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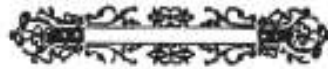
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DR. LUIS RAZETTI

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INDEX

EDITORIAL

The Intersection of the COVID-19 Pandemic and Population Health
Ferry Efendi 8867

ORIGINAL ARTICLES

Mental health management in online learning media during the COVID-19 pandemic for students at private
Ade Irma Suryani, Linardita Ferial, Kustia Anggereni, Febri Maryani, Annysa Nur Mala Sari 8869

Home contact support in prevention of transmission of tuberculosis in west Lombok based on the theory
of the health belief model
Agus Supinganto, Ni Ketut Metri, Irwan Budiana, Suharmanto Suharmanto 8875

Self-efficacy and COVID-19 prevention behaviour among adolescents: A cross-sectional study
Angraini Dwi Kurnia, Evita Rohmaniah, Nur Lailatul Masuroh, Nur Melizza, Yoyok Bakti Prasetyo,
Dewi Rury Arindari 8882

Comparison of knowledge of mothers with stunted and severely stunted toddlers before and after education
with brainstorming and audiovisual methods
Roosi Rachma Kemala, Ayling Sanjaya 8890

Sustainable services for the elderly in Malang city: A qualitative study
Cici Indah Setiowati, Yati Sri Hayati, Nurul Muslihah 8896

Correlation of self-efficacy and self-management among patients undergoing hemodialysis with
intradialytic hypertension complications
Dhian Luhuh Rohmawati, Nymas Khoriah Fadhlita, Rony Tri Hantoro 8905

The effect of maternal role identity application on cognitive development in stunting children
aged 1-3 years in a public health center
Diyah Arini, Nursalam Nursalam, Mahmudah Mahmudah, Esti Yunita Sari, Fatimah Dwi Cahyani 8913

The experience of nurses conducting nursing assessments of occupational diseases in the farm: A
phenomenological study
Eko Prasetya Widiyanto, Arista Maisyaroh, Rizeki Dwi Fibriansari 8921

Relationship between stigma and quality of life in people with pulmonary tuberculosis in East Java, Indonesia
Eppy Setiyowati, Erika Martining Wardani, Nur Ainiyah, Siti Damawiyah, Ni Njoman Juliasih 8928

Synthesis of manganese nanoparticles of Oolong tea extract by sonication method for a natural oral contrast
media on magnetic resonance cholangiopancreatography
Fatimah Fatimah, Neni Susilaningsih, Hermina Sukmaningtyus, Agus Subagio 8936

