ANTIBACTERIAL EFFECTS OF *ziziphus mauritiana* (lam) LEAF EXTRACT AGAINST *Vibrio cholerae*

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Abstract

Introduction: In Indonesia, treatment using leaves as traditional medicine is still firmly integrated with the community to overcome various health problems experienced, one of which is treatment using Bidara leaves (*Ziziphus mauritiana*). Nowadays, in addition to chemical drug treatment, many researchers have also developed herbal medicine. Bidara leaf is a potential source of antibacterial agent. **Methods**: The method used well diffusion. Antibacterial activity has been shown in the inhibition zone. Total samples were 25 samples divided into five groups; groups treated with Purple Eggplant peel extract 25 %, 50 %, 75%, positive control (erythromycin), and negative control. Data were analyzed with the Kruskal-Wallis test. **Results**: Bidara leaf extract inhibited V. cholerae growth characterized by an inhibition zone that formed. Means of inhibition zone diameter were statistically significantly different between groups (p-value = 0.001. **Conclusions**: Bidara leaf extract with 25%, 50%, and 75% concentrations has the same ability as nystatin in inhibiting the growth of V. cholera.

Keywords*: Ziziphus mauritiana*; Bidara leaf extract; *Vibrio cholerae*; Antibacterial

Abstrak

Pendahuluan: Di Indonesia, pengobatan dengan menggunakan tanaman herbal sebagai obat tradisional masih melekat erat pada masyarakat untuk mengatasi berbagai masalah kesehatan yang dialami, salah satunya adalah pengobatan dengan menggunakan daun bidara (*Ziziphus mauritiana*). Saat ini selain pengobatan dengan menggunakan obat-obatan kimia, banyak juga peneliti yang mengembangkan tentang pengobatan herbal. Daun bidara berpotensi sebagai sumber antibakteri. **Metode:** Metode yang digunakan adalah difusi sumur. Aktivitas antibakteri ditunjukkan dari diameter zona hambat. Jumlah sampel sebanyak 25 sampel yang dibagi menjadi lima kelompok; kelompok perlakuan ekstrak kulit terong ungu 25%, 50%, 75%, kontrol positif (eritromisin) dan kontrol negatif. Data dianalisis dengan uji Kruskal-Wallis. **Hasil:** Ekstrak daun bidara menghambat pertumbuhan V. cholerae yang ditandai dengan terbentuknya zona hambat. Rerata diameter zona hambat berbeda signifikan antar kelompok (p-value = 0,001). **Kesimpulan:** Ekstrak daun bidara dengan konsentrasi 25%, 50%, dan 75% memiliki kemampuan yang sama dengan nistatin dalam menghambat pertumbuhan V. *cholerae*

Kata Kunci: *Ziziphus mauritiana*; Daun bidara; *Vibrio cholerae*; Antibakteri

INTRODUCTION

Cholera is a disease caused by the toxigenic serogroup of V. *cholerae*, which can cause dehydration and rapid death. Cholera is closely related to poverty, poor sanitation, and a lack of clean drinking water. Thus, the number of cholera cases in Africa and southern Asia, is approximately for about 99% of cases worldwide (1). V. *cholerae* infection causes symptoms such as excessive watery diarrhea without fever or abdominal pain, and early in the duration of the disease often causes nausea and vomiting(2). In Indonesia, treatment using leaves as traditional medicine is still firmly integrated with the community to overcome various health problems experienced, one of which is treatment using Bidara leaves (*Ziziphus mauritiana*).

A previous study reported that the ethanolic extract of the leaves of *Ziziphus mauritiana* has phytochemical compounds including tannins, saponins, and flavonoids

which act as antioxidants and antibacterial. The mechanism of saponins as an antibacterial is to reduce surface tension which causes increased permeability of cell damage, so intracellular bacterial compounds will come out. These compounds diffuse through the outer membrane and cell wall, then bind to the cytoplasmic membrane and reduce stability. This causes the cytoplasm to leak out of the cell leading to cell death. Saponins work as antimicrobials because saponin compounds can perform an inhibitory mechanism by forming complex compounds with cell membranes through hydrogen bonds to destroy the permeability properties of bacterial cell walls and cause bacterial cell death. It was proved that the ethanolic extract of *Ziziphus mauritiana* leaves against S. *aureus* with optimal concentration at 40%, which showed antibacterial activity was 1.68 ± 0.03 mm (3). Another study using methanol extract *Ziziphus mauritiana* from its root could induce inhibition zone towards *E. coli* and *Klebsiella pneumoniae* (4). Isolated alkaloid from *Ziziphus mauritiana was reported had antimicrobial* activity against *M. tuberculosis* (5). This findings provide futher evidence that Bidara is a potential herb as antibacterial. However, no previous study has demonstrated the role of *Ziziphus mauritiana*, especially its leaves, against V. *cholerae*.

