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Antibacterial Activity of Ethanol Extracts from Leaves and Flowers of Katang-Katang *Ipomoea pes-caprae* (L) R.Br Against *Pseudomonas aeruginosa*

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Abstract

The resistance character and infection pathogenicity of bacteria to humans is currently increasing, so there is a need for new alternative drugs. The bioactive compounds contained in katang-katang plant are one of the new sources of antibacterials obtained from nature. This study used leaves and flower samples of Katang-katang (Ipomoea pes-caprae (L)R.Br) obtained from the coastal area of Bengkulu City. The purpose of this study was to determine the phytochemical compounds of the leaves and flower extracts of Katang-katang using color reaction test method and to determine the antibacterial activity of the extracts of the leaves and flowers of Katang-katang against the testing pathogenic bacteria (Pseudomonas aeruginosa) at concentrations of 10%,25%,50%,75% using disc diffusion method. The leaves and flowers ethanolic extracts of the Katang-katang were proven contain secondary metabolites such as alkaloids, flavanoids, saponins, tannins and steroids. Antibacterial effectiveness of the ethanol extract from leaves was classified as moderate at a concentration of 50% with a measured inhibition zone of 6.30 mm and a concentration of 75% with an inhibition zone size 8.13 mm. In contrast, effectiveness of the

antibacterial extracted from flower was classified as weak at a concentration of 50% with an inhibition zone 4.50mm and moderate at a concentration of 75% with a measured inhibition zone size of 6.40 mm. The experiment revealed that the leaves and flowers of the Katang-katang have antibacterial potential against P. aeruginosa which could be developed as a medicinal ingredient in the future

Keywords: *Antibacterial, Ethanolic extract, Katang-Katang, Pseudomonas aeruginosa*

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INTRODUCTION

Indonesia is the world's largest archipelagic country, consisting of 17,508 islands with a coastline stretching over 81,000 km² and approximately 3.1 km² of sea area (Mattiro et al., 2023). The island of Sumatra, specifically in the Province of Bengkulu, is home to a lengthy coastal area known as Pantai Panjang, or Long Beach. Pantai Panjang spans approximately 7 km with a gently sloping shoreline and extensive stretches of white sand (Nugraha et al., 2013). The coastal region of Pantai Panjang in Bengkulu is characterized by sandy beaches forming dunes, and along the coastline, it is covered by vegetation commonly referred to as coastal vegetation (Mahfudz, 2012). Based on preliminary observations the dominant vegetation in Pantai Panjang were coastal pine trees and creeping plants such as Katang-katang (*Ipomoea pes-caprae* (L)R.Br*).*

Katang-katang plant, also known as beach morning glory or sea spinach, is a creeping plant belong to *Convolvulaceae* family, characterized by oval-shaped leaves and pinkish-purple flowers. Katang-katang is recognized as a sand stabilizer and play a crucial role in coastal ecosystem rehabilitation (Nayak et al., 2017). Additionally, coastal people utilizes katang-katang leaves as a first-aid for jellyfish stings (Wardhani & Poedjirahajoe, 2020). Katang-katang is also employed as a remedy for reducing inflammation, pain, hemorrhoids, urinary disorders, and pain associated with gonorrhea (Akinniyi et al., 2022). According to research of Saimima & Manuhuttu (2021), katang-katang exhibits pharmacological activity as an antimicrobial agent against *Escherichia coli*.

Besides *E. coli, a*nother important pathogenic bacteria commonly found in the human body is *Pseudomonas aeruginosa*, a Gram-negative and rod-shaped morphology. This bacterium commonly found in human with nosocomial pneumonia, urinary tract infections (UTIs), meningitis, and diarrhea (Newman et al., 2022). The *P. aeruginosa* is a normal microbiome of the human intestines and skin, capable of causing urinary tract infections and pneumonia due to ventilator use (Qin et al., 2022). Study of antibacterial of ethanol extracts from Katang-katang has not been conducted. Therefore, this research is aimed to investigate the secondary metabolite contained in 96% ethanol extracts Katang-katang and test their antibacterial effectiveness against *P. aeruginosa*.