Factor analysis of the exile of women giving birth in the Forest Fenita Pimama Sari Indah, Riris Andriati, Rita Dwi Pratiwi, Nurwulan Adi Ismaya, Ika Rohmawati	S944
Milieu therapy based on local virtue influence on community acceptance on post restriction mental disorder patients at home Ganif Djuwadi, Dyah Widodo	S951
Perceived constraints and impacts of online learning experiences by Indonesian university students during COVID-19 Devia Putri Lenggogeni, Hema Malini, Dewi Eka Putri, Bunga Permata Wenny	S957
Effect of Aloe vera gel on wound healing process for diabetic foot ulcers: A pilot study Hema Malini, Yance Komela Sari, Elvi Oktarina	S967
Coping strategies of healthcare providers on social stigma due to COVID-19 using the Roy adaptation model approach Ana Zakiyah, Ika Ainur Rofi'ah, Enny Virda Yuniarti, Arief Andriyanto	S974
Experience of Kaali Community in reducing pain and swelling because of filariasis disease Irsanty Collelin, Aminuddin Aminuddin, Andi Fatmawati Syamsu, Dafrosia Darmi Manggasa	S981
Knowledge and reliance on the availability of voluntary counseling and testing (VCT) services relating to the utilization of VCT services by the Man who has Sex with Man community Isna Ovari, Silvia Nora Anggreini, Fitra Wahyuni, Rima Novita	S990
Phytochemical screening, stability test formulation and physical gel ethanol extract of <i>Jatropha</i> leaves (<i>Jatropha curcas L.</i>) as a gel compress preparation for post-ischemic stroke patients Luluk Widarti, Suprianto Suprianto, Siti Maimuna	S997
The influence of religious coping on family resilience in communicating and solving problems during the COVID-19 pandemic Yoyok Bakti Prasetyo, Faridi Faridi, Nur Lailatul Masruroh, Nur Melizza, Muhammad Hafiz Maulana	S1011
Relationship between exclusive breastfeeding and stunting among children aged 2-5 years in Indonesia Nurus Safaah, Esti Yunitasari, Ferry Efendi, Sunanita Sunanita, Suhartono Suhartono	S1019
An analysis of elderly experience using the GALASEMA application Pepin Nahariani, Shanti Rosmaharani, Fin Noviana, Ririn Probowati	S1025
The relationship between workload and nurses' performance in carrying out nursing care in inpatient at Hospital, Riau Province, Indonesia Ennimay Ennimay, Raja Fitriana Lestari, Nancy Hidayah Oktari	S1031
Psychological experience of women post total abdominal hysterectomy bilateral salpingo-oophorectomy Ramdya Akbar Tukan	S1038
Web-Based public health center management information system using Codeigneter and Ajax techniques at Public Health Canter Reno Renaldi, Yesica Devis, Lita Lita, Muhammad Dedi Widodo, Rizer Fahlepi	S1046
The effect of warm ginger on the frequency of nausea and vomiting among pregnancy women Ririn Ariyanti, Selvia Febrianti, Zulfa Rahmalia Khariani, Tantry Sulistyowati	S1054
Effectiveness of 3D Pageflip Professional electronic module to prevent urolithiasis recurrent among urolithiasis patients Riris Andriati, Tukimin bin Sansuwito, Kosheila Ramuni, Bibi Florina Abdullah	S1060
The relationship of skin integrity picture in stroke patients with the use of anti-decubitus mats at Hospital Aulia Asman, Sena Wahyu Purwanza, Ainul Mufidah, Ida Yanriatutid, Yulian Heiwer Matongka, Estelle Lilian Mua, Robi Adikari Sekeon, Meilin Anggreyni, Denny Susanto	S1068
The role of C-Reactive Protein in the level severity of hyperemesis gravidarum Ni Made Rosiyana, M. Aryadi Arsyud, Saidah Syamsuddin, Ni Luh Emilia, Estelle Lilian Mua, Robi Adikari Sekeon, Meilin Anggreyni, Suratno Kaluku, Bayu Azhar, Sena Wahyu Purwanza	S1074
The effect of progressive muscle relaxation therapy on anxiety of hemodialysis patients in Pekanbaru, Indonesia Silvia Nora Anggraini, Awaliyah Ulfah Ayudytha Ezdha, Dwi Elka Fitri, Isna Ovari, Lita Lita, Nanda Oktaviani	S1081

