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Screening Flavonoids of Ethyl Acetate Extract from Matoa Tree Bark (*Pometia pinnata*) Using UV-Vis Spectrophotometry Method

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Abstract: Matoa tree bark is a plant that contains flavonoid chemical compounds believed to be a traditional medicine in various alternative treatments. Therefore, it is important to measure its flavonoid content. Ethyl acetate extracts were prepared using the maceration method at durations of 3 x 24 hours. The aim of this study was to determine the flavonoid compound content and levels in matoa tree bark (*Pometia pinnata*) using UV-Vis spectrophotometry. Qualitative analysis was carried out using NaOH reagent and the wilstater cyanidin test, both of which showed positive results for containing flavonoid compounds, indicated by an orange color change. Quantitative analysis using a regression quercetin standard curve with an R^2 value of 0,9844 revealed a flavonoid content of 2.712%. These results indicate that matoa tree bark extract has a high flavonoid content and has the potential to be developed as a herbal medicine formulation.

Keywords: Flavonoids; Matoa Tree Bark; Secondary Metabolites; UV-Vis Spectrophotometer.

Introduction

The matoa plant (*Pometia pinnata*) is widely used in traditional medicine. Its compounds and activities have the potential to be developed as a phytopharmaceutical that can replace synthetic drugs [1]. The use of matoa as a traditional medicine is inextricably linked to its chemical compounds and biological activity. This plant possesses a wide range of biological activities, including antioxidant, antibacterial, antiviral, anti-inflammatory, anti-allergic, and anticancer properties. This diversity of biological activities is related to the secondary metabolites found in the matoa plant [2].

The active compounds produced by the matoa plant have been tested for their efficacy, where previous research has been conducted on the bark of the matoa tree with ethanol extract containing flavonoids, tannins, terpenoids and saponins, which have antidiabetic activity [3]

The leaves and bark of the matoa tree (*Pometia pinnata*) contain phytochemicals in the form of secondary metabolites such as flavonoids, saponins, and tannins. These secondary metabolites are known to inhibit bacterial growth and can be used as antimicrobial agents by inhibiting bacterial protein synthesis, damaging cell walls, and inhibiting energy metabolism [4]. Secondary metabolite compounds are defined as antioxidant compounds that play a role in fighting free radicals [5]. One of the contents of the matoa tree bark skin is flavonoids, which are natural phenolic compounds that act as antioxidants and have the potential to be used as medicine [6].

The process of extracting secondary metabolites from the matoa tree bark can be done through extraction. It's a process for separating compounds using a specific solvent [7]. Extraction is necessary to obtain the desired active compounds contained in the matoa tree bark. Selecting the right solvent is crucial because it can affect the effectiveness and stability of the active ingredients, as well as the antioxidant
activity, yield, and levels of secondary metabolites in the matoa bark extract [8]

Furthermore, solvent selection also improves extraction efficiency, with selectivity, toxicity, polarity, and ease of evaporation being key factors to consider. Ethyl acetate was chosen as the solvent due to its semipolar nature and low toxicity, which is expected to be able to extract both polar and nonpolar compounds [9]. The second reason for choosing ethyl acetate as a solvent is that it is a solvent that is generally not dangerous and does not absorb water from its environment through retention or adsorption [10].

Based on this background, this study utilises matoa tree bark because it is considered a novelty, in addition to the leaves and fruit, specifically to determine the presence of flavonoid compounds and their levels in the ethyl acetate extract of matoa tree bark (*Pometia pinnata*).

Research Methods

The sample used in this study was the bark of the matoa tree (*Pometia pinnata*) taken from Campaka Village, Pasongsongan District, Sumenep Regency. The sample taken was the outermost part of the matoa tree bark.

Tools and materials

The tools used in this study included analytical scales, measuring cups, Erlenmeyer flasks, measuring flasks, test tubes, desiccators, micropipettes, water baths, a set of rotary evaporators, UV-Vis spectrophotometers, stirrers, and maceration containers.

The materials used in this study were the bark of the matoa tree (Pometia pinnata), distilled water, $AlCl_3$,

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CH_3COOK 1M, ethyl acetate, concentrated HCl, filter paper, methanol, n-hexane, Mg powder, and tissue.

Extraction Procedure

The sample powder was extracted using the maceration method. A total of 100 g was soaked in 900 mL of ethyl acetate solution. The sample was macerated for 3 x 24 hours and stirred periodically [11]. The macerate obtained was then filtered using a Buchner funnel lined with Whatman No. 41 filter paper. The solvent liquid was collected, and then the dregs were macerated again with the same solvent until a clear liquid was obtained. The maceration results were collected and then evaporated using *a rotary evaporator* at low temperature. (50 ºC) until the ethyl acetate extract is obtained.

