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Antioxidant and Antibacterial Activities of Ethanol Extract of Matoa (*Pometia pinnata*) Leaves

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ABSTRACT
A Plants used for traditional medicine are matoa (*Pometia pinnata*). This plant is spread in almost every area in the province of Papua. The use of plants as medicinal ingredients is closely related to the content of chemical compounds contained in these plants, especially related to the active compounds contained in plants. Based on previous research, matoa leaf extract contains alkaloids, flavonoids, steroids, tannins, glycosides, and saponins. This study aims to determine the antioxidant and antibacterial activity of Pometia pinnata leaf extract. In the early stages of this research, the extraction process was carried out using the reflux method using ethanol solvent, testing the antioxidant activity using the DPPH and ABTS methods, as well as total phenol and flavonoids. Meanwhile, testing the antibacterial activity with the Minimum Inhibitory Concentration method against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. From the results of the antioxidant activity test, the IC50 value was obtained in the DPPH method of 41.83 ± 0.17 µg/mL and also in the antibacterial test, it could inhibit the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria.

Keywords: *Pometia pinnata, Extract, Antibacterial, Antioxidant*

INTRODUCTION

Indonesia is a country known for its extraordinary natural wealth. All kinds of plant products in Indonesia can be utilized for the benefit of society. The Indonesians have used various ingredients from plant parts such as leaves, roots, fruit, wood, and tubers to gain health and cure various diseases (Sa'diyah et al., 2020).

There are many uses for secondary metabolites, including antioxidants, antibacterials, anticancer, blood anticoagulants, and inhibiting carcinogenic effects. Secondary metabolites can also be used as environmentally friendly pest control agents. Several secondary metabolites are alkaloids, terpenoids, flavonoids, steroids and others (Tunasamy et al., 2019).

Antioxidants are believed to be able to ward off free radicals so that antioxidants can prevent degenerative diseases that free radicals may cause. Antioxidants are compounds that can protect cells from damage caused by free radicals (Francenia et al., 2019). Antioxidants will interact by stabilizing free radicals to prevent damage due to any possible free radicals. Natural antioxidants are flavonoid compounds, a group of polyphenolic compounds that can be derived from plants such as tea, fruits and vegetables. Flavonoid compounds can work directly to reduce free radicals (Dalimunthe et al., 2018).

Antibacterials are compounds used to control the growth of harmful bacteria by killing or suppressing the growth or reproduction of bacteria. Controlling the growth of microorganisms aims to prevent the spread of disease/infection and eradicate microorganisms in infected hosts. Antibacterial substances are divided into two groups, namely antibacterials that can inhibit bacterial growth (bacteriostatic) and antibacterials that can kill bacteria (bacteriocides) (Evbuomwan et al., 2018).

Pometia pinnata is one of the typical Papuan plants which has spread to various parts of Indonesia, especially in Kalimantan. The parts of the P. pinnata plant known to be efficacious as traditional medicines are the leaves and bark. P. pinnata leaf decoction is believed by the people of Papua to relieve hypertension. Secondary metabolite compounds contained in Matoa leaves are Flavonoids, Tannins and Saponins. The activity is contained in flavonoid compounds as antibacterial, antioxidant and antifungal. Antioxidant compounds function to capture free radicals in the body (Munirah et al., 2020).

Some of the research results found the presence of secondary metabolites in the ethanol extract of matoa stem bark, such as flavonoids, tannins, terpenoids and saponins. Based on Martiningsih's research, matoa leaves contain phenolic and flavonoid compounds. Phenolic compounds are active compounds of secondary metabolites known to have several properties, such as astringents, antidiarrheals, antibacterials, and antioxidants. *Flavonoid compounds* are compounds found in foods derived from plants with anti-inflammatory, antioxidant, antiallergic and antiviral effects. Other studies have found that matoa leaves contain saponin compounds that have antimicrobial activity (Darwis, 2022).

Based on the results of this description, the researchers were interested in testing the antibacterial and antioxidant activity of the ethanol extract of matoa (*Pometia pinnata*) leaves.

METHODS

This research was conducted using experimental methods, including collecting plant materials, making simplicia, preparation of extract, examining total phenols and total flavonoids and examining antioxidant activity using the DPPH (*1,1-diphenyl-2-picrylhydrazyl*) and ABTS (*2,2'-azinobis 3-ethylbenzthiazoline-6-sulfonic acid*) method using a UV-Visible spectrophotometer. Preparation of bacterial cultures and testing of antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria using the agar diffusion method.

Preparation of simplicia

Matoa leaves are washed thoroughly, drained, then dried in a drying cupboard with a temperature of 40-50^{0}C until the leaves are dry and weigh the dry weight of the sample. Furthermore, the sample is mashed or powdered using a blender, then sieved, and the sieve results are stored in a closed container at room temperature (Alara et al., 2019).