METHOD

Sampling and Extract Preparation

A total of 2 kg each of leaves and flowers of Katang-katang were collected from the Pantai Panjang area, Ratu Agung sub-district, Bengkulu City, Bengkulu province. The samples were then cleaned and washed with running water, and drained. Subsequently, it were sliced and dried using a drying facility lined with clear plastic (greenhouse effect) to prevent direct exposure to sunlight on the surface of the drying samples. Once dried, the samples were ground into powder using a blender and sieved using a 40-mesh sieve. Extraction was carried out using the maceration process with 96% ethanol. A total of 250 g of powdered plant material was soaked in 2.5 liters of alcohol for 3 x 24 hours, followed by re-maceration with the same solvent for 2 x 24 hours. The macerated mixture was filtered and then concentrated using a Rotary Evaporator at a temperature of 50°C/80 rpm until a concentrated extract was obtained (Tetti et al., 2014).

Phytochemical Screening

The content of phytochemical compounds, including secondary metabolite which thought to inhibit microbial growth, were tested qualitatively with color reactions.

Alkaloid Identification

A total of 0.5 grams of each extract was placed into separate reaction tubes, then 2 mL of 2N HCl was added. Subsequently, 1 mL of each filtrate was taken and placed into reaction tubes 1, 2, and 3. Then, 2 drops of Mayer's reagent were added to tube 1, Wagner's reagent to tube 2, and Dragendorff's reagent to tube 3. Positive results were indicated by the formation of a white precipitate in tube 1, a brown precipitate in tube 2, and an orange precipitate in tube 3 (Oktavia et al., 2020).

Flavonoid Identification

A total of 0.5 grams of extract was added to a reaction tube. Then, 0.1 gram of Mg powder and 5 drops of concentrated HCl solution were added. If the color of the solution changes to red, yellow, or orange, it indicates the presence of flavonoids (Oktavia et al., 2020).

Saponin Identification

A total of 0.5 grams of extract was added to a reaction tube. Then, 10 mL of distilled water was added, and the mixture was vigorously shaken. The formation of foam or bubbles that persist for more than 10 minutes and do not disappear upon the addition of HCl indicates the presence of saponin compounds (Oktavia et al., 2020).

Tannin identification

A total of 0.5 grams of extract was placed into a reaction tube. Then, 3 drops of 5% FeCl solution were added. A dark blue or bluish-black color indicates the

presence of tannin compounds. Additionally, 1 mL of the test solution was added to a reaction tube, followed by the addition of 3 drops of 10% gelatin. The formation of a white precipitate indicates the presence of tannin.

Steroid Identification

A total of 0.5 grams of extract was placed into a reaction tube, followed by the addition of 3 drops of anhydrous CH_3COOH (acetic acid). Then, 3 drops of concentrated H_2SO_4 (sulfuric acid) were added slowly to the tube's wall. Subsequently, a formation of a bluish-green ring indicates the presence of steroids (Oktavia et al., 2020).

Antibacterial Activity Test

The antibacterial activity of Katang-katang's leaves and flower extracts was evaluated using the disc diffusion method with 6 treatments and 3 repetitions. The tested extract concentrations were 10%, 25%, 50%, and 75%. The diameter of the inhibition zones was measured using a caliper, measured vertically and horizontally from the clear zones formed around the paper discs, then subtracted by the diameter of the paper disc. The classification of inhibition zone diameters was categorized according to Davis & Stout (1971) as presented in Table 1 (Ouchari et al., 2019).

Tabel 1. Classification of antimicrobial activity based on the size of the inhibitory zone

Inhibitory zone	Category
>20mm	Very strong
10-20mm	Strong
5-10mm	Moderate
<5mm	Weak

Data Analysis

The data analysis was conducted using the statistical data management application SPSS 25 (Statistical Product and Service Solution) with Analysis Of Variance (ANOVA). If there were significant differences in the data, the analysis was continued with the Duncan's multiple range test (Dahlan, 2014).

RESULTS AND DISCUSSION

Ethanol Extract Yield of Katang-katang Leaves and Flowers *(I.pes-caprae)*

The preparation of Katang-katang simplisia was carried out by collecting fresh green leaves and fresh blooming flowers as shown in Figure 1(a) and 1(d) respectively, each amounting to 2 kg. These samples were then cleaned and washed with running water before being drained. Subsequently, the samples were sliced and dried. The dried samples were then ground using a blender and sieved through a 40-mesh sieve (Handoyono et al., 2020). The obtained dry weight was 450 grams as depicted in Figure 1(b) and 1(e).

