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Antibacterial activity of ethanolic extract of *Averrhoa bilimbi* L. fruit against *Salmonella typhi*

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ABSTRACT

Averrhoa bilimbi L. fruit has been used in Bali traditional medicine to treat typhoid fever. The fruit contains flavonoids and triterpenoids which are considered to have antibacterial activity. This study aimed to investigate antibacterial activity of ethanolic extract of *A. bilimbi* L. fruit. The ethanolic extract was prepared by maceration and antibacterial activity was determined by the disc diffusion method against *Salmonella typhi* cultured on Mueller hinton agar (MHA). Ciprofloxacin was used as positive control and sterile distilled water as negative control. The results showed that the ethanolic extract of *A. bilimbi* L. fruit at various concentrations of 25; 50; 75 and 100% have an inhibition zone diameter of 0 ± 0; 13.000 ± 1.414; 18.750 ± 1.500 and 20.250 ± 1.707 mm, respectively. In addition, ciprofloxacin at concentration of 1 mg/L has an inhibition zone diameter of 34.250 ± 1.892 mm. In conclusion, the ethanolic extract of *A. bilimbi* L. fruit has strong antibacterial activity against *S. typhi.*

ABSTRAK

Buah belimbing wuluh (*A. bilimbi* L.) telah digunakan sebagai obat tradisional Bali untuk demam tifoid. Buahnya mengandung flavonoid dan triterpenoid yang dipercaya mempunyai aktivitas antibakteri. Penelitian ini bertujuan untuk mengkaji aktivitas antibakteri ekstrak etanol buah *A. bilimbi* L. Ekstrak etanol dibuat dengan cara maserasi dan aktivitas antibakteri ditentukan dengan metode difusi cakram terhadah S. typhi yang ditumbuhkan dengan agar hinton Mueller (MHA). Siprofloksasin digunakan sebagai kontrol positif dan air destilasi steril sebagai kontrol negatif. Hasil penelitian menunjukkan ekstrak etanol *A. bilimbi* L. pada berbagai konsentrasi yaitu 25; 50; 75 dan 100% berturut-turut mempunyai diameter zona hambatan sebesar 0 ± 0; 13,000 ± 1,414; 18,750 ± 1,500 dan 20,250 ± 1,707 mm. Ditambahkan, siprofloksasin sebagai kontrol positif pada konsentrasi 1 mg/L mempunyai diameter zona hambatan sebesar 34,250 ± 1,892 mm. Dapat disimpulkan, ektrak etanol buah *A. bilimbi* L. mempunyai aktivitas antibakteri kuat melawan *S. typhi.*

INTRODUCTION

Typhoid fever is a bacterial infection caused by *Salmonella typhi* which can spread throughout the body, affecting many organs and resulting in systemic infections. Without appropriate treatment, this infection can cause serious complications and can be life-treatening.[1] Typhoid fever remains a public health problem in developing countries especially among the people who live in poor sanitation and lack of clean water supply.[2] It was estimated, typhoid fever caused 13.5 million illnesses worldwide in 2010 with the highest incidence was recorded in Africa and Asia.[3,4] In Indonesia, the incidence rate of typhoid at 148.7 per 100000-years in the age group 2–4 years old, 180.3 in the age group 5–15 years old and 51.2 in those over years of age.[5]

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Since 2010, the typhoid control program has been implemented in Indonesia, however the implementation has not been optimal, yet, due to some challenges such as limited the budged and the emergence of bacterial resistance.[6] Some antibiotics have been recommended to treat of the typhoid fever such as ceftriaxone, ciprofloxacin, azithromycin, and cephalosporins.[7] However, multidrug-resistant *S. typhi* (MDR-ST) was reported in some endemic areas of typhoid fever in the world.[2,7,8] Since these issues with the MDR-ST have been reported, explorations of medicinal plants to discover and develop a new effective antibiotic a gainst *S. typhi* were conducted.

