A REVIEW OF PHYTOCHEMICAL PROPERTIES, ANTIBACTERIAL ACTIVITY, AND TOXICITY STUDY OF Averrhoa bilimbi LEAVES AND FRUIT

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ABSTRACT

Background: Averrhoa bilimbi or starfruit is a plant that is commonly found in Indonesia. A. bilimbi is widely used as herbal therapy against various diseases, especially those caused by bacteria. Objective: This article is intended to provide information regarding the chemical content of A. bilimbi, its antibacterial potential, and its safety level. Methods: The preparation of this article used a systematic literature review method of articles that have been published in both national and international scientific journal. Result: Leave and fruit of A. bilimbi is known to contain several classes of primary and secondary metabolites, namely carbohydrates, proteins, fats, flavonoids, alkaloids, glycosides, saponins, tannins, triterpenoids, and steroids. A. bilimbi also contains several macro and micro minerals including calcium (Ca), magnesium (Mg), and zinc (Zn). A. bilimbi could inhibit the growth of various Gram positive and negative bacteria which have been proven through in vitro testing with diffusion and dilution methods. Based on the acute toxicity study, it was found that 80% ethanol extract of A. bilimbi fruit had an LD₅₀ value of above 5,000 mg/kg BW so that it is practically non-toxic. Based on sub-chronic toxicity of A. bilimbi up to a dose of 500 mg/kg BW there was no death in the test animals and shows a toxic effect. Conclusion: The leaves and fruit of A. bilimbi have antibacterial activities. The compounds contained in A. bilimbi are thought to have a role in its antibacterial activity. Based on several toxicity studies, the ethanol extract of A. bilimbi has been proven safe to use.

Keywords: Averrhoa bilimbi, Starfruit, Antibacterial, Chemical Properties, Toxicity.

INTRODUCTION

Averrhoa bilimbi or more commonly known as starfruit is a plant of the Oxalidaceae family that originates from Southeast Asia and is widely cultivated or found throughout Sri Lanka, Indonesia, the Philippines, Bangladesh, Malaysia, Myanmar, and Zanzibar[¹,²]. However, in Indonesia, the use of A. bilimbi is still not optimal considering its various properties and abundant existence. Based on its morphology, A. bilimbi leaves have alternate arrangement, pinnate, and ovate to oblong shape. Fruit is obovoid or ellipsoid shape, measuring 5.5-7 cm long[³]. Traditionally A. bilimbi is used for the treatment of thrush, cough, acne, tinea versicolor, and digestive dysfunction[⁴]. Based on its empirical benefits, there have been many studies related to the antibacterial effects of A. bilimbi.

Research on the antibacterial effect of herbal ingredients has been developed due to the increasing incidence of bacterial resistance. Bacterial resistance to certain
antibiotics is a global problem faced by both developed and developing countries due to the irrational use of antibiotics so that many efforts have been made to overcome this problem. One of the efforts made is the discovery of new drugs from herbal plants that have the potential to act as antibiotics [5,6].

Based on the research, the leaves and fruit of A. bilimbi have been shown to have antibacterial activities. The activities cannot be separated from a chemical compound that plays an active role as an antibacterial agent such as flavonoids, tannins, glucosides, formic acid, citric acid, and several minerals such as calcium and potassium [7,8]. Therefore, this article review is expected to provide information about the chemical compounds contained in A. bilimbi, its potential as an antibacterial, and its safety level based on toxicity study.

METHODS
The method used in compiling this article is a systematic literature review. Articles were collected both published in national and international scientific journals online from Google scholar and Scopus portal. Literature was original article with testing phytochemical content, antibacterial activity, and toxicity tests on Averrhoa bilimbi.

RESULTS AND DISCUSSION
This section discusses the results of research related to the phytochemical content of A. bilimbi, its antibacterial activity which has been tested through the diffusion method where the observed parameters are the inhibition diameter and the dilution method where the observed parameters are the MIC and MBC values. Besides, the results of acute and sub-chronic toxicity tests on tested animals, as well as the brine shrimp lethality test (BSLT) toxicity test on shrimp larvae were also explained.