Learning experience about human anatomy of health students during the pandemic COVID-19: A phenomenological study Siska Mayang Sari, Rani Lisa Indra, Rian Ordila, Sekani Niriyah, Raja Fitriana Lestari, T. Abdur Rasyid, Fadli Anggara	S1087
Live experience of people with diabetes mellitus on self-management during COVID-19 in remote a rea of Indonesia Angelina Roida Eka, Lusia Henny Mariati, Maria Getrida Simon, Claudia Fariday Dewi, Kornelia Romana Iwa, Fransiska Yuniati Demang, Yohana Hepilita, Yuliana Reginaldis Rosali Krowa	S1094
Effect of the standard operating procedure Box Method to increase self-confidence and basic nursing skills of nursing students during the COVID-19 pandemic Lidwina Dewiyanti Wea, Paskaliama Hilpriska Danal, Oliva Suyen Ningsih	S1101
Risk factors of increased blood pressure among adolescents in rural areas of Indonesia Oliva Suyen Ningsih, Lidwina Dewiyanti Wea, Heribertus Handi	S1108
The correlation between working shifts and nurse's motivation in the implementation of handover in the surgical documentation room Susi Erianti, Asfeni Asfeni, Cut Siti Nurhafiza	S1114
Correlation between parental verbal violence behaviour and the aggressiveness of adolescents Sylvi Harmandillah, Dadang Kusbiantoro, Nurul Hikmatul Qowi, Trijati Puspita Lestari, Nur Hidayati, Tri Indra Aji Putra	S1119
Optimizing shift scheduling and work-life balance to improve job satisfaction among female nurses Tita Rohita, Nursalam Nursalam, Krisna Yetti, Kuntarti Kuntarti, Dedeng Nurkholik, Idyatul Hasanah	S1126
Differences in extraction methods to antidiarrheal activity in vitro and in vivo in unripe Kayu banana fruit (<i>Musa paradisiaca</i> L. Var. Kayu) Arista Wahyu Ningsih, Edo Pratama, Siti Komariyah, Diah Putri Astuti, Ivan Charles S. Klau, Dewi Rahmawati	S1133
Relationship between cholesterol levels and smoking behavior among active smokers Farida Anwari, Martina Kurnia Rohma, Acivrida Mega Charisma, Iif Hanifa Nurrosyidah, Arif Rahman Nurdianto, Galih Satrio Putra, Dimas Dimas	S1147
Antibacterial activity of Indonesian Bidara Upas Tuber (<i>Merremia Mammosa</i> L.) against pathogen bacteria Iif Hanifa Nurrosyidah, Ervina Oktalia Eka Saputri, Sayyidah Mufidatunnisa, Farida Anwari, Arista Wahyu Ningsih	S1153
Immunomodulator activity test of ethanol extract of Sappan Wood (<i>Caesalpinia Sappan</i> L.) in mice (<i>Mus Musculus</i>) infected by <i>Staphylococcus aureus</i> Martina Kurnia Rohmah, Farida Anwari, Arif Rahman Nurdianto, Elisa Dwi Febrianti, Juvita Anggraini, Arista Wahyu Ningsih	S1160
Family support and peer support related to the physical activity of the prospective bride and groom Wiwit Kurniawati, Yati Afyanti, Asa Akmelia, Neni Fidya Santi	S1169
The effectiveness of mindfulness-based stress reduction on parental stress during COVID-19 pandemic: A randomized controlled trial Yurike Septianingrum, Febta Liffa Arnawi, Chilyatiz Zahroh, Siti Nur Hasina, Andikawati Fitriyani, Nety Mawarda Hatmanti, Erika Martining Wardani, Siti Damawiyah	S1175
REVIEW ARTICLES	
Impact of diabetes self-management education in middle-aged patients with type 2 diabetes mellitus: A systematic review Emilia Ermingwati Akotit, Ferry Efendi, Yulis Setiya Dewi	S1183
Factors of medication adherence among adult patients with tuberculosis: A literature review Farida Nur Qomariyah, Lukawee Piyabanditkul, Donwiwat Saensorn	S1196
Relationship quality of nursing work life and burnout among nurses: A systematic review Tita Rohita, Nursalam Nursalam, Muhammad Hadi, Ferry Efendi, Dedeng Nurkholik, Idyatul Hasanah	S1205
The effectiveness and usability of electronic partograph for obstetric care: A systematic review Widya Maya Ningrum, Rahayu Bodi Utami, Yeny Ristaning Belawati, Tita Rohita, Karniati Devi Purnamasari	S1215