Averrhoa bilimbi L. (Oxalidaceae) is one of medicinal plants that widely used traditionally by Balinese community to treat various illness including typhoid fever. The use of *A. bilimbi* L. as traditional medicine to reduce fever was documented in *lontar* manuscript called Usada Taru Pramana.[9] *In vitro* study reported that *A. bilimbi* L. fermented extract in combination with bacteriocin has activity against multidrug-resistant *Escherichia coli* (MDR *E. coli*).[10] Furthermore, the ethanolic extract of *A. bilimbi* L. has activity against all MDR bacteria.[11] The ethanolic extract of *A. bilimbi* L. also had antioxidant and antibacterial activities against *Salmonella sp*., *E. coli*, and *Staphylococcus aureus* bacteria.[12] This study aimed to evaluate antibacterial activity of ethanolic extract of *A. bilimbi* L. fruit against *S. typhi*. The results of this study could be used as scientific evidence to support the traditionally use of *A. bilimbi* L. fruit to treat typhoid fever.

MATERIALS AND METHODS

Preparation of ethanolic extract

The ethanolic extract of *A. bilimbi* L. fruit was prepared by maceration. The *A. bilimbi* L. fruit was washed by tap water and air dried. The fruit then chopped into thin slices and air dried for three days. The dried fruit slices were powdered by using a blender. 100 g of dried powder was macerated by using 60 mL of 96% ethanol for three days with stirring conducted once a day. Macerate was separated by filtered. Remaceration with 40 mL of 96% ethanol was then conducted for two days. All macerates were collected and evaporated using a rotary evaporator to obtain a thick extract.

Antibactarial activity testing

Antibacterial activity of the ethanolic extract of *A. bilimbi* L fruit against *S. typhi* was conducted using the disc diffusion method at the Biopesticide Laboratory and Clinical Microbiology Laboratory, Faculty of Medicine, Universitas Udayana, Bali. *Salmonella typhi* was obtained from the collection of the Department of Clinical Microbiology, Faculty of Medicine. The inocula were prepared by dissolving 1 to 3 *S. typhi* colonies from 24-h culture of Nutrient agar in sterile saline (0.9% NaCl). The turbidity was then adjusted to match 0.5 standard McFarland turbidity (10^8 CFU/mL). Muller Hinton Agar media (20 mL) was poured into each of the 90 mm Petri dishes. The *S. typhi* colonies suspension (100 μL) was uniformly spread on the MHA medium using a Pasteur pipette and allowed to dry. Six mm diameter wells were bored on the MHA medium using a sterile borer. Fifty μL of each of the ethanolic extract solutions in various concentrations (25; 50; 75 and 100%) were carefully added into designated wells on the surface of the MHA media containing the *S. typhi*. Fifty μL of sterile distilled water and 50 μL of ciprofloxacin (1 mg/L) were used as negative and positive control, respectively. The Petri dishes were kept for 30 min and then incubated at 37±1°C for 24h. The Petri dishes were observed for *S. typhi* growth and the inhibition zones diameters (mm) was measured using a ruler. The antibacterial activity test was

conducted in four repetitions in different experiment. This study was approved by the Research Ethic Committee, Faculty of Medicine, Universitas Udayana/Sanglah Central General Hospital, Denpasar, Bali (ref. no. 155/UN.14.2.2.VIII.6/2018).

Data analysis

The antibacterial activity is expressed by the inhibition zone diameter. The antibacterial is considered weak; medium; strong or very strong if the inhibition zone diameters are 5; 5-10; 10-20 and > 20 nm, respectively. The data of inhibition zone diameter were presented as mean ± standard deviation (SD) and analyzed statistically using the Kruskal Wallis test and followed by the U-Mann Whitney test. The Statistical Package for the Social Sciences (SPSS) Inc. version 21.0 software was used. A p value < 0.05 (a 95% confidence level) was considered significant.

RESULT

FIGURE 1 shows inhibition zone of the ethanolic extract of *A. bilimbi* L. fruit at various concentrations. No inhibition was observed at the negative control and at concentration of 25% (T1) indicating no antibacterial activity. In addition, the highest inhibition was observed at the positive control (ciprofloxacin), whereas for ethanolic extract of *A. bilimbi* L., the highest inhibition was observed at concentration of 100% (T4).