Qualitative Test

NaOH Reagent Test

NaOH reagent was added to 10 mg of the extract. A positive reaction indicates flavonoids if the solution turns yellow or brown [12].

Wilstater Cyanidin Test

A total of 10 mg of the thick extract was then heated for approximately 5 minutes. After heating, 0.1 g of Mg metal and 5 drops of concentrated HCl were added to each. If the solution turns orange-yellow to red, it is positive for flavonoids [11].

Quantitative Test

Preparation of Quercetin Standard Solution and Quercetin Standard Curve Measurement

Weigh 2.5 mg of quercetin standard dissolved in 25 mL of methanol for analysis, used to make 100 ppm. From a concentration of 100 ppm, several different concentrations were made, namely 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm by pipetting 1 ml, 2 ml, 3 ml, 4 ml, and 5 ml, respectively, and dissolved with methanol (for *analysis*) up to 10 ml. Then pipetted as much as 0.5 mL of each concentration of quercetin solution diluted with 1.5 mL of methanol (for analysis), then added 0.1 mL of 10% AlCl3, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. After that, it was left at room temperature for the operating time (30 minutes) [13].

Standard quercetin solutions with concentrations of 10, 20, 30, 40, and 50 ppm that had been incubated were measured for maximum wavelength absorbance in the wavelength range of 400-450 nm [13].

Determination of Maximum Wavelength (λ_{max})

The maximum absorption wavelength was determined by scanning a concentration of 100 ppm, which was prepared by adding 0.5 mL of a 100 ppm solution, then 0.1 mL of 10% AlCl3, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. At a wavelength of 400-450 nm, the measurement was performed using a UV-Vis Spectrophotometer. This wavelength was used to measure the absorption of the ethyl acetate extract sample of the bark of the matoa tree (*Pometia pinnata*) [13].

Preparation of Sample Solution and Determination of Total Flavonoid Content

Weigh 1 mg of the sample extract and dissolve it in 10 mL of methanol in an Erlenmeyer flask, then stir for 30 minutes. The solution is taken in as much as 0.5 mL and then placed into a test tube. To this, 0.1 mL of 10% $AlCl_3$, 0.1 mL of 1 M Na Acetate, 1.5 mL of methanol, and 2.8 mL of distilled water are added. Shake and incubate for 30 minutes at room temperature [14], [15]

The incubated sample solution was measured using a UV-Vis spectrophotometer. The absorbance of the solution was read at a maximum wavelength of 444 nm. The absorbance results were then calculated using a linear regression equation from the previously measured standard curve of quercetin to obtain the flavonoid concentration contained in the extract [13].

Data analysis

Data were obtained by measuring absorbance at the maximum wavelength. Flavonoid content was calculated based on a standard curve and expressed in grams. Total flavonoid content was calculated using the following formula:

$$\text{Flavonoid Content} = \frac{\text{C x V x Fp}}{\text{m}} \text{ x } 100\%$$

Information:
C : Sample concentration (mg/L)
V : Volume of sample solution (L)
Fp : Dilution factor
m : Mass of extract (mg)

Composition references used must consist of a minimum of 60% of primary references (journals, proceedings) and a maximum of 40% of secondary references (books) published in the last 10 years. [16], [17].

Results and Discussion

Extraction *Pometia pinnata*

The dried matoa tree bark was ground using a blender and then sieved using a 50 mesh. The sieved results were then extracted using ethyl acetate, which aims to extract secondary metabolite compounds such as flavonoids [16]. The maceration process was repeated three times over a 24-hour period to achieve optimal results [18]. The resulting macerate was filtered using a Boucher funnel, and then the solvent was evaporated. One of them was evaporation using a rotary evaporator at a temperature of 50 ºC and a speed of 78 rpm to obtain a concentrated extract of matoa tree bark. The treatment of ethyl acetate vapor pressure of 197 mmHg (50 ºC) aims to easily form a thick extract, where ethyl acetate is in a vacuum and easily evaporates [19]. After obtaining a thick, blackish-green extract, the yield was calculated to yield an average percentage of 1.359%. This yield measurement is intended to determine the amounts of

secondary metabolites carried by the solvent, but cannot determine the type of compound carried by the solvent [20], [16], [21].

Table 1. Results of the yield of ethyl acetate extract of the matoa tree bark

Simplex (gram)	Extract (grams)	Yield (%)
100 grams	1.359 grams	1.359 %

Qualitative Test Results

Qualitative test extract skin stem tree longan using two methods, which are using NaOH and *Wilstater cyanidin* reagents.

Table 2. Results of qualitative tests of the ethyl acetate extract of the matoa tree bark

Group Test	Reagent	Color	Information
Flavonoid	NaOH	Brown yellow	(+)
	Magnesium + HCl	Orange	(+)

Qualitative Test Using NaOH Reagent

Matoa tree bark extract positively contains flavonoid compounds, because the color changes to brownish yellow after being dripped with NaOH.

