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Antibacterial Activity Test of Extract and Fraction of Jabon Stem Bark (*Anthocephalus cadamba* (Roxb) Miq.) Againts *Staphylococcus aureus* dan *Escherichia coli*

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ABSTRACT

The stem bark of the Jabon plant (*Anthocephalus cadamba* (Roxb) Miq.) is a plant used empirically as a traditional medicine in the Jayapura City area. This study aims to determine the antibacterial activity and effective concentration of Jabon stem bark extract and fraction (*Anthocephalus cadamba* (Roxb) Miq.) against the test bacteria. The method used in this study is the Kirby-Bauer disc diffusion method to measure the diameter of the inhibition zone of each treatment. The results showed that the extract and fraction had antibacterial activity. The effective concentration of the extract was demonstrated at a concentration of 500 ppm with a strong category against the test bacteria. In comparison, the fractions were shown at a concentration of 1000 ppm with a moderate category against the test bacteria. This study concludes that the extract and fraction of the stem bark of Jabon have antibacterial activity and have the potential to be further developed as an antibacterial agent.

Keywords: Stem Bark Jabon, Extract, Fraction, Antibacterial

INTRODUCTION

Indonesia is a nation abundant in biodiversity. This attracts the public to the use of medicinal plants for traditional healing. Experts continue to develop by testing and researching various plants with medicinal properties so that they can be designed to cure certain diseases. The community's experience and knowledge about all the uses of medicinal plants have been collected to be used as the basis for research development on medicinal plants.

Jabon is one of the medicinal plants used in traditional healing. Jabon as a traditional medicine in Indonesia has not been widely reported, on the contrary, in India and Bangladesh, Jabon is a traditional medicine for various diseases such as analgesic-antiinflammatory (Kumar et al., 2013), febrifugal, antidiuretic, anthelmintic, anti-cataract, blood cleanser, astringent (Brinker & J., 2006; C.P. Khare, 2006; Dubey et al., 2011), and antidiabetic (Marles & Farnsworth, 1995; Ahmed et al., 2011).

In Jayapura, especially in the Koya Koso area, the Jabon plant, especially the bark of the stem, is used to treat strep throat. It is used by boiling the bark of the trunk as much as one sheet, about an inch long, of 10-12 cm, taken from the tree at a part of 1 meter – 1.5 meters from the ground. Boil with approximately 2 liters of water, boiling for 35-40 minutes.

Qualitative phytochemical analysis detected that the methanol extract of white Jabon bark was positive for containing flavonoids, a group of phenolic compounds, alkaloids, triterpenoids, saponins, and glycosides (Sari et al., 2008). Jabon leaf ethanol extract showed the presence of phenolic compounds that have anti-inflammatory activity, flavonoid, and tri-terpenoid (Pant et al., 2012).

Several studies related to the Jabon plant have been conducted, including water extract from Jabon leaves, which shows analgesic and anti-

inflammatory activities (Ambujakshi H.R., Antony S.T., Kanchana Y., Patel R., Thakkar H., 2009; Bachhav, R.S., Buchake V.V., Aher S.S., Rode R.R., 2009).

Methanol extract of jabon bark also has analgesic, antipyretic, and anti-inflammatory activities (Mondal et al., 2009; Chandrashekar et al., 2010). Ethyl acetate fraction extract from Jabon leaves, ethanol extract from Jabon leaves and fruits, and methanol extract from Jabon bark also have antioxidant activity (Chandel et al., 2011; Chandel et al., 2012; Veramaneni et al., 2013).

Methanol extract of white Jabon bark has antiproliferative activity against cervical cancer and breast cancer cells (Sari et al., 2008). Jabon bark has also been characterized by its phenolic compound content (Nadiyah et al., 2018). Jabon leaf ethanol extract shows antihyperglycemic activity by inhibiting the α-glucosidase enzyme (Anisah et al., 2015).