The side effects of synthetic/chemical drugs have made people turn to herbal medicine because they have minimal side effects and maximum therapeutic effects. Nowadays, in addition to chemical drug treatment, many researchers have also developed herbal medicine. Bidara leaf is a potential source of antibacterial agents. Meanwhile, according to a previous report from a systematic review, antibiotic therapy with antibiotics such as Erythromycin could be effective for treating cholera (6). This study aims to determine the effectiveness of Bidara leaves extract compared with Erythromycin against the growth inhibition of V. *cholerae in vitro*.

MATERIAL AND METHODS

This research is true experimental research with the treatment of giving ethanol extract of Bidara leaves (*Ziziphus mauritiana*) to V. *cholerae*. Study aim is to test the effectiveness of the inhibition of this extract on the growth of V. *cholerae* using the method disc diffusion, using extract concentrations of 25%, 50%, and 75%. This research was conducted from December 2019 - January 2020 at the Laboratory of Microbiology, Faculty of Pharmacy, Universitas Hasanuddin, Indonesia.

Bidara Leaf (*Ziziphus mauritiana)*

Bidara, also known as *Ziziphus mauritiana* is a tropical fruit tree belonging to the family Rhamnaceae **Figure 1**. This species is believed to have originated in the Indo-Malaysian region of Southeast Asia. However, it is widely naturalized worldwide from South Africa to subcontinents such as India, China, Indomalaya, Australia, and the Pacific Islands. In Indonesia, this plant grows wild throughout Java and Bali at altitudes below 400 meters above sea level. The plant endures extreme temperatures and thrives in moderately dry conditions with between 6 and 88.5 inches (15-225 cm) of annual rainfall. Leaves variable, alternate, in 2 rows, oblong-elliptic, 2.5-6 x 1.5-5 cm, with tip rounded or slightly notched base; finely wavy-toothed on edges, shiny green and hairless above; dense, whitish, soft hairs underneath (7,8).

Figure 1. (a) Plant of *Ziziphus mauritiana* (b) Leaves and flowers, (c) fruit of *Ziziphus mauritiana* (8)

Procedure

Extracts Preparation

Samples were taken from Bidara leaves (*Ziziphus mauritiana*) in various areas such as Makassar, Palangga Gowa, Soppeng, and the Polewalimandar region in South Sulawesi. Bidara leaves obtained were cleaned and washed with clean running water. Then cut into small pieces and stored for ± three days to prevent damage to the bioactive compounds that are sensitive to direct sunlight until the Bidara leaves are ready to be extracted. The extract of Bidara leaves was put into two jars, then soaked with 96% ± 2.5 L ethanol solvent in each jar, then covered with aluminium foil and left for three days while stirring occasionally. Then evaporated using a rotary evaporator to obtain a thick extract of Bidara leaves.

Preparation of V. *Cholerae* Medium

V. *Cholerae* was inoculated on Muller-Hinton Agar medium in a petri dish then incubated at 37ºC for one day in an incubator.

Determination of antibacterial activity using the Disc Diffusion method

Sterile paper discs and Erythromycin discs 500 units/mg were placed with sterile tweezers. Then the extract solution Bidara leaves with a concentration of 25%, 50%, and 75% was dropped on the disc paper as much as 25 µl using. After being left for 1 hour for the solution to absorb into the disc paper, the V. *Cholerae* medium was tightly closed and incubated at 37ºC for 24 hours. Observations were made on the growth medium by measuring the zone of inhibition formed around the disc paper using a ruler.

Phytochemical Test of Extract

The phytochemical test of qualitative analysis was conducted. Test the active ingredient flavonoid content, with 0.1 grams of the Bidara leaves extract with 10 ml ethanol, then add a few drops of concentrated HCl and 0.05 grams of magnesium metal. The presence of flavonoids is indicated by the formation of yellow in 3 minutes. For the presence of tannin active ingredients, 1 ml of Bidara leaves extract is reacted with a solution of $FeCl_3$ 10%, if there is a change in color to dark blue or greenish-black, shows the presence of tannin. Test the content of the active ingredient Alkaloid, extract of the Bidara leaves extract is reacted with 1 ml of Wagner's reagent, and a brown precipitate is formed. For the presence of saponin, 1 gram of Bidara leaves eggplant peel extract is soaked and boiled for 2-3 minutes over a bunsen fire. Then, shake firmly. Positive if the stable foam is formed (9)

Statistical Analysis

The inhibition zone diameter was presented as the mean and standard deviation. The data was not normally distributed with the Shapiro-Wilk test. Kruskal-Wallis H test was used to determine the mean differences of diameter inhibition zone among groups. SPSS for Mac software was used for data analysis.

RESULTS

Phytochemicals Screening

A phytochemical test of the Bidara leaf extract was done to identify the content of the active compound in the extract. Phytochemical screening carried out in this study was screening for flavonoids, alkaloids, tannins, and saponins. The data obtained can be seen in **Table 1**.