Figure 1. Qualitative Test Results Using NaOH Reagent

The chrysin compound, which is a derivative of the flavone compound, undergoes base decomposition when NaOH is added to it, producing a yellow acetophenone-like molecule due to the breaking of bonds in the isoprene structure. This proves that the matoa tree bark extract contains flavonoid compounds.

Extract skin stem tree longan positive flavonoid compounds, because they undergo a change in colour to become yellow-brownish after NaOH is added. The cristin compound, which is a derivative of the flavone compound, decomposes into a yellow molecule like acetophenone when NaOH is added due to the breaking of bonds in the isoprene structure. This proves that the matoa tree bark extract contains flavonoid compounds [17].

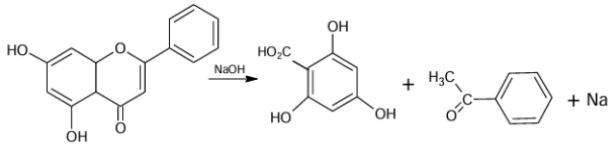

Figure 2. Reaction of flavonoids with NaOH

Wilstater Cyanidin Qualitative Test

The results of qualitative tests on samples of matoa tree bark extract with the addition of Mg powder and concentrated HCl showed positive flavonoid content, as indicated by the formation of a reddish-orange color in the solution [12], [22].

Figure 3. Wilstatter cyanidin Qualitative Test Results

In the flavonoid color reaction test, the purpose of adding Mg powder and concentrated hydrochloric acid is to reduce the benzopyrone nucleus in the flavonoid structure and produce a flavyllium salt. The Mg powder and HCl react to form bubbles and produce H_2 gas [6].

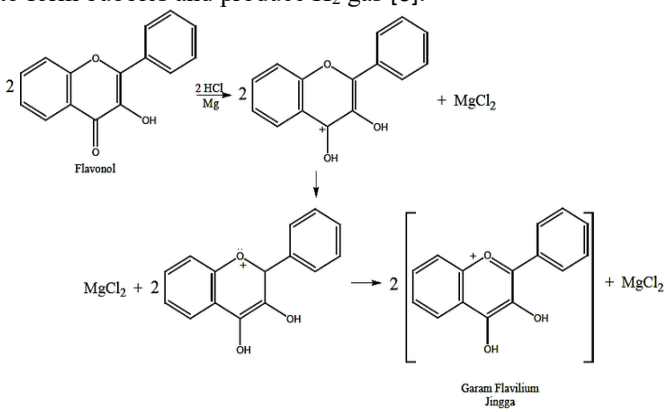

Figure 3. Reaction of flavonoids with Mg powder and concentrated HCl

Quantitative Test

Quantitative test of flavonoid content from ethyl acetate extract of matoa tree bark and determination of flavonoid content using spectrophotometry UV-Vis. It's used for content determination because it is easy to use, short processing time, and provides valid results [17]. The content determination is done by first determining the maximum wavelength. The determination of the maximum wavelength aims to identify the absorption range that can be produced, as indicated by the absorption value of the standard comparison solution. The absorption is measured with a UV-Vis spectrophotometer in the wavelength range of 400-800 nm [21]. In the wavelength region of 400-450 nm, the maximum wavelength is 444 nm.

Preparation of Quercetin Standard Curve

A standard curve was created by correlating the concentration of the standard solution with the absorbance results. The standard curve was created at different

concentrations of 10, 20, 30, 40, and 50 ppm, measured at a maximum wavelength of 444 nm, and yielded the absorbance and average values as shown in Table 3.

Table 3. Concentration and Absorbance of Quercetin

Concentration (ppm)	Absorbance			Average
	A_1	A_2	A_3	
5	0	0	0	0
10	0.157	0.157	0.156	0.157
20	0.241	0.241	0.241	0.241
30	0.461	0.461	0.460	0.461
40	0.523	0.523	0.523	0.523
50	0.658	0.658	0.657	0.657

contain flavonoid compounds. Explain about results or external researchers who discuss the difference between results and theoretical or other relevant research. Explanation can utilize tables, images, and charts to make the article's content easier for the reader to understand.