The therapeutic effects of various herbal plants for antibacterial purposes have been extensively studied and developed. Research and development of herbal plants have shown their potential as a source of new medicines (Andriani, 2023). Unlike modern allopathic drugs, which contain a single active ingredient aimed at a specific target, herbal medicine comprises multiple active compounds that work synergistically across various action targets (Lestari et al., 2023).

Antibacterial compounds are compounds that can inhibit or kill bacteria. Several classes of metabolite compounds from plants that have antibacterial activity are flavonoids (Shamsudin et al., 2022), tannins (Farha et al., 2020), alkaloids (Karunakaran et al., 2022), saponins (Sonfack et al., 2021), and steroids (Vollaro et al., 2020). These natural antibacterial compounds can be an alternative treatment to reduce the incidence of resistance due to antibiotics or antibacterial (Pandey & Mishra, 2010).

The antibacterial activity of Jabon plants, specifically Jabon leaves, has been reported to have antibacterial properties with MIC values of 0.078 mg/ml for *B. cereus*, 0.156 mg/ml for *Escherichia coli*, and 0.039 mg/ml for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Aspergillus niger*. The methanol extract of Jabon leaves exhibits antibacterial activity against *Staphylococcus aureus* (11.5 mm) and *Escherichia coli* (11 mm) at a concentration of 900 µg/µL (Zahan et al., 2019).

The pharmacological activity of Jabon stem bark has yet to be widely reported, particularly antibacterial activity against *S.aureus* and *E.coli* bacteria, so this study is important to be conducted. Given this context, this study aims to assess the antibacterial activity of the extract and fractions of Jabon stem bark.

RESEARCH METHOD

This experimental study was conducted at the Microbiology Laboratory and Research Laboratory in the Pharmacy Department of Cenderawasih University.

Equipment

The equipment needed in this study is an analytical scale, measuring glass, petri dish, beaker glass, micropipette, autoclave, laminar air flow, rotary evaporator, and oven.

Materials

The materials used in this study are Jabon stem bark simplicia, *S.aureus* and *E.coli* bacteria, Ciprofloxacin, aquadest, disk paper, n-Hexane, ethyl acetate, ethanol, Nutrient Agar (NA).

Simplicia Powder and Extract Preparation

The *Anthocephalus cadamba* (Roxb) Miq stem bark was collected and wet sorted to separate the impurities; then, the outer bark was peeled off and rinsed with clean running water; the sample was then chopped and dried without direct sunlight, initially covered with a black cloth. Then, the dried sample was smoothed using mesh No. 60 and weighed by the weight of the resulting simplicia.

Simplicia powder (500 g) from the stem bark of *Anthocephalus cadamba* (Roxb) Miq was extracted with 96% ethanol using the maceration method at 1:5. The procedure was repeated twice with the same solvent volume. It was then filtered with filter paper, concentrated using a rotary evaporator, and processed with a water bath. The viscous extract was calculated as a percentage of its yield.

Fractination Preparation

Fractionation involves separating compounds from the extracted material according to the polarity levels. The fractionation method was Liquid-Liquid Extraction (LLE), using n-Hexane, ethyl acetate, and water as solvents. Due to their varying polarity characteristics, these solvents were applied continuously. Fractionation was performed using a 15-gram thick extract dissolved in ethanol and water (ethanol and water ratio, 1:1) as much as 30 mL. Next, it was put into a separate funnel, and then 30 mL of n-Hexane was added and beaten slowly.

After being left alone, a separation occurred between the n-Hexane fraction and ethanol water. The process was repeated multiple times until the

solution became clear. Fractionation continued with 30 mL of ethyl acetate using the same procedure as n-Hexane. The n-Hexane, ethyl acetate, and water fractions were evaporated using a water bath. The fraction results were calculated as the yield percentage of the fractionated extract sample using the following formula (Payung et al., 2021).

