ciocalteu reagent, which will produce a blue-colored complex that gives strong absorption at a wavelength of 765 nm. The increase in blue color intensity is proportional to the amount of phenolics in the sample [13].

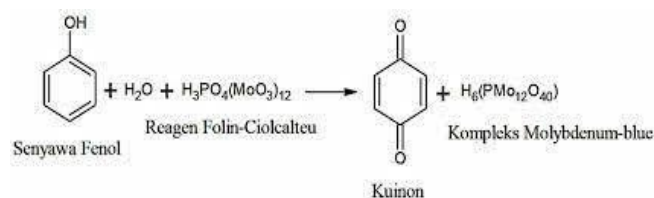


Figure 1. Reaction of phenol compounds with Folin-Ciocalteu reagent

The maximum absorption wavelength used a gallic acid standard as a comparison because gallic acid is a phenolic compound that has high stability, purity, is inexpensive, easily obtainable, and available in nature [8]. The results of determining the maximum wavelength of gallic acid at a concentration of 40 µg/mL was 762 nm with an absorbance value of 0.261. After obtaining the maximum wavelength for gallic acid, the absorbance of gallic acid at concentrations of 40, 50, 60, 70, and 80 µg/mL was measured to determine the gallic acid calibration curve with Folin-Ciocalteu reagent, which yielded the following linear regression equation (Figure 2.)

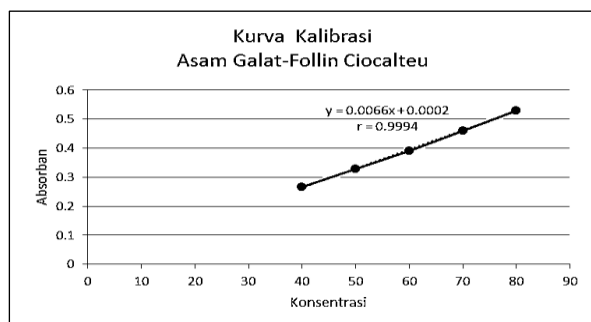


Figure 2. Gallic Acid Calibration Curve

The regression equation obtained was $(y) = 0.0002 + 0.00658x$ with a correlation coefficient $r = 0.9994$, a detection limit (BD) of $1.8905 \mu\text{g/mL}$, and a quantitation limit of $6.3024 \mu\text{g/mL}$. The detection limit is the smallest amount of analyte that can be detected by the instrument, while the quantitation limit is the smallest amount of analyte that can be accurately and precisely measured [9]. The linear equation is intended to measure the total phenolic content in the sample by measuring the sample's absorbance and then applying it to the regression equation. The total phenolic content obtained from the ethanol extract of matoa leaves was $13.98\% \text{ w/w}$.

Table 1. Results of Total Phenolic Content Calculation

Repeat	Absorbant	Concentration ($\mu\text{g/mL}$)	Phenolic content (%)	SD	KV (%)
1	0.470	71.399	14.17		
2	0.456	70.271	13.74	1.08	1.53
3	0.465	70.436	14.02		
Avarage		70.436	13.98		

In addition to determining the total phenolic content, this study also evaluated the antioxidant activity of the ethanol extract of matoa leaves. The antioxidant activity of the ethanol extract of matoa leaves was determined using the DPPH method. The principle of this method is the hydrogen scavenging reaction by DPPH from antioxidant compounds. Before testing antioxidant activity, the maximum absorption wavelength was determined using a control solution. This was done to determine at which wavelength DPPH gives the highest absorbance. For this determination of maximum absorption wavelength, a DPPH solution with a concentration of $35 \mu\text{g/mL}$ was used. The results obtained from UV-Vis Spectrophotometry readings at wavelengths of 400-800 nm showed an absorbance of 0.373 and a maximum absorption wavelength of 521 nm.

Antioxidant activity testing using the DPPH method is performed to determine how effectively a sample can inhibit the stable DPPH radical by donating a hydrogen atom. Samples with antioxidant activity will reduce DPPH to DPPH-H [14]. The reduction will be indicated by a color change of DPPH followed by a decrease in DPPH absorbance. The stronger the antioxidant activity of the sample, the more faded the resulting DPPH color.

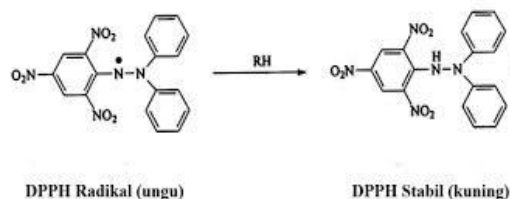


Figure 3. Reaction of DPPH and Antioxidant

Table 2. Results of Matoa Leaf Ethanol Extract Antioxidant Activity Determination

Concentr ation of extract ($\mu\text{g/mL}$)	Control Absorbant	Absorbant Sampel+ DPPH	% inhibition	IC ₅₀ ($\mu\text{g/mL}$)
100	0,646	0,351	45,665	
120	0,646	0,305	52,786	112,8
140	0,646	0,268	58,786	54
160	0,646	0,228	58,513	
180	0,646	0,184	71,517	

The antioxidant activity of the sample solution is expressed in terms of the IC₅₀ parameter. IC₅₀ is a value that indicates the ability of an extract concentration (ppm) to inhibit 50% of free radical activity [15]. A smaller IC₅₀ value indicates a greater antioxidant ability of the compound used. The research results showed that the antioxidant activity of the ethanol extract of matoa leaves had an IC₅₀ value of $112.854 \mu\text{g/mL}$, while the IC₅₀ value of gallic acid used as a comparison was $7.922 \mu\text{g/mL}$. A compound is said to have strong antioxidant activity if its IC₅₀ value is less than $50 \mu\text{g/mL}$, moderate if IC₅₀ is between $50-100 \mu\text{g/mL}$, weak if IC₅₀ is between $101-250 \mu\text{g/mL}$, very weak if IC₅₀ is between $250-500 \mu\text{g/mL}$ and inactive if $>500 \mu\text{g/mL}$ [16]. From this classification, it can be concluded that the ethanol extract of matoa leaves has medium antioxidant activity. Based on the IC₅₀ value, gallic acid showed higher antioxidant activity compared to the ethanol extract of matoa leaves because it is a pure compound, whereas the ethanol extract of matoa leaves is still a mixture of several compounds. Various compounds contained in the extract may or may not have antioxidant activity, but in this case, it does not guarantee that the pure compounds contained in the extract have much better antioxidant activity than gallic acid. Based on these research results, the equivalence of matoa leaf ethanol extract to gallic acid was 14.2456 mg , meaning that 1 mg of gallic acid is equivalent to 14.2456 mg of matoa leaf ethanol extract.

CONCLUSION

Based on the research conducted, the following conclusions can be drawn that The total phenolic content obtained from the ethanol extract of matoa leaves (*Pometia pinnata*) using the folin-ciocalteu method measured with a uv-vis spectrophotometer was $13.98\% \text{ w/w}$. The antioxidant

activity obtained from the ethanol extract of matoa leaves (*Pometia pinnata*), with an IC₅₀ of 112.854 µg/ml, is classified as medium.

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