Figure 1. Yield Results of Katang-katang Leaves and Flower Extracts

The preparation of 96% ethanol extracts from Katang-katang leaves and flowers utilized 250 grams of powdered simplisia yielded from maceration, followed by re-maceration with 96% ethanol solvent for 6 days. The extract was concentrated using a Rotary Evaporator, resulting in a leaves extract of 35 grams (14% yield) and a flower extract 36.7 grams, represented 15% yield (Figure 1(c) and 1(f)). The ideal yield for concentrated extract should not be less than 10%.

Phytochemical Screening of Katang-katang Leaves and Flower Extract

Phytochemical screening serves as an initial step in identifying compound contents within crude drugs or plants, aiding in understanding their chemical structure, biosynthesis, botanical distribution, and biological functions. The chemical mixture resulting from secondary metabolites in plants encompasses numerous types of natural compounds and it can be classified into several groups of substances including Saponins, Steroids, Tannins, Flavonoids, and Alkaloids (Nuskiya et al., 2022). The results of the phytochemical screening of Katang-katang leaves and flowers can be seen in Tables 2 and 3 below:

Table 2. Phytochemical Test Results of Katang-katang Leaves Extract

Phytochemical test	Reagent	Result	Remarks
Alkaloid	Mayer	+	White sediment shown
	Wagner	-	No brown sediment
	Dragendroff	+	Orange sediment shown
Flavonoid	HCl, Mg	+	Color change to yellowish

Saponin	Akuades, HCl	+	Foam/bubble on the surface of the tube
Tanin	$FeCl_3$	+	Color change to blackish
	Gelatin	+	White sediment shown
Steroid	H_2SO_4, CH_3COOH	+	Color change to bluish-green

Remarks:
+ : Indicate positive result;
- : Indicate negative result

Table 3. Phytochemical Test Results of Katang-katang Flower Extract

Phytochemical test	Reagent	Result	Remarks
Alkaloid	Mayer	-	No sediment
	Wagner	-	No sediment
	Dragendroff	-	No sediment
Flavonoid	HCl, Mg	+	Color change to brick-red
Saponin	Aquades	+	Foam/bubble on the surface of the tube
Tanin	$FeCl_3$	+	Change color to blackish green
	Gelatin		
Triterpenoid	H_2SO_4, Acetic acid anhidrat	+	Brownish or violet ring shown

Remarks:
+ : Indicate positive result
- : Indicate negative result

The difference in the phytochemical test results of Katang-katang leaves in previous studiesof Saimima & Manuhuttu, (2021), which utilized methanol, ethyl hexane, and hexane solvents, yielded positive results in the third solvent test containing saponin, tannin, and terpenoid compounds. However, in the alkaloid test using Mayer's reagent, negative results were obtained. Subsequently, according to Aditiyarini et al., (2022) who similarly employed ethanol solvent, yielded positive outcomes in the phytochemical analysis for saponins and tannins. However, negative results were observed in the alkaloid analysis using Mayer's and Dragendroff's reagents, as well as in the flavonoid analysis with sulfuric acid and the steroid assessment. The qualitative phytochemical examination outcomes of beach morning glory leaves and flower extracts are depicted in Figures 2 and 3. The phytochemical analysis in this study exhibited several disparities from previous research findings, attributable to various factors including the geographical region where the plants were grown, as well as the extraction process, which took into consideration factors such as the age or condition of the leaves. Additionally, differences in the solvents utilized were also considered (Sani et al,. 2014). Meanwhile, the phytochemical analysis Katang-katang flowers cannot be directly compared to previous studies as there is no record of the previous studies.