Antibacterial Activity Test

The antibacterial activity test of the *Anthocephalus cadamba* (Roxb) Miq stem bark extract and fraction was conducted using the Kirby-Bauer Disc Diffusion method against *S.aureus* and *E.coli*. Extracts and fractions were made in six concentrations of 50, 100, 250, 500, 750, and 1000 ppm. Nutrient Agar is used as media.

The disc papers were soaked in a test solution and then positioned on the surface of the medium where the bacteria had grown. Three times, replication is performed. Petri dishes are incubated at 37°C for 24 hours, after which the inhibition zone is examined. The positive control was the antibiotic ciprofloxacin, while the negative control was aquadest (Warsiti et al., 2019).

Data Analysis

The collected data were analyzed using a one-way ANOVA test and the Tukey test.

RESULTS AND DISCUSSION

Anthocephalus cadamba (Roxb) Miq stem bark was collected from Koya Koso, Jayapura City. This study begins with collecting materials and preparing to make simplicia, extract, and fraction of *Anthocephalus cadamba* (Roxb) Miq stem bark.

Extraction result of *Anthocephalus cadamba* (Roxb) Miq

Extraction of simplicia *Anthocephalus cadamba* (Roxb) Miq.) carried out by maceration method by directly extracting simplicia stem bark of *Anthocephalus cadamba*(Roxb) Miq.) with ethanol of 96%. The maceration technique was selected due to its simplicity, maximum contact between the solvent and the material, and absence of heating, which reduces the likelihood of the active substance being damaged or decomposed.

The solvent used was 96% ethanol, a polar and non-toxic solvent; mold and yeast are challenging to grow, volatile, and can filter compounds that can provide antibacterial activity, including alkaloids, flavonoids, tannins and saponins, solvents that are often used for extraction and are easy to obtain (Retnaningsih et al., 2019).

The extraction results from the stem's bark *Anthocephalus cadamba* (Roxb) Miq. was obtained as much as 64,17 g with a percentage yield of 12.8%. In this context, the extraction result is represented as % yield (rendement), indicating the efficiency of the solvent in extracting specific components from the original material. It will also benefit the extraction methods of plants studied under various conditions (Adam et al., 2019). In addition, the % yield of a sample is essential to determine the number of extracts obtained during the extraction process.

Data on rendement results are linked to the active compounds in a sample, indicating that an increase in rendement correlates with a higher quantity of active compounds in the sample (Hasnaeni, 2019). The high number of yields produced indicates a sample's high number of active compounds (Harbone, 1987).

Previous research showed that the percentage value of *Anthocephalus cadamba* (Roxb) Miq yield using the maceration method was 7.6% (Alam et al., 2011) and 4.62% (Anisah et al., 2015); meanwhile, by using the soxhletation method of 6.32% (Alam et al., 2011). The difference in yield values can be caused by factors such as the ratio of materials to solvents, extraction methods, extraction time, and where plants grow (Ramayani Laksmi et al., 2022).

Fractination result of *Anthocephalus cadamba* (Roxb) Miq

The purpose of the fractionation stage is to separate compounds based on different degrees of polarity in two solvents with varying degrees of polarity. Fractionation by extraction of liquids is carried out by shaking. The separation principle in the fractionation process is based on the difference in polarity levels and type weights between two fractions (Makalunsenge et al., 2022).

Table 1 shows the results of different fraction yields of each solvent, where this difference is due to the polarity level of the compounds contained in the bark extract also different. The yield of the ethanol-water fraction is 41.33 %. This figure is more than the other two types of fractions, namely n-Hexane and ethyl acetate, which means that in the stem bark *Anthocephalus cadamba* (Roxb) Miq fraction tends to be polar, slightly non-polar, 4.66 %, and some of them are semi-polar 20.66 % so that in the fractionation process the extracted sample is more absorbed into the polar solvent, namely the ethanol-water fraction.