Preparation of extract

Extract preparation was carried out under reflux with 96% ethanol solvent. Identification of matoa leaves was carried out by sorting the leaves from the color of the fresh leaves. After sorting the leaves, they are washed thoroughly using clean water; then, the leaves are chopped into small pieces to facilitate drying. Drying was carried out for several days at room temperature. The dried matoa leaves were then crushed using a blender, and 70 grams of simplicia powder was taken in 700 mL of absolute ethanol solvent. The reflux apparatus was assembled and then heated to 78^{0}C for 5 hours. Let the round bottom pumpkin cool down, and then filter it. After separating the filtrate and residue, the residue was refluxed three times. The reflux filtrate was thickened using a rotary evaporator at 40^0 C until a thick extract was obtained (Evbuomwan et al., 2018).

DPPH radical scavenging activity

A total of 3.5 ml of DPPH solution was added to 0.5 ml of the sample. Variation of concentration is then mixed with the extract. Absorbance was seen after 60 minutes with methanol as a blank. Absorbance was measured at a wavelength of 517 nm (Nazliniwaty et al., 2021).

ABTS radical scavenging activity

ABTS stock solution (2 ml of ABTS stock solution (0.01 M) was added to 58 ml of phosphate buffer pH 6.9, then incubated for 12 hours and diluted with ethanol to achieve an absorbance of 0.70 at length wave 734 nm. As an evaluation of antioxidant activity, ABTS solution was diluted with ethanol to obtain an absorbance of 0.700 (± 0.020) at a wavelength of 734 nm.2 ml of ABTS solution was mixed with 100 µL of the sample solution in a cuvette, and the decrease in absorbance was measured after 6 minutes (Nazliniwaty et al., 2021).

Determination of Total Phenol

Total phenol content using Folin-Ciocalteu reagent. Homogenize 100 µL of the test sample and 100 µL of the Folin-Ciocalteu reagent. After 4 minutes, 2 ml of 7.5% Na_2CO_3 was added and then incubated for 2 hours. Absorbance was measured at a wavelength of 765 nm. The comparator was gallic acid—Total Phenol Concentration (TPC) based on gallic acid equivalent (mg GAE/g) (Yazid et al., 2020).

Determination of Total Flavonoids

The Aluminum Chloride ($AlCl_3$) method determined the total flavonoid content using a spectrophotometer. A total of 2 ml of extract (10 mg/ml) or standard quercetin solution (25-200 µL/ml) was added with 2 ml of 2% $AlCl_3$ solution and 2 ml of 12 mM potassium acetate. Samples were incubated for one hour at room temperature. The absorbance was measured at a maximum wavelength of 425 nm against the blank using a UV-Vis spectrophotometer. The total concentration of flavonoids obtained was expressed as mg QE (Quercetin Equivalent)/g sample (Nazliniwaty et al., 2022).

Minimum Inhibitory Concentration of Bacteria

Antibacterial activity testing against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria from the ethanol extract of matoa leaves was carried out using the agar diffusion method using paper discs tested in 3 repetitions. 0.1 ml of inoculum was taken using a micropipette mixed homogeneously with 15 ml of Mueller Hinton Agar in a sterile petri dish and then left until the media solidified. On the compacted media, put paper discs dripped with each concentration of ethanol extract of Matoa leaves, positive control, negative control, and solvent control of 30 µl. The extract concentration used was 200 mg/ml; 100mg/ml; 50mg/ml; 25mg/ml; 12.5 mg/ml. Then incubated at 37^0C for 24 hours. Furthermore, the diameter of the inhibition area around the paper disc was measured using a caliper (Sunday et al., 2018).

Statistical Analysis

Data on IC_{50} values and the diameter of the inhibition zone on bacterial growth are presented in the average standard deviation (±) value (Oluyege et al., 2019).

RESULTS AND DISCUSSION

Based on Table 1. *Pometia pinnata* reflux extract with ethanol solvent using the antioxidant test method DPPH (41.83 ± 0.17 µg/mL) and ABTS (151.02 ± 0.03 µg/mL). Total phenol content of *Pometia pinnata* reflux extract with ethanol solvent (197.21 ± 0.21 mg GAE/g) and total flavonoid content (28.73 ± 0.07 mg QE/g). This result shows that the best IC_{50} of *Pometia pinnata* reflux extract with ethanol solvent is found in the antioxidant activity test using the DPPH method. The DPPH test method is known to be a simple method due to the high solubility of the reagent in polar and non-polar solvents (Satria et al., 2017).

