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Antibacterial and antioxidant activities of ethanol extract of *Artocarpus lacucha* Buch-Ham leaves

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Abstract

Phytochemicals with antimicrobial and antioxidant properties have tremendous potential in suppressing both plant and human diseases. Screening and identifying such compounds from diverse plant species is the first step toward realizing they are medicinal. Mobe plant (*Artocarpus lacucha* Buch-Ham.) is a species of *Artocarpus* from the family Moraceae and contains a lot of phenolics (flavonoids and phenolic acid). Flavonoids, phenolic acids, and phenolic derivatives are phenolic compounds derived from plant flavonoids that can be used as antitumor, antibacterial, antifungal, anticancer, and anti-inflammatory drugs. This study aimed to determine the antibacterial and antioxidant activities, total flavonoid, and total phenolic content of *Artocarpus lacucha* Buch-Ham leaves. The extract was prepared using ethanol with the reflux method. The antibacterial activity was determined with the minimum inhibitor concentration method. The concentration of 300 mg/mL up to 25 mg/mL can inhibit the growth of *Staphylococcus aureus, Staphylococcus epidermidis, Cutibacterium acnes, Pseudomonas aeruginosa*, the Minimum Inhibitory Concentration (MIC) itself is at the smallest concentration that has been able to inhibit bacterial growth. The number of bacteria is < 10 colonies. The antioxidant activity was determined with the 1,1- diphenyl-2-picrylhydrazyl (DPPH) method. Total flavonoid and total phenolic contents were determined with colourimetric methods. Antioxidant activity from the DPPH assay measured as IC_{50} value was 22.83±0.56 µg/mL. The extract contained high levels of total phenolic (300.77±4.56 mg GAE/g) and total flavonoid (15.56±0.32 mg QE/g). The results revealed that ethanol extract of *Artocarpus lacucha* Buch-Ham leaves has antibacterial and antioxidant potential.

1. Introduction

Secondary metabolites are produced at specific growth rates or conditions. This group of compounds is produced in limited quantities, not continuously, and only for particular purposes. The ability of plants to carry out photosynthesis causes the secondary metabolite products produced by plants to be very different from the secondary metabolites produced by other organisms (Kabera *et al*., 2014).

Phytochemicals with antimicrobial and antioxidant properties have tremendous potential in suppressing both plant and human diseases. An antioxidant is an electron-donating molecule or an inhibitor of a reduction reaction. Antioxidants are compounds that have small molecular weights and can inactivate the process of oxidation reactions by inhibiting the formation of free radicals and reactive molecules. The benefits of antioxidants are neutralizing free radicals, preventing degenerative diseases (Prasad *et al*., 2009). Other than antibacterial obtained from natural ingredients, such as plants, spices, or microorganisms, many antibacterial have recently been discovered from natural ingredients such as plants, spices, or microorganisms (Eleazu, 2016).

Secondary metabolites have evolved into plant survival compounds by interfering with pharmacological targets, growing because humans are interested in seeing their potential for human benefit through biotechnology and biomedicine. Its main field is phytomedicine, and thousands of plants have been used around the world to cure various diseases (Wink, 2010).

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In the family moraceae, the mobe plant (*Artocarpus lacucha* Buch-Ham.) is a variety of *Artocarpus*. Many phenolic compounds can be found in this product (flavonoids and phenolic acid). Antitumor and antibacterial antifungal anticancer and anti-inflammatory drugs derived from plant flavonoids are found in Mobe leaves, as are flavonoids, phenolic acids, or phenolic derivatives (Hossain, 2016). This study aimed to determine the antioxidant and antibacterial activity of the ethanol extract of *Artocarpus lacucha* Buch-Ham leaves.

2. Materials and methods

2.1 Materials

The materials used in this study were mobe leaves (*Artocarpus lacucha* Buch-Ham.). The collection of plant material was carried out purposively, that is, without comparing with the same plants from other areas. Mobe leaves (*Artocarpus lacucha* Buch-Ham.) were taken from the Huta Tinggi Village area, Laguboti District, Toba Samosir Regency, North Sumatra. The taxonomic identification of plants was carried out at the Herbarium Medanense Laboratory, FMIPA USU Medan, dimethylsulfoxide (Merck®), ethanol (Merck®), methanol (Merck®), distilled water (Merck®), aluminium chloride (Merck®), sodium acetate (Merck®), DPPH (2,2-diphenyl-1-picryl-hydrazil) (Sigma Aldrich), sodium bicarbonate (Merck®), folin-ciocalteu (Merck®), Mueller Hinton Agar (Sigma Aldrich), *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 27853, *Cutibacterium acnes* ATCC 6919, and *Pseudomonas aeruginosa* ATCC 9027(Culti-LoopsTM), Spectrophotometer (UV-Vis) using PharmaSpec 1700 (Thermo®)