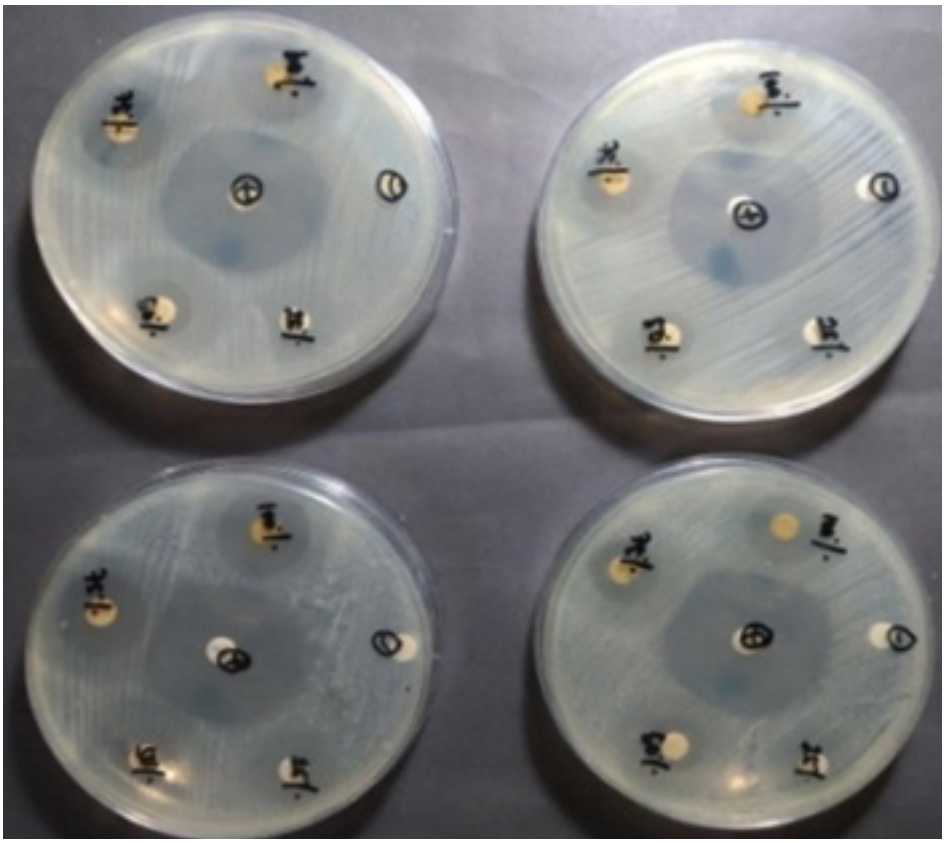

FIGURE 1. *Salmonella typhi* bacteria have been inoculated on MHA media with the addition of discs according to the concentration of the treatment. C (-) = negative control; C (+) = positive control (ciprofloxacin); T1 = *A. bilimbi* L. concentrations of 25%; T2 = *A. bilimbi* L. concentrations of 50%; T3 = *A. bilimbi* L. concentrations of 75%; and T4 = *A. bilimbi* L. concentrations of 100%.

TABEL 1 shows inhibition zone diameter of the ethanolic extract of *A. bilimbi* L. fruit at various concentrations. The highest inhibition zone diameter was observed at the ciprofloxacin as positive control, whereas for ethanolic extract of *A. bilimbi* L. fruit, the highest inhibition zone diameter was observed at concentration of 100% (T4). However, no significantly different in the inhibition zone diameter between T4 (20.250 ± 1.707 mm) and T3 (18.750 ± 1.500 mm) was observed (p> 0.05). Based on the criteria, the ethanolic extract of *A. bilimbi* L. fruit at concentration of 50 and 75% was categorized to have strong antibacterial activity, whereas at concentration of 100% was categorized to have very strong antibacterial activity.