Antibacterial activity result with Kirby-Bauer disc diffusion

The antibacterial activity test results obtained show that extracts, n-Hexane fractions, ethyl acetate fractions, and ethanol-water fractions from the stem

bark of *Anthocephalus cadamba* (Roxb) Miq can inhibit the growth of *S. aureus and E. coli* bacteria by showing the presence of inhibitory power around the disc.

According to the findings in Tables 2 and 3 from the bacterial activity test, higher concentrations lead to more potent inhibitory effects in each group. Both their concentration and type influence the effectiveness of antibacterial agents in inhibiting microorganisms. As the concentration of an antibacterial agent increases, its inhibitory power also increases. The higher the concentration of antibacterial ingredients, the more active substances are contained, increasing the number of inhibiting bacteria and creating a more expansive clear zone. (Monica Sandy et al., 2021).

Tabel 1. Fractination result from the stem's bark *Anthocephalus cadamba* (Roxb) Miq.

No.	Fraction	Extract (g)	Fraction (g)	% yield
1	n-Hexane	15	0,7	4,66
2	Ethyl acetate	15	3,1	20,66
3	Ethanol-water	15	6,2	41,33

Table 2. Results of Testing Antibacterial Activity by Diffusion on *Staphylococcus aureus*

No.	Sample	Concentra-tion (ppm)	Inhibition (mm) ± SD
1	Extract	50	7,39 ± 0,06
		100	8,55 ± 0,05
		250	9,48 ± 0,05
		500	10,58 ± 0,08
		750	11,53 ± 0,05
		1000	12,47 ± 0,08
2	n-Hexane fraction	50	5,32 ± 0,20
		100	5,52 ± 0,18
		250	6,32 ± 0,26
		500	7,07 ± 0,24
		750	8,10 ± 0,56
		1000	9,41 ± 0,21
3	Ethyl acetate fraction	50	6,75 ± 0,26
		100	6,84 ± 0,50
		250	6,92 ± 0,15
		500	7,59 ± 0,02
		750	8,50 ± 0,05
		1000	9,67 ± 0,16
4	Ethanol-water fraction	50	5,81 ± 0,18
		100	6,14 ± 0,46
		250	7,04 ± 0,46
		500	7,22 ± 0,42
		750	8,19 ± 0,56
		1000	9,08 ± 0,46
5	K+	5µg	24,08 ± 0.00
6	K-	-	00.00 ± 0.00

Table 2 and 3 show the diameter of the inhibition zone of the extract, fraction, positive control, and negative control. The results of the ANOVA test indicated a significant relationship among the treatment groups ($p<0.05$).

The results of Tukey's test revealed that the extract, n-Hexane fraction, ethyl acetate fraction, and ethanol-water fraction effectively inhibited the test bacteria *S.aureus* and *E.coli*.

However, the extracts at 750 ppm and 1000 ppm exhibit varying antibacterial effects in inhibiting the growth of *S.aureus and E.coli*. The analysis results for the extracts at 750 and 1000 ppm concentrations indicated no significant difference ($p>0.05$).

Nonetheless, the extract at a concentration of 1000 ppm was more effective in inhibiting the growth of *S.aureus* and *E.coli* because it had the most significant inhibition power, namely 12.47 mm

(strong category) in *S.aureus* and 12.69 (strong category) mm in *E.coli*.

Table 3. Results of Testing Antibacterial Activity by Diffusion on *Escherichia coli*