2.2 Preparation of extraction

The preparation of simplicia powder was carried out at the Pharmacognosy Laboratory of the Faculty of Pharmacy, University of North Sumatra. A total of 2 kg of fresh mobe (*Artocarpus lacucha* Buch-Ham.) leaves were cleaned of dirt by washing them one by one with clean running water, then drained, thinly sliced, weighed, then dried by drying in a drying cabinet. Mobe leaves are put in a drying cabinet at a temperature of 40±5°C. Mobe leaves are considered dry (simplicia) when they are brittle (crushed into pieces), then powdered using a blender and weighed the dry powder (simplicia powder). Simplicia powder is stored in a well-closed container protected from sunlight, heat, and humidity. Simplicia powder of *Artocarpus lacucha* Buch-Ham leaves (500 g) was extracted using ethanol absolute with the reflux method (Dalimunthe *et al*., 2018; Urip *et al*., 2018; Hasibuan *et al*., 2020).

2.3 Free radical scavenging activity test

An aliquot (1 mL) of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution containing 200 µg/mL was added to the solution. A UV-Vis spectrophotometer was used to measure the absorbance of the mixture after it had been incubated for 30 mins at a maximum wavelength of 516 nm (DPPH). All assays were performed in triplicate to ensure accuracy. (Naziliniwaty *et al*., 2021; Urip *et al*., 2021).

2.4 Determination of Total Phenol Concentration

The sample's total phenol concentration (TPC) was determined using a folin reagent. Briefly, 100 µL of *Artocarpus lacucha* Buch-Ham extract (500 µg/mL) were mixed with 7.9 mL of distilled water and 0.5 mL of folin-ciocalteu's reagent (1:10 v/v) and mixed with vortex for 1 min. A 20% aqueous sodium bicarbonate solution was added after mixing, and the mixture was allowed to stand for 90 mins while being shaken intermittently. The absorbance was measured at 775 nm using a spectrophotometer. Total phenolic concentration is expressed as gallic acid equivalent in mg per gram of extract. The methanol solution was used as a blank. All assays were carried out in triplicate (Rosidah *et al*., 2008). The equation to determine the total phenolic concentration:

$$C\,(GAE) = \frac{c \times V}{M} \times F$$

Where C (GAE): Concentration of phenolic as gallic acid equivalent, c: concentration determined from a standard curve (µg/mL), V: volume which used in the assay (mL), M: mass of the sample which used in the assay (g), and F: dilution factor.

2.5 Determination of total flavonoid concentration

As previously reported, spectrophotometric measurements of the extracts' total flavonoids determined their flavonoid content. Briefly, 2 mL of extract in methanol was mixed with 0.10 mL of 10% aluminium chloride ($AlCl_3.6H_2O$), 0.10 mL of sodium acetate ($NaC_2H_3O_2.3H_2O$) (1 M), and 2.80 mL of distilled water. After incubation for 40 mins, absorbance was measured at 432 nm using a spectrophotometer. To calculate the concentration of flavonoids, we prepared a calibration curve using quercetin as standard. The flavonoid concentration is expressed as quercetin equivalents in mg per gram of extract. All assays were carried out in triplicate (Jamuna *et al*., 2012). The equation to determine total flavonoid concentration:

$$C\,(QE) = \frac{c \times V}{M} \times F$$

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Where C (QE): Concentration of flavonoid as quercetin equivalent, c: concentration determined from a standard curve (μg/mL), V: volume used in the assay (mL), M: mass of the sample used in the assay (g), and F: dilution factor.

2.6 Determination of minimum inhibitory concentration

The extracts' antibacterial activity was determined using the agar well plate diffusion assay and the minimum inhibitory concentration (MIC) method. The examination used Mueller Hinton Agar (MHA) as the test medium and pure bacteria cultures (*S. aureus, S. epidermidis, C. acnes*, and *P. aeruginosa*) with numerous treatment adjustments. Negative blanks were prepared using a dimethylsulfoxide combination. (DMSO, Merck®) (Kuspradini *et al*., 2019).

3. Results and discussion

3.1 Antioxidant activity of ethanol extract Artocarpus lacucha Buch-Ham

The stabilization by delocalization on aromatic rings makes DPPH a stable radical. There is no dimerization of the radicals that DPPH can trap. Deep violet is formed in the DPPH radical solution due to a strong absorption band centred at 516 nm. It turns colourless to pale yellow upon reduction with a hydrogen donor. The decline in absorbance is proportional to the concentration of antioxidants (Abuin *et al*., 2002). Total phenolic activity is figuring out the amount of phenolic content in the samples. Phenolic compounds contained in the plants have redox properties, allowing them to act as antioxidants (Shoib and Shaid, 2015). Metal oxide reduction products have a blue colour and a broad light absorption spectrum with a maximum wavelength of 750 -770 nm. Several flavonoids (polyphenolic compounds) are potential ROS enhancers because they efficiently chelate trace metals. Flavonoid concentrations were measured using a colourimetric assay method with minor modifications. In a nutshell, rutin was used as a standard to establish a linear calibration, as shown in Table 1.