TABLE 1. Antibacterial activity of the ethanolic extract of *A. bilimbi* L. fruit against *S. typhi*

Group	Replication				Inhibition zone (mean ± SD mm)	Activity
	1	2	3	4		
C (+)	33	37	34	33	34.250 ± 1.892[a]	Very strong
C (-)	0	0	0	0	0 ± 0[b]	-
T1	0	0	0	0	0 ± 0[b]	-
T2	15	12	12	13	13.000 ± 1.414[c]	Strong
T3	20	17	18	20	18.750 ± 1.500[d]	Strong
T4	21	18	20	22	20.250 ± 1.707[d]	Very strong

Note: *= there is a significant difference (p<0.05); different letter = there is a significant difference (p<0.05); same letter = no significant difference (p>0.05); C (-) = negative control; C (+) = positive control (ciprofloxacin); T1 = *A. bilimbi* L. concentrations of 25%; T2 = *A. bilimbi* L. concentrations of 50%; T3 = *A. bilimbi* L. concentrations of 75%; and T4 = *A. bilimbi* L. concentrations of 100%.

DISCUSSION

Averrhoa bilimbi L. (Oxalidaceae) is a commonly cultivated and planted plant species in Indonesia. The fruit of *A. bilimbi* L. has a highly sour flavor due to the high concentration of oxalic acid, which exceeds 70%.[13] Additionally, the fruit of *A. bilimbi* L. includes bioactive substances that may be utilized to make herbal medication (*Usada*), including saponins, flavonoids, triterpenoids, tannins, and peroxides, formic acid, glucose, calcium oxalate, and sulfur.[14] The fruit's bioactive chemicals were shown to inhibit the growth of disease-causing bacteria, including *S. typhi*, which causes typhoid fever.

The result of this study showed that the ethanolic extract of *A. bilimbi* L. fruit at a 25% concentration does not has an antibacterial activity against *S. typhi*. It may due to the low concentration of bioactive compounds at 25% concentration. This is consistent with previous study, which demonstrated that antibacterial activity of *A. bilimbi* L. depends on the appropriate concentration of its bioactive compounds.[15] Furthermore, the antibacterial activity of the ethanolic extract of *A. bilimbi* L. fruit at concentrations between 50-100% showed dose-dependent antibacterial activity against *S. typhi.*

The extract of *A. bilimbi* L. was reported to have bioactive compounds with various biological activities including antibacterial, antioxidant, antimicrobial, antiaging, and antioxidative.[16,17] Furthermore, the antibacterial activity of *A. bilimbi* L. fruit is due to its bioactive compounds such as alkaloids, saponins, flavonoids, and tannins.[18] Antibacterial activity of flavonoids through the interaction between alcohol in flavonoids with lipid compounds and amino acids of bacteria that causes the bacterial cell damage.[19-21]

The flavonoids are also reported inhibit *S. typhi* growth by forming complex with extracellular and dissolved protein which causes the bacterial cell membrane damage.[16] Whereas tannin worked by lysing cell walls, inactivating enzymes, and genetic functions of *S. typhi* bacteria.[22]

The antibacterial activity of *A. bilimbi* L. has been reported in the previous studies. Cold powder preparation containing ethanolic extract of *A. bilimbi* L. leaf actives against *Propionibacterium acnes*.[23] Gel preparation containing ethyl acetated extract of *A. bilimbi* L. fruit also actives against *P. acnes* and *S. aureus*.[24] Moreover, the essential oil isolated from *A. bilimbi* L. fruit has an antibacterial, antioxidant, and antibiofilm activities potential.[25] Another study reported that the active compound identified in ethanolic extract of *A. bilimbi* L. fruit which active against *E. coli* and *S. aureus* is terpenoids.[26]

CONCLUSION

The ethanolic extract of *A. bilimbi* L. fruit has strong antibacterial activity in concentration-dependent manner against *S. typhi*. Further study is recommended to isolate antibacterial active compounds which especially active against *S. typhi.*

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