No.	Sample	Concentration (ppm)	Inhibition (mm) ± SD
1	Extract	50	7,60 ± 0.08
		100	8,65 ± 0,12
		250	9,57 ± 0,07
		500	10,65 ± 0,13
		750	11,64 ± 0,04
		1000	12,69 ± 0,06
2	n-Hexane fraction	50	5,65 ± 0,19
		100	5,78 ± 0,53
		250	6,35 ± 0,18
		500	7,24 ± 0,31
		750	8,25 ± 0,45
		1000	9,70 ± 0,44
3	Ethyl acetate fraction	50	6,79 ± 0,34
		100	6,93 ± 0,33
		250	6,99 ± 0,17
		500	7,60 ± 0,18
		750	8,56 ± 0,03
		1000	9,71 ± 0,25
4	Ethanol-water fraction	50	5,87 ± 0,49
		100	6,26 ± 0,40
		250	7,07 ± 0,57
		500	7,33 ± 0,40
		750	8,21 ± 0,56
		1000	9,70 ± 0,44
5	K+	5µg	27.62 ± 0.08
6	K-	-	00.00 ± 0.00

Keterangan: (K+) Ciprofloxacin, (K-) Steril aquadest

Ethanol is a universal solvent capable of dissolving almost any organic compound in the sample, whether polar or non-polar (Karepu et al., 2020). This results in the extract group becoming the most active group by forming the most significant inhibition compared to the fractional group. Each fraction group showed the most significant antibacterial effect at 1000 ppm with mild category.

This study showed that extracts, n-Hexane, ethyl acetate, and ethanol-water fractions of *Anthocephalus cadamba* (Roxb) Miq stem bark had the potential to inhibit bacterial growth. However, they were not as effective as Ciprofloxacin in inhibiting the growth of *S. aureus and E. coli*, as the diameter of the inhibition zone was more significant than that of the extracts and the n-Hexane, ethyl acetate, and ethanol-water fractions of *Anthocephalus cadamba* (Roxb) Miq stem bark.

According to the study findings, the antibacterial substances tested indicated that the compounds from the extract and fractions of the stem bark of *Anthocephalus cadamba* (Roxb) Miq were more effective against *E.coli* than against *S.aureus*. This is attributed to differences in cell wall structure, as gram-negative bacteria have fewer peptidoglycan layers than gram-positive bacteria, which possess a thicker and more rigid peptidoglycan layer. Because of this structural difference, gram-negative bacteria are more susceptible to physical shocks such as administering antibacterial compounds.

Also, high lipid levels in the cells of gram-negative bacteria will increase the permeability of active substances into the cell. In contrast, the thick cell wall of gram-positive bacteria, composed of many layers of peptidoglycan (about 70%), makes it more resistant to the penetration of active substances (Mpila et al., 2012; Shafina Hanum et al., 2022). In previous studies, it was also reported that the antibacterial activity of *Piper betel* leaf extract (Ngamsurach & Praipipat, 2022) and Eucalyptus *globulus* (Bachir & Benali, 2012) were more effective against *E.coli* bacteria than *S.aureus*.

The antibacterial activity of Jabon stem bark, both extracts and fractions, is due to its several

groups of compounds that have antibacterial activity, namely alkaloids, flavonoids, saponins, and tannins. Alkaloids interfere with the constituent components of peptidoglycan in bacterial cells, preventing the complete formation of the layer and resulting in cell death (Hasanah & Gultom, 2020).

Flavonoids inhibit nucleic acid synthesis, cell membrane function, and energy consumption (Zhou et al., 2023)—tannins cause protein denaturation that causes disturbances in bacterial metabolism (Rahayu, 2019). Saponins act as an antibacterial by denaturing proteins. Since the active component on the surface of saponins resembles a detergent, they can be used as antibacterials by reducing the surface tension of the bacterial cell wall and compromising the permeability of the bacterial membrane (Nugraha et al., 2019).

CONCLUSION

From this research, we can conclude that extracts and fractions of *Anthocephalus cadamba* (Roxb) Miq stem bark have antibacterial activity against *S. aureus* and *E. coli*. The extract of *Anthocephalus cadamba* (Roxb) Miq stem bark showed the most significant activity at 1000 ppm against both bacteria with a strong category. Meanwhile, *Anthocephalus cadamba* (Roxb) Miq stem bark fractions showed the greatest inhibitory activity at 1000 ppm with a mild category.

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