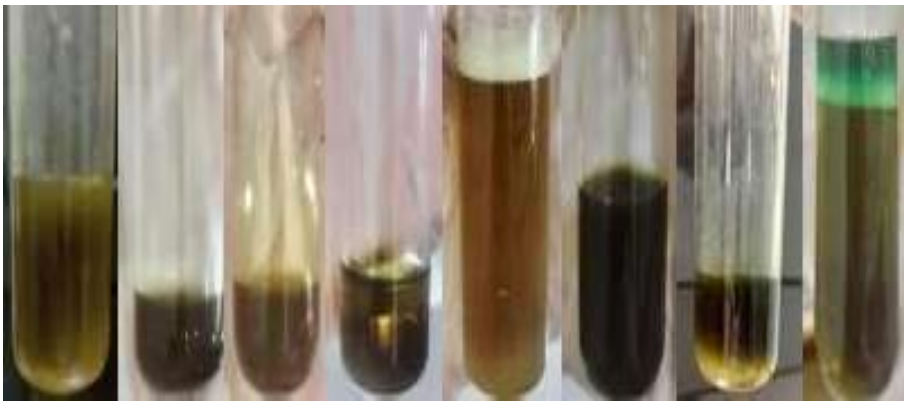

Figure 2. Phytochemical Test Results of Katang-katang Leaves Extract; (a) Alkaloid test (Mayer) (b) Alkaloid test (Wagner) (c) Alkaloid test (Dragendroff) (d) Flavonoid test (e) Saponin test (f) Tannin test (g) Tannin + Gelatine test (h) Steroid test

Figure 3. Phytochemical Test Results of Katang-katang Flower Extract; (a) Alkaloid test (Mayer) (b) Alkaloid test (Wagner) (c) Alkaloid test (Dragendroff) (d) Flavonoid test (e) Saponin test (f) Tannin test (g) Steroid test

Antibacterial Activity

The antibacterial activity test of ethanol extracts from Katang-katang leaves and flowers against *Pseudomonas aeruginosa* was conducted using 6 treatments, as illustrated in Figure 4 and Tables 4 and 5. Ciprofloxacin was utilized as the K+ control, yielding an inhibition zone diameter of 30.83 mm, categorized as very strong. This occurred because Ciprofloxacin is one of the antibiotics with the highest sensitivity to Gram-negative bacterial isolates. Fluoroquinolone antibiotics work by inhibiting bacterial nucleic acid synthesis, causing damage to the bacterial chromosome (Baggio & Rajah, 2021). Conversely, the inhibition zone for K- using 40% DMSO did not exhibit any clear zones, as DMSO lacks antimicrobial properties. Additionally, DMSO serves as a solvent capable of dissolving both polar and non-polar compounds.

Table 4. Antibacterial Activity of Katang-Katang Leaves Extract against *P.aeruginosa*

Treatment(%;g/ml)	Average of inhibition zone (mm) ± SD	Inhibition category
75%	8,13 ± 0,10[c]	moderate
K- (DMSO 40)	0,00 ± 0,00[a]	No inhibition
10%	0,00 ± 0,00[a]	No inhibition
25%	0,00 ± 0,00[a]	No inhibition
50%	6,30 ± 0,75[b]	moderate
K+ (Ciprofoxacin)	30,83 ± 0,62[d]	strong

Table 5. Antibacterial Activity of Katang-Katang Flower Extract against *P.aeruginosa*

Treatment(%;g/ml)	Average of inhibition zone (mm) ± SD	Inhibition category
K- (DMSO 40)	0,00 ± 0,00[a]	No inhibition
10%	0,00 ± 0,00[a]	No inhibition
25%	0,00 ± 0,00[a]	No inhibition
50%	4,50 ± 2,29[b]	Weak
75%	6,40 ± 1,84[c]	Moderate
K+ (Ciprofoxacin)	20,4 ± 1,15[d]	Strong

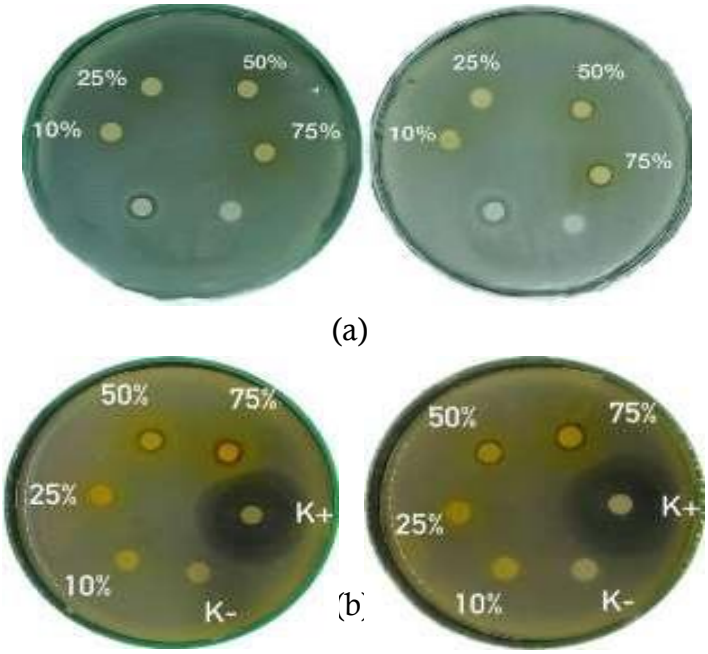

Figure 4. Antibacterial Test Results of Katang-katang Leaves Extract (a); Antibacterial Test Results of Katang-katang Flower Extract (b) against *P.aeruginosa*.

The results antibacterial tests reveal that the leaves and flower extracts of Katang-katang possess the ability to inhibit the growth of *Pseudomonas aeruginosa*, as indicated by the presence of clear zones around the paper discs. At a concentration of 50%, the inhibition zone diameter was measured at 6.30 mm, while at a concentration of 75%, it was 8.13 mm. These results are categorized as moderate inhibition for the growth of *P. aeruginosa*. Additionally, for flower extracts, at a concentration of 50%, the inhibition zone diameter was 4.50 mm, categorized as weak, while at a concentration of 75%, it was 6.40 mm, falling into the moderate category. Moreover, at concentrations of 10% and 25% for both tested extracts, no clear zones were observed, indicating an inability to inhibit the growth of *P. aeruginosa*. Therefore, in can be concluded that the most effective concentrations of plant extract in inhibiting *P. aeruginosa* are 50% and 75%.

The Katang-katang leaves contain compounds that contribute to their effectiveness against Gram-negative pathogenic bacteria, including *Escherichia coli*. Previous studies have reported pharmacological activities found in Katang-katang plants, such as antimicrobial properties. This was demonstrated by the appearance of clear zones in the testing of ethyl acetate extracts of Katang-katang leaves at concentrations of 25% (14.5 mm), 50% (34.3 mm), 75% (38.2 mm), and 100% (39.3 mm) (Saimima & Manuhuttu, 2021). However, research conducted on the Gram-negative bacterium such as *P. aeruginosa* indicates smaller inhibition zone results.

The results of antibacterial test were then analyzed using SPSS by conducting Analysis of Variance (ANOVA). This analysis aimed to determine the significance value, indicating whether the ethanol extracts of Katang-katang leaves and flowers significantly influenced the growth of *P. aeruginosa*. Based on the ANOVA analysis, the significance value obtained was 0.000, which is less than 0.05. This indicates that the ethanol extracts of Katang-katang leaves and flowers as well as the positive control group used in the experiment, have an effect on the growth of *P. aeruginosa*. Following the ANOVA analysis, a post-hoc test, namely the Duncan test, was conducted. The Duncan test is performed to determine which concentration groups have similar or dissimilar mean values by assigning notations to each concentration group (Dahlan, 2014).

CONCLUSION

From the results of the conducted research, it can be concluded that the leaves and flowers of the Katang-katang plant positively contain secondary metabolite compounds such as alkaloids, flavonoids, saponins, tannins, and steroids. The antibacterial effectiveness of ethanol extracts from Katang-katang leaves against *P. aeruginosa* falls into the moderate category at a concentration of 50%, with an inhibition zone diameter of 6.30 mm, and at a concentration of 75%, resulting in an inhibition zone diameter of 8.13 mm. Meanwhile, the 96% ethanol extract from Katang-katang flowers contains flavonoids, saponins, tannins, and steroids. The most effective concentration of the 96% ethanol extract from Katang-katang flowers in inhibiting the growth of the pathogenic bacteria *P. aeruginosa* is at a concentration of 50%, with an inhibition zone diameter of 4.50 mm, categorized as weak, and at a concentration of 75%, resulting in an inhibition zone diameter of 6.4 mm, categorized as moderate. The

leaves and flowers of the Katang-katang plant exhibit antibacterial potential against *P. aeruginosa*, which could be further explored for future medicinal purposes.

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