Table 1. Phytochemical Screening Results of Bidara leaf extract (*Ziziphus mauritiana*)

Phytochemical Test	Results	Interpretation
Flavonoid	Yellow colour in 3 minutes	Positive
Alkaloid	No brown sediment	Positive
Tannin	Blackish green	Positive
Saponin	Stable emulsion	Positive

It has been shown that the active substances contained in Bidara leaf extract based on phytochemical test qualitative analysis are positive containing Flavonoids, Alkanoid, Saponins, and Tanins (Table 1).

Antibacterial activity of Bidara leaf extract

Bidara leaf extract test results against V. *Cholerae* showed antibacterial activity. This is evidenced by the formation of an inhibition zone around the disc paper that has been given the extract.

Kruskal-Wallis test was conducted to determine if there were differences in the diameter of the inhibition zone between groups. Means of the diameter of the inhibition zone were significantly different between groups. Means of diameter of inhibition zone were statistically significant different between groups (p-value = 0.001).

Table 2. The results of the diffusion test for the antibacterial activity of Bidara Leaf extract against V. *Cholerae*

Groups	Diameter of Inhibition Zone (mm) in petri dish						P – value*
	I	II	III	IV	V	Means ± SD	
Positive control (Eritromisin)	30.2	26.7	20.2	27.9	21.4	25.3 ± 4.3	0.001
Negative control	6.0	6.0	6.0	6.0	6.0	6.0 ± 0.0	
Biadara leaf extract 25 %	9.2	7.2	8.3	6.7	8.2	7.9 ± 0.9	
Bidara leaf extract 50 %	8.1	6.5	10.2	9.3	7.5	8.3 ± 1.4	
Bidara leaf peel extract 75 %	18.4	16.2	17.3	14.8	13.5	16.0 ± 1.9	

*Data expressed as mean ± SD and p-value with Kruskal-Wallis test. Positive control (erythromycin), negative control (DMSO)

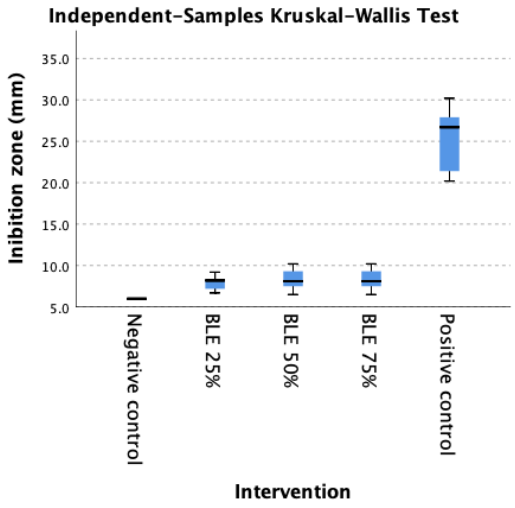

Figure 2. Boxplot average means inhibition zone among groups

DISCUSSION

Traditionally, for the last several decades, Bidara (*Ziziphus mauritiana*) has been widely used as a medicinal remedy to treat various types of diseases. The leaves were reported to have an anthelmintic, antipyretic, and effect in remedying stomatitis, asthma, typhoid fever (10). Our results showed that the Bidara leaf extract inhibited V. *Cholerae* growth characterized by an inhibition zone that formed (**Table 2**, **Figure 2**). This clearly demonstrated the consistency with the previous study showing the ability of Bidara extract as antidiarrheal. The plant could reduce pendular movement of jejunum and contraction of ileum by inhibition of acetylcholine in rats (11).

The ability of Bidara leaf extract to inhibit the growth of V. *Cholerae* is due to the active compound in this plant. These chemical compounds include flavonoids, alkaloid tannins, and saponins (**Table 1**). These findings are supported up by evidence from previous studies. Other studies reported that triperpenoids, such as saponins, colubrinic acid, alphitolic acid, maslinic acid, oleanolic acid (12).

The leaves of *Ziziphus mauritiana* were rich of flavonoids (13). In the literature, there is broad theoretical and experimental evidence to support the role of flavonoids as antibacterial. Flavonoid mechanisms for inhibiting the growth of V. *Cholerae* by causing cell-membrane damage, inhibition on various synthase involving the nucleic acid synthase involving the bacterial respiratory chain, or the cell envelope synthesis (14). Another study reported that flavonoids can modulate the Toll-like receptor pathway in Salmonella-induced mice This condition leads to the activation of proinflammatory cytokines such as Interleukin-6 (15). Another research found that flavonoids have antibacterial activity against *E. coli* through inhibition of DNA gyrase, inhibiting the nucleic acid synthesis and cytoplasmic membrane function (16).

Saponin was also reported found in the Bidara leaf. The mechanism of action of saponins as an antibacterial is protein denaturation. Because the surface-active substances of saponins are similar to detergents, saponins can be used as antibacterial where the surface tension of the bacterial cell wall will be lowered, and the permeability of the bacterial membrane is damaged (17).

CONCLUSIONS

Bidara leaf extract contains active substances flavonoids, alkaloid, tannins, and saponins. Bidara leaf extract with 25%, 50%, and 75% concentrations has the same ability as erythromycin in inhibiting the growth of V. *Cholerae*.

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