Tabel 1. The results IC_{50} value of the antioxidant test method, total phenols and total flavonoids on the ethanol extract of *Pometia pinnata*

Ethanol extract	DPPH	ABTS	Total phenolic	Total Flavonoid
Pometia pinnata	41.83 ± 0.17 µg/mL	151.02 ± 0.03 µg/mL	197.21 ± 0,21 mg GAE/g	28.73 ± 0.07 mg QE/g

The principle of the DPPH method is that The principle of the DPPH method is that compounds that do not react with antioxidants (remaining) will be read as absorbance values at a wavelength of 516 nm in methanol solvent and can be seen organoleptically through a color change from purple to bright purple or light yellow. The decrease in color intensity is related to the number of DPPH electrons that capture the hydrogen atoms. The color change is due to reduced conjugated double bonds in DPPH. The principle of the ABTS method is based on the binding process of the stable ABTS+ radical with antioxidants compared to Trolox. Reduction causes loss of ABTS color. The loss of color is measured at λmax 750 nm (Ifedibalichucwu et al., 2020).

Total phenol using the Folin-Ciocalteau reagent is based on measuring the total antioxidant capacity in a sample based on electron transfer, which reduces the Folin-Ciocalteau reagent from an antioxidant source to form a blue chromophore. This blue color indicates a broad light absorption with a maximum wavelength of 765 nm. The higher the phenol concentration in the system, the greater the light absorption intensity. The standard of comparison widely used in this method is gallic acid. The total antioxidant capacity can be calculated and expressed as gallic acid equivalent (GAE). Total levels of flavonoids were determined using chlorimetry. The principle of this method is based on the addition of $AlCl_3$, which will form a stable acid complex with the C-4 ketone group, as well as the C-3 or C-5 hydroxyl groups of flavone and flavonoids. $AlCl_3$ forms stable acid complexes with orthodihydroxyl groups on rings A or B. Flavonoid compounds will have a maximum absorption at a wavelength of 432 nm (Razali et al., 2019; Lee et al., 2020).

Based on Table 2, it can be seen that at a concentration of 200 µg/mL up to a concentration of 12.5 µg/mL, it can inhibit the growth of both bacteria, namely, *Staphylococcus aureus* bacteria and *Pseudomonas aeruginosa* bacteria, Minimum Inhibitory Concentration (MIC) is present at the smallest concentration capable of inhibiting bacterial growth and the number of bacteria < 10 colonies. The MIC is the lowest concentration of a bacterium expressed in mg/L (µg/mL) under strictly controlled in vitro conditions; this MIC also completely prevents the growth seen in the microbial strains tested (Habtom et al., 2019; Abike et al., 2020).

Table 2. Minimum Inhibitory Concentration Result of *Pometia pinnata* ethanol extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa* bacteria

Bacterial	Inhibitory zone diameter (µg/mL)				
	200	100	50	25	12.5
Staphylococcus aureus	17.31 ± 0.13	15.28 ± 0.07	14.62 ± 0,05	10.41 ± 0.16	10.16 ± 0.11

Pseudomoas aeruginosa	15.28 ± 0.06	11.32 ± 0.14	10.61 ± 0.05	10.19 ± 0.10	9.12 ± 0.18

The reason for choosing the pour method in the antibacterial test in this study is because the pour method is suitable for facultative, microaerophilic, and anaerobic microorganisms. In addition, this method has the advantage of being simple, not consuming many resources, and being easy and economical (Teia et al., 2021; Khan et al., 2013). Secondary metabolite compounds possessed by plants, such as alkaloids, flavonoids, glycosides, saponins, tannins, and steroids/triterpenoids, are chemical compounds that have the potential as antibacterials. The mechanism of action of flavonoids as antibacterials for phenol derivatives is by inhibiting the synthesis of nucleic acids. The mechanism of action of tannins as an antibacterial is to inhibit the enzyme reverse transcriptase and DNA topoisomerase so that bacterial cells are not formed. The mechanism of action of saponins as antibacterials is that they can cause protein and enzyme leakage in cells (Reygaert, 2018; Pumpaluk et al., 2017).

Alkaloids work as an antibacterial by interfering with the constituent components of peptidoglycan in bacterial cells so that the cell wall layer cannot form completely. Steroid/triterpenoid compounds inhibit bacterial growth through interactions with cell membrane phospholipids which are permeable to lipolytic compounds causing decreased membrane integrity and resulting in brittle cells and lysis (Thanh and Tran., 2017; Karfi et al., 2021).

CONCLUSIONS

Based on the results obtained, *Pometia pinnata* extract obtained antioxidant and antibacterial activity. The best antioxidant activity of *Pometia pinnata* extract with ethanol solvent was found in the DPPH method antioxidant activity test with an IC50 value of 41.83 ± 0.17 µg/mL. Meanwhile, *Pometia pinnata* extract with ethanol solvent inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa bacteria*.

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