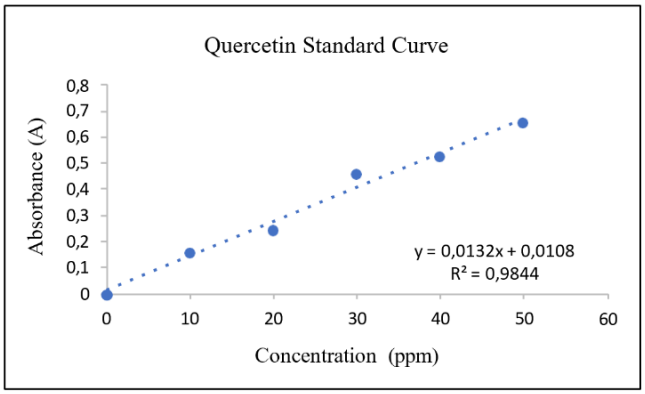

Figure 4. Quercetin Standard Curve

Based on the results of the standard curve determination, a linear equation was obtained: y = 0.132x + 0.0108 with a correlation coefficient of R^2 = 0.9844. The value (R^2) approaching one indicates that the regression equation is linear [21]. Therefore, it can be said that there is a very strong correlation between absorption and concentration [23], [24], [10]. The total flavonoid content was determined using a colorimetric reaction after the sample was reacted with $AlCl_3$ in an acidic medium. The function of adding $AlCl_3$ is to form a stable acid complex with the C-4 ketone group and also with the C-3 or C-5 hydroxyl group of flavones and flavonols [21]. When $AlCl_3$ is added to the sample, a complex is formed between aluminium chloride and quercetin, resulting in a wavelength shift towards visible light and a more yellow color of the solution. The function of adding acetic acid is to maintain the wavelength in the visible range [18].

Determination of Level

The determination of total flavonoid content in the ethyl acetate extract of matoa tree bark was determined using UV-Vis spectrophotometry at a wavelength of 444 nm. The data obtained were as shown in Table 4.

A Matoa tree bark extract sample solution was prepared by weighing 1 mg of the sample extract and then dissolving it in 10 mL of methanol, followed by stirring for 30 minutes. Then, 0.5 mL of the solution was taken and placed into a test tube, to which 0.1 mL of 10% AlCl3, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water were added, yielding an absorbance of 0.025 in the matoa tree bark extract. The absorbance data results were entered into the linear regression model that had been obtained, namely, y = 0.0132x + 0.0108. From the results of this study, a flavonoid concentration of 2.712 mg/L and a total flavonoid content of 2.712% were obtained in the matoa tree bark extract. Research conducted by [18], which examined the determination of flavonoid levels of matoa leaf extract, the flavonoid levels of matoa leaf extract were known to have a total flavonoid content of 57.55%, and research conducted by Islamiati [25], tested the flavonoid levels of matoa leaf ethyl acetate extract, which had a total flavonoid content of 6.19%. Therefore, it can be concluded that the flavonoid levels of matoa tree bark ethyl acetate extract are lower than those of matoa leaf extract. Differences in total flavonoid content are likely influenced by the cultivar used in the study, sampling location, and harvest time [26],[27]. Leaves contain more flavonoids than bark because they are the main organs exposed to sunlight and where photosynthesis occurs, so they need more protection from oxidative stress and UV radiation [28], [29]. These factors can affect the formation of active substances in the harvested plant parts, thereby affecting flavonoid levels [18]. When measuring flavonoid compounds, the addition of $AlCl_3$ to the sample solution forms a complex that shifts the wavelength towards the visible light range, while the addition of potassium acetate maintains the wavelength within the visible range. Before measurement, an incubation process was carried out for 30 minutes so that the reaction between the standard quercetin solution and the added reagent could run well [10], [30].

Table 4. Flavonoid Levels in Matoa Tree Bark

Sample	Replication	Absorbance (y)	Content (mg/L)	Average total flavonoids (%)
Matoa tree bark	1	0.025	2.712	
	2	0.025	2.712	2.712%
	3	0.024	2.636	

The LC-MS characterization aims to determine the compounds identified in the ethyl acetate extract of matoa leaves. This study still has limitations in the method for determining the presence of flavonoid compounds in matoa tree bark. However, previous research has identified twelve compounds in the ethanol extract of matoa leaves. The identified flavonoid compounds are epigallocatechin and apigenin-7-O-diglucuronide, with a total flavonoid content measured at 1.384% [31]

Conclusion

Screening flavonoids of ethyl acetate extract from matoa tree bark (Pometia pinnata) using the spectrophotometry UV-Vis method resulted in a yield of

1.359%. Qualitative testing using NaOH and Wilstater Cyanidin reagents showed positive results for the presence of flavonoids as indicated by a yellow/orange color change in the sample. A calibration curve was obtained from the quercetin standard solution, yielding a regression equation of $y = 0.0132x + 0.0108$, with an R^2 value of 0.9844. Quantitative testing obtained a flavonoid content of 2.712%. However, there are limitations in the method for determining the presence of flavonoid compounds, such as the LC-MS method, which separates sample components based on differences in polarity.

Author's Contribution

Ratno Budiyanto: Collected data, analyzed data, and arranged language processing. Taufiqurrahman: Assisted researcher and prepared discussion. Ach. Kholis: As a supervisor, provided direction and ideas.

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