3.2 Minimum inhibitory concentration

Staphylococcus aureus, S. epidermidis, C. acnes, and P. aeruginosa can be inhibited at concentrations ranging from 300 mg/mL to 25 mg/mL. The Minimum Inhibitory Concentration (MIC) is the lowest concentration that can stop bacteria from growing, and the number of bacteria in less than ten colonies. The MIC of antimicrobial substances that can inhibit bacterial growth after 24 hours of incubation and no known bacterial colonies grow is determined by counting the number of bacterial colonies that grow, as shown in Table 2 (Tortora *et al*., 2010).

The colourimetric method determined the total flavonoid and phenolic content of the ethanol extract of *Artocarpus lacucha* Buch-Ham with $AlCl_3$ represented as quercetin equivalent. While the total phenolic content was carried out using a colourimetric method with Folin-Ciocalteu reagent. The data in Table 1 shows that the ethanolic extract of *Artocarpus lacucha* Buch-Ham contains flavonoid levels of 15.56±0.32 mg QE/g, while the phenolic 300.77±4.56 mg GAE/g.

Antioxidants are electron-donor compounds with low molecular weights that can stop oxidation reactions from progressing by preventing the formation of radicals (Lü *et al*., 2010). The DPPH radical reduction method was used to determine the antioxidant activity. This method is simple, easy, and effective for screening the radical scavenging activity of several compounds (Molyneux, 2003). Due to the hydrogen atom transfer reaction by antioxidant compounds, testing with the DPPH radical reduces the purple DPPH revolutionary compound to yellow, and the DPPH radical becomes stable (Kulisic *et al*., 2004; Nur *et al*., 2017). The results obtained showed that the ethanol extract of *Artocarpus lacucha* Buch-Ham gave a very strong inhibition characterized by the IC_{50} value of 22.83±μg/mL.

Flavonoid phenolic compounds can act as antioxidants, such as flavonols and flavones. The number and location of the OH group, which helps to neutralize

Table 1. Result of antioxidant activity (IC_{50}) with DPPH, total phenolic, and total flavonoid method.

No	Extract	IC_{50}	Total phenolic	Total flavonoid
1	*Artocarpus lacucha* Buch-Ham	22.83±0.56 μg/mL	300.77±4.56 mg GAE/g	15.56±0.32 mg QE/g

Values are presented as mean±SD of triplicates (n = 3).

Table 2. The minimum inhibitory concentration.

No	Bacteria	Inhibition zone diameter (Concentration mg/mL)				
		300	200	100	50	25
1	*Staphylococcus aureus*	12.17±0.12	10.80±0.10	10.17±0.06	9.23±0.05	8.77±0.06
2	*Staphylococcus epidermidis*	11.67±0.06	11.40±0.10	10.93±0.06	9.37±0.12	8.87±0.15
3	*Cutibacterium acnes*	12.57±0.12	11.37±0.11	10.73±0.06	9.73±0.05	8.57±0.06
4	*Pseudomonas aeruginosa*	12.87±0.12	11.67±0.11	9.73±0.12	8.87±0.15	7.97±0.16

Values are presented as mean±SD of triplicates (n = 3).

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free radicals, has a significant impact on flavonoids' activity. Flavonoids' ability to donate electrons is linked to suppressing free radicals. The relationship between total phenol content and antioxidant activity is due to this. The ability of antioxidants to donate electrons in suppressing the development of free radicals is proportional to the total value of phenols and flavonoids. The main compounds in the role of antioxidants are flavonoids' phenolic components (Apak *et al*., 2007; Sandrasari, 2009).

The antibacterial activity was demonstrated by forming an inhibitory zone around the paper disc, as shown in Table 2. The formation of an inhibition zone indicates an indication of antibacterial activity. It can be seen from the results of antibacterial activity tests on extracts with varying concentrations of 300, 200, 100, 50, and 25 µg/mL for the test bacteria *S. aureus, S. epidermidis, C. acnes, P. aeruginosa*. The composition of active compounds in each extract influences the difference in the bacterial inhibitory activity of each extract. Flavonoid compounds can damage cell walls causing cell death, whereas flavonoids have antibacterial activity by interfering with the metabolic function of microorganisms by damaging cell walls and denaturing proteases—microorganism cells (Priya *et al*., 2010). Apart from flavonoids, the content of other compounds such as tannins can also damage cell membranes. Tannin compounds can damage the formation of bacterial conidia. Besides, the high density of bacterial cells may also affect the work of the active antibacterial substances contained in the extract (Heinrich *et al*., 2010).

4. Conclusion

Based on the results obtained, it can be concluded that the ethanol extract of *Artocarpus lacucha* Buch-Ham leaves has a strong antioxidant activity of 22.83±0.56 µg/mL and the concentration of 300 mg/mL up to a concentration of 25 mg/mL can inhibit the growth bacterial.

Conflict of interest

The authors declare no conflict of interest in conducting this study.

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