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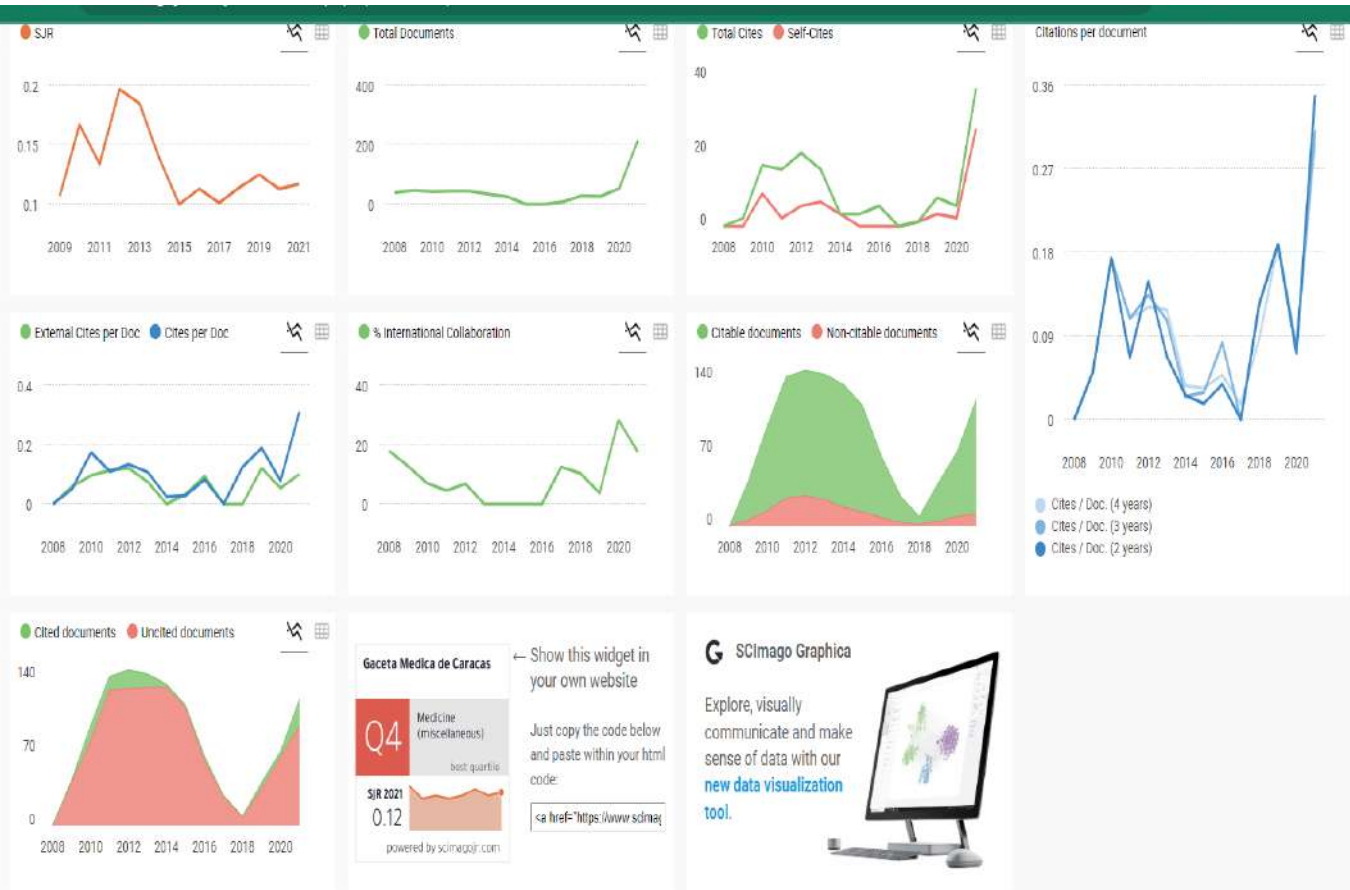
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Antibacterial activity of Indonesian Bidara Upas Tuber (*Merremia Mammosa* L.) against pathogen bacteria

by lif Hanifa Nurrosyidah ,

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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

Lif Hanifa Nurrosyidah^{1a*}, Ervina Oktalia Eka Saputri^{2a}, Sayyidah Mufidatunnisa^{3a},
Farida Anwari^{4a}, Arista Wahyu Ningsih^{5a}

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 indigenous 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 metilicina (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 metilicina (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, along with 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 121°C.

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 37°C 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 37°C. 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 1×10^7 CFU/mL - 1×10^8 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 31-37°C 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 (*Merremiamamosa*L.).

ANTIBACTERIAL ACTIVITY OF INDONESIAN BIDARA UPAS TUBER

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

10
 +: 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 <i>Pseudomonas aeruginosa</i> SD	Category	Bacteria inhibition zone <i>Streptococcus sp</i> ±SD	Category	Bacteria inhibition zone <i>E. coli</i> 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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PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

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

Iif Hanifa Nurrosyidah^{1a*}, Ervina Oktalia Eka Saputri^{2a}, Sayyidah Mufidatunnisa^{3a},
Farida Anwari^{4a}, Arista Wahyu Ningsih^{5a}

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 indigenous 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 *Mycobacterium 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 121°C.

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 37°C 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 37°C. 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 1×10^7 CFU/mL - 1×10^8 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 31-37°C 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.).

ANTIBACTERIAL ACTIVITY OF INDONESIAN BIDARA UPAS TUBER

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	Category	Bacteria inhibition zone	Category	Bacteria inhibition zone	Category	Bacteria inhibition zone	Category
	<i>Pseudomonas aeruginosa</i> SD		<i>Streptococcus sp</i> ±SD		<i>E. coli</i> ESBL±SD		MRSA±SD	
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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