1. Phytochemical Content Averrhoa bilimbi
Averrhoa bilimbi is known to contain primary metabolites and secondary metabolites. The 70% ethanol extract of A. bilimbi leaves qualitatively contains several groups of chemical compounds namely alkaloids, saponins, tannins, phenolic, flavonoids, triterpenoids, steroid, and glycosides [9]. In addition, 70% ethanol extract of A. bilimbi fruit also contains carbohydrates, protein, fat, vitamin A, vitamin C, and vitamin E [10]. The results of phytochemical screening from the water extract of A. bilimbi fruit showed that the content of several groups of chemical compounds such as carbohydrates, proteins, flavonoids, tannins, and hydrolyzed tannins. The chloroform extract of A. bilimbi fruit contains tannins and flavonoids. Meanwhile, the methanol extract of A. bilimbi fruit contains protein and flavonoids. Further identification of the methanol extract of the fruit of A. bilimbi with methods Thin Layer Chromatography (TLC) showed that the extract contains compounds bitter, volatile oil, valepotrait, coumarin, flavonoids, and terpenes [11]. A. bilimbi fruit is also rich in macro and micro mineral content. Macro-minerals contained in A.bilimbi fruit are calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), and sodium (Na). Meanwhile, the micro minerals contained in A.bilimbi are zinc (Zn), iron (Fe), copper (Cu), and manganese (Mn) [12].

A study conducted by Auw et al. (2014), identified 15 compounds, 3 of which were new compounds from the ethanol extract of 70% A. bilimbi leaves. The twelve known compounds are β-amyrin, β-amyrenone, β-sitosterol, phytol, aurantiamide benzoate, stigmastanol, 4-hydroxycinnamic acid, trans-cinnamic acid, (S)-dehydrovomifoliol, (6S,7aR)-looliolide, phloretic acid, and carambolaflavone. The three new known compounds are 7-O-methylapigenin-6-C-(2'-O-α-L-rhamnopyranosyl)-β-L-fucopyranoside, 7-O-methyl-
apigenin 6 - C - (4" - O - α - L rhamno-pyranosyl)-β-L-fucopyranoside, and 2'-C-β-fucopyr-anosyl 3',4,4' trihydroxydihydrochalcone - 5' - O - α arabino-furanosid[13]. Kurian et al. (2018) conducted phytochemical screening and isolation and identification of flavonoids from the methanol extract of A. bilimbi fruit. The results of phytochemical screening showed that the extract was positive for alkaloids, flavonoids, triterpenoids, tannins, phenolic compounds, and amino acids. The results of the isolation and identification of flavonoids showed that the fruit of A. bilimbi contained dihydromyricetin, namely (2R, 3R) - 3, 5, 7-trihydroxy - 2 - (3, 4, 5- trihydroxyphenyl) 2,3-dihydrochromen - 4-one with molecular formula C15H12O8 [14]. Abraham (2016) identified 20 compounds contained in the methanol extract of A. bilimbi fruit using the LC-MS method. These chemical ingredients are umbelliferone, salicylic acid, trans-veratrol, dihydromyricetin, methyl salicylate, boswellic acid, eriocitrin, hydroxy aristolochic acid, benzyl cinnamate, cinnnamaldehyde, hydroxy citric acid lactone, benzyl alcohol, phenethyl amine, leaf alcohol, caffeolmalic acid, ascorbic acid, tartaric acid, citric acid, xylose, and tyrosine[15].

2. Antibacterial Activities In Vitro With Diffusion Method

Averrhoa bilimbi which has been studied in vitro by diffusion method can inhibit pathogenic bacteria, both Gram-positive and Gram-negative bacteria. The ethanol extract of A. bilimbi leaves had activity against several bacteria such as Bacillus cereus, Staphylococcus aureus, Pseudomonas sp., Enterobacter sp, Escherichia coli, Shigella dysentriae, and Propionibacterium acnes [9, 16, 17, 18]. It is known that 70% ethanol extract of A. bilimbi leaves gives better results in the inhibition of B. cereus, S. aureus, Pseudomonas sp., and Enterobacter sp. compared to ethyl acetate extract. Best inhibition against B. cereus and Enterobacter sp. was given by ethanol extract of 70% A. bilimbi leaves with a concentration of 20% (ϕ11.43 and ϕ6.32 mm). While the best inhibition against S. aureus and Pseudomonas sp. was given by ethanol extract of 70% A. bilimbi leaves with a concentration of 25% (ϕ8.8 and ϕ4.94 mm) [16]. The 70% ethanol extract of A. bilimbi leaves studied by Pendit et al. (2016) gave the best test results for S. aureus and E. coli bacteria at 100% extract concentrations (ϕ13.13 and ϕ8.63 mm) [17]. The 70% