Antibacterial activity of Indonesian Bidara Upas Tuber (Merremia Mammosa L.) against pathogen bacteria

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Antibacterial activity of Indonesian Bidara Upas Tuber (*Merremia Mammosa L.*) against pathogen bacteria

Actividad antibacteriana del tubérculo de Bidara Upas de Indonesia (*Merremia Mammosa L.*) contra bacterias patógenas

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SUMMARY

Introduction: *Infectious diseases are still a problem in developing and tropical countries such as Indonesia. This study aims to determine whether the antibacterial substances contained in Bidara Upas tubers affect the growth of Streptococcus sp., Pseudomonas aeruginosa, Escherichia coli Extended-spectrum β-lactamase and Methicillin resistance Staphylococcus aureus (MRSA).*
Methods: *The method used in this study was an experimental laboratory using various extract concentrations of 25 %, 50 %, 75 %, and 100 % and positive control of meropenem and chloramphenicol and negative control (DMSO 5 %). The greater the concentration of the extract of the Bidara Upas tuber, the wider the zone of inhibition. The tuber of Bidara Upas has antibacterial activity against Streptococcus sp., Pseudomonas aeruginosa, E. coli Extended-spectrum β-lactamase (ESBL), and Methicillin-Resistant Staphylococcus aureus (MRSA).*
Results: *The results of the Mann Whitney and Kruskal Wallis test showed that antibacterial activity affects E. coli ESBL and MRSA with sig. ($\alpha < 0.05$). This indicates that there is no significant difference in the use of various concentrations of extracts capable of inhibiting bacterial growth. This study concluded that the greater the concentration of the extract of the Bidara Upas tuber, the wider the zone of inhibition, and the tuber of Bidara Upas had antibacterial activity against Streptococcus sp., Pseudomonas aeruginosa, Escherichia coli ESBL, and MRSA.*
Conclusion: *Indonesian Bidara Upas tuber extract can inhibit pathogen bacteria Streptococcus sp., Pseudomonas aeruginosa, E. coli ESBL, and MRSA with strong activity based on the resulting inhibition zone. The indegenous Indonesian Bidara Upas tuber has potential as an antibacterial agent and can be developed in further research related to in vivo activity tests and activity tests on other pathogenic bacteria.*

Keywords: *Antibacterial, Bidara Upas Tuber, E. coli ESBL, MRSA, Pseudomonas aeruginosa, Streptococcus sp.*

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RESUMEN

Introducción: *Las enfermedades infecciosas siguen siendo un problema en los países en desarrollo y tropicales como Indonesia. Este estudio tiene como objetivo determinar si las sustancias antibacterianas contenidas en los tubérculos de Bidara Upas afectan*

el crecimiento de Streptococcus sp., Pseudomonas aeruginosa, Escherichia coli, β-lactamasa de espectro extendido y Staphylococcus aureus resistente a la meticilina (MRSA).

Métodos: *El método utilizado en este estudio fue experimental utilizando varias concentraciones de extracto al 25 %, 50 %, 75 % y 100 % y control positivo de meropenem y cloranfenicol y control negativo (DMSO 5 %). Cuanto mayor es la concentración del extracto del tubérculo Bidara Upas, más amplia es la zona de inhibición. El tubérculo de Bidara Upas tiene actividad antibacteriana contra Streptococcus sp., Pseudomonas aeruginosa, E. coli BLEE y Staphylococcus aureus resistente a la meticilina (MRSA).*

Resultados: *Los resultados de la prueba de Mann-Whitney y Kruskal Wallis mostraron que la actividad antibacteriana afecta a E. coli BLEE y MRSA con sig. (P < 0,05). Esto indica que no existe una diferencia significativa en el uso de varias concentraciones de extractos capaces de inhibir el crecimiento bacteriano. Este estudio concluyó que cuanto mayor era la concentración del extracto del tubérculo de Bidara Upas, más amplia era la zona de inhibición y el tubérculo de Bidara Upas tenía actividad antibacteriana contra Streptococcus sp., Pseudomonas aeruginosa, E. coli BLEE y MRSA.*

Conclusión: *El extracto de tubérculo de Bidara Upas de Indonesia puede inhibir las bacterias patógenas Streptococcus sp., Pseudomonas aeruginosa, E. coli ESBL y MRSA con una fuerte actividad basada en la zona de inhibición resultante. El tubérculo Bidara Upas autóctono de Indonesia tiene potencial como agente antibacteriano y puede desarrollarse en futuras investigaciones relacionadas con pruebas de actividad in vivo y pruebas de actividad en otras bacterias patógenas.*

Palabras clave: *Antibacteriano, Bidara Upas Tuber, E. coli ESBL, MRSA, Pseudomonas aeruginosa, Streptococcus sp.*

INTRODUCTION

Infectious diseases are the main cause of high mortality rates in developing countries. According to the World Health Organization (WHO), infection was the cause of death in 3.5 million people every year, mostly poor children and children living in low and middle-income countries (1). The COVID-19 pandemic has increased the death rate from infectious diseases in the world and Indonesia (2). One of the diseases that many Indonesian people have suffered since ancient times is an infectious disease (3-6). Infectious diseases can be caused by pathogenic microorganisms, such as *Streptococcus sp.*, *Pseudomonas aeruginosa*, *E. coli* ESBL, and Methicillin-Resistant *Staphylococcus aureus* (MRSA) (7-10).

The development of antibacterial drugs from natural ingredients is needed to reduce the occurrence of antibiotic resistance. One of them is a natural medicine that comes from plants. One of the plants that have been widely used is the Bidara Upas plant *(Merremia mammosa)*. Bidara Upas (*Merremia mammosa* (Lour)) is a medicinal plant from Indonesia that can be found in Meru Betiri National Park. This plant from the Convolvulaceae tribe can be used as an anti-inflammatory, analgesic, wound healing, treating snake bites, cancer, leprosy, syphilis, typhus, diphtheria, inflammation, and diabetes (11,12).

This plant contains various secondary metabolites that can be used as medicine (13-15). Bidara Upas contains alkaloids and tannins as antibacterial compounds (13). Research that has been carried out on Bidara Upas, among others, extracts of Bidara Upas tubers can inhibit the growth of various good pathogenic bacteria such as *Microbacterium tuberculosis*, *Salmonella typhi*, *Staphylococcus aureus* (16-18). Research on Bidara Upas tubers has been carried out by several previous researchers with a focus on different pathogens such as *Staphylococcus aureus*, *Escherichia coli*, and Brine Shrimp Lethality Test (15) used *Mycobacterium tuberculosis* (19). However, in this study, researchers used bacteria such as *Streptococcus sp.*, *Pseudomonas aeruginosa*, *E. coli* ESBL, and Methicillin-Resistant *Staphylococcus aureus* (MRSA).

Based on the above background, the researcher has conducted this study to assess the antibacterial activity contained in the Bidara Upas tuber, which functions to inhibit or prevent the growth of bacteria, and to see the potential of the Bidara Upas content in inhibiting antibacterial against the bacteria *Streptococcus sp.*, *Pseudomonas aeruginosa*, *E. coli* ESBL and Methicillin-Resistant *Staphylococcus aureus* (MRSA).

METHODS

Plant Sources and Determination

The Indonesian Bidara Upas tuber plant used in this study was obtained and determined in Batu Materia Medica, Batu, East Java. These tubers were determined based on the results of the determination of kingdom, division, class, order, family, genus, species, and the common name of Bidara Upas tubers in Batu Materia Medica.

Preparation of Fermentation Media

MHA media was prepared by dissolving 1.33 g of MHA media into 35 mL of distilled water. Also, sterilization was carried out by autoclaving for 15 minutes at 121ºC.

Sample Preparation

Bidara Upas tuber powder, as much as 800 g, was extracted with ethanol using the maceration method. Bidara Upas tuber powder was soaked in 6 liters of ethanol for three days with regular stirring. The thick extract of Bidara Upas tuber was obtained as much as 31.1 g. Bidara Upas tuber extract was tested for phytochemical screening and antibacterial activity tests. The thick extract of Bidara Upas tuber was made in 4 concentration series (100 %, 75 %, 50 %, and 25 %) using a 5 % DMSO solution. DMSO 5 % solution was prepared by dissolving 1 mL of concentrated DMSO into sterile distilled water up to 100 mL. Each concentration series was made by adding 5 % DMSO solution into several grams of thick extract of Bidara Upas tuber until the volume was 3 mL.

Inoculum Preparation

Amount of 1 dose of each pure bacterial culture was inoculated on the surface of the media so that it was slanted, then incubated in an incubator at 37ºC for 24 hours. One loop of the rejuvenated bacterial culture on MHA media was suspended in a tube containing 5 mL of NB media and incubated for 24 hours at 37ºC. The bacterial suspension was diluted using sterile 0.9 % NaCl until the turbidity was equivalent to a standard solution of 0.5 Mc. Farland (liquid culture whose turbidity is equivalent to 0.5 Mc. Farland has a population of 1×107 CFU/mL - 1×108 CFU/mL).

Antibacterial Activity Test of Ethanol Extract Using Disc Method

The antibacterial test method used in this study was the disc diffusion method (Kirby-Bauer test). Prepare a Petri dish that has been sterilized, pour 1 mL of the bacterial suspension to be inoculated, then pour 35 mL of MHA media on each Petri dish and shake it around like a figure eight and wait until it solidifies. Place the paper discs soaked in each treatment using the tip of sterile tweezers. Each Petri dish consisted of 6 paper discs, and in each plate contained each treatment group (negative control, positive control, extract concentration 100 %, extract concentration 75 %, extract concentration 50 %, and extract concentration 25 %). After the disc, the paper is embedded in the Petri dish and then closed. The mouth of the Petri dish is passed through the fire and then wrapped. Then incubated at 31-37ºC for 24 hours. Each sample's zone of inhibition (mm) was measured (20).

RESULTS

Bidara Upas is a woody vine with a stem length of 3-6 m. The stem is a bit slippery, small, and purplish red. Bulbs are in the ground, elongated round shape. The tuber skin is brownish-yellow, thick, and gummy. The tuber flesh is white (21).

Figure 1. Indoensian Bidara Upas (*Merremia mammosa L.*).

Based on Table 1, the extract of Indonesian Bidara Upas tuber (*Merremia mammosa L.*) contains alkaloids, flavonoids, saponins, tannins, and terpenoids.

Table 1

Phytochemical screening results of Bidara Upas tuber (*Merremia mammosa* L.) Extract

Screening test	Results	Information
Alkaloids	+	Mayer formed a yellow precipitate Dragendorph formed a red precipitate, Wagner formed a brown precipitate
Flavonoids	+	Change color to orange
Saponins	+	Stable foam is formed for 10 minutes
Tannins	+	Change color to dark green
Terpenoids	+	Change color to red
Steroids	-	Does not turn green

+: Indicates presence of secondary metabolites
-: Indicates the absence of secondary metabolites

Phytochemical screening in this study was conducted to determine the content of secondary metabolites in the ethanol extract of Bidara Upas tubers. This study is following research by Pereda et al. in 2009 (22) which showed that Bidara Upas tubers contain secondary metabolites of alkaloids, flavonoids, saponins, tannins, phenolics and triterpenoids.

The results obtained from the antibacterial activity test of the tuber of Bidara Upas (*Marremia mammosa*) against the bacterium *Pseudomonas aeruginosa* found that a concentration of 25 % had an average inhibition zone of 1.75 mm, which was included in the resistant category. The 50 % concentration has an average inhibition zone of 2.25 mm, including the resistance category, the 75 % concentration has an average inhibition zone of 2.5 mm, including the resistance category, and the 100 % has an average inhibition zone of 3.5 mm, which included in the category of resistance and negative control did not form an inhibition zone. While the positive control, namely the antibiotic chloramphenicol, showed an average diameter of the inhibition zone of 1.25 mm, which means this antibiotic, according to the Clinical and Laboratory Standards Institute (CLSI),

Table 2

Antibacterial activity test results

Sample	Bacteria inhibition zone *Pseudomonas aeruginosa* SD	Category	Bacteria inhibition zone *Streptococcus sp*±SD	Category	Bacteria inhibition zone *E. coli* ESBL±SD	Category	Bacteria inhibition zone MRSA±SD	Category
K(-)	0±0.00^{b}	-	0±0.00^{b}	-	0±0.00^{b}	-	0±0.00^{b}	-
K(+)	1.25±0.50^{b}	Resistant	1.75±0.95^{b}	Resistant	10±0.00^{b}	Resistant	9.5±1.00^{b}	Resistant
25 %	1.75±0.50^{a}	Resistant	1.5±0.57^{a}	Resistant	1±0.81^{a}	Resistant	1±0.00^{a}	Resistant
50 %	2.25±0.50^{a}	Resistant	2.5±0.57^{a}	Resistant	2±0.81^{b}	Resistant	4.25±2.21^{a}	Resistant
75 %	2.5±0.57^{a}	Resistant	3.75±0.95^{a}	Resistant	2.5±0.57^{a}	Resistant	4.25±0.95^{a}	Resistant
100 %	3.5±0.57^{a}	Resistant	4.75±0.05^{a}	Resistant	4.5±1.73^{b}	Resistant	6.75±2.87^{a}	Resistant

a = Significant difference
b = The difference is not significant
K- = Negative Control
K+ = Positive Control
K25 % = Concentration 25 %
K50 % = 50 % concentration
K75 % = 75 % concentration
K100 % = 100 % concentration

chloramphenicol if the diameter of the inhibition zone <12 mm is categorized as resistant so that the bacteria are resistant to the ethanol extract of the Bidara Upas tuber. For the test results of the antibacterial activity of Bidara Upas tubers (*Marremia mammosa*) against *Streptococcus sp*. bacteria, it was found that a concentration of 25 % had an average inhibition zone of 1.5 mm including the resistant category, a concentration of 50 % had an average inhibition zone of 2.5 mm which included in the resistant category, 75 % concentration had an average inhibition zone of 3.75 mm which was included in the resistant category, and 100 % concentration had an average inhibition zone of 4.75 mm which was included in the resistant category. Negative control did not form a zone of inhibition. While the positive control, namely the antibiotic chloramphenicol, showed an average diameter of the inhibition zone of 1.75 mm, which means this antibiotic is classified as Resistant.

The results obtained from the antibacterial activity test of Bidara Upas (*Marremia mammosa*) tubers against *E. coli* ESBL bacteria found that a concentration of 25 % had an average inhibition zone of 1 mm, which was included in the weak category. The 50 % concentration has an average inhibition zone of 2 mm, which is included in the resistant category, the 75 % concentration has an average inhibition zone of 2.5 mm, which is included in the resistance category, and the 100 % has an average inhibition zone of 4.5 mm which is included in the category of resistance. And negative control did not form an inhibition zone. While the positive control, namely the antibiotic meropenem showed an average diameter of the inhibition zone of 10 mm, which means this antibiotic, according to the Clinical and Laboratory Standards Institute (CLSI), meropenem if the diameter of the inhibition zone of 15 mm is categorized as resistant so that the bacteria are resistant to the ethanol extract of the Bidara Upas tuber. The test results of the antibacterial activity of Bidara Upas tubers (*Marremia mammosa*) against MRSA bacteria found that a concentration of 25 % formed an inhibition zone of 1 mm categorized as resistant. In comparison, concentrations of 50 % and 75 % formed an inhibition zone of 4.25 mm categorized as resistant. A concentration of 100 % formed an inhibition zone of 4.25 mm. the 6.75 mm inhibition zone was categorized as resistant, and the negative control did not form an inhibition zone. While the positive control, namely the antibiotic meropenem, showed an average diameter of the inhibition zone of 9.5 mm, which means this antibiotic is classified as Resistant so that MRSA bacteria are resistant to the ethanol extract of Bidara Upas tuber. It can be concluded that the higher the concentration, the wider the inhibition zone formed.

For the test results of the antibacterial activity of Bidara Upas tuber (*Marremia mammosa*) against MRSA bacteria, it was found that a concentration of 25 % formed an inhibition zone of 1 mm which was categorized as resistant, while concentrations of 50 % and 75 % formed an inhibition zone of 4.25 mm which was categorized as resistant, and a concentration of 100 % formed an inhibition zone of 4.25 mm, and a concentration of 100% formed an inhibition zone of 6.75 mm inhibition zone categorized as resistant, and the negative control did not form an inhibition zone. While the positive control, namely the antibiotic meropenem, showed an average diameter of the inhibition zone of 9.5 mm, which means this antibiotic is classified as Resistant so that MRSA bacteria are resistant to the ethanol extract of Bidara Upas tuber. It can be concluded that the higher the concentration, the wider the inhibition zone formed. While the positive control, namely the antibiotic meropenem, showed an average diameter of the inhibition zone of 9.5 mm, which means this antibiotic is classified as Resistant so that MRSA bacteria are resistant to the ethanol extract of Bidara Upas tuber. It can be concluded that the higher the concentration, the wider the inhibition zone formed.

DISCUSSION

Bidara Upas tubers were obtained. Results from phytochemical screening showed that the ethanolic extract of Bidara Upas tubers was positive for alkaloids, flavonoids, saponins, tannins, and terpenoids. While the steroid compound test showed negative results. Ethanol extract of Bidara Upas tuber (*Marremia mammosa*) can provide antibacterial activity against *Streptococcus sp., Pseudomonas*

aeruginosa, *E. coli* ESBL and MRSA at all concentrations. And this study proves that the higher the concentration of Bidara Upas tuber extract given, the larger the diameter of the inhibition zone formed around the paper disc.

This study is in accordance with research conducted in previous research stated the presence of alkaloids and flavonoids in Bidara Upas tuber extract (23) and Bidara Upas tubers contain secondary metabolites of alkaloids, flavonoids, saponins, tannins, phenolics, and triterpenoids (22).

The activity of the ethanolic extract of Bidara Upas tuber in inhibiting the growth of gram-positive bacteria *Streptococcus sp*. and MRSA was more sensitive than the gram-negative bacteria *Pseudomonas sp*. and *E. coli* ESBL. This is in accordance with the statement that the inhibition zone of gram-positive bacteria is larger than that of gram-negative bacteria (24). This indicates that the extract is more sensitive to gram-positive bacteria. In this case, *Streptococcus sp*. and MRSA were gram-positive bacteria that were more sensitive to the extract than *Pseudomonas sp*. and *E. coli* ESBL. This difference in activity is due to differences in the structure and components of the bacterial cell wall. The peptidoglycan layer on the cell wall of gram-negative bacteria is thinner, whereas, in Gram-positive bacteria, the peptidoglycan layer is thicker. In addition, the components that make up the cell wall of gram-negative bacteria are more complex because they have an additional outer membrane layer, so it is easier to penetrate the cell walls of gram-positive bacteria than gram-negative ones. In the calculation of the clear zone of the two Petri dishes, it can be seen that the clear zone of the samples in the *Streptococcus sp*. and *E. coli* ESBL cultures was wider than the positive control.

CONCLUSION

Ethanol extract of Bidara Upas tuber (*Marremia mammosa*) can provide antibacterial activity against *Pseudoemonas aeruginosa*, *Streptococcus sp*., *E. coli* ESBL, and MRSA at all concentrations characterized by the formation of a clear zone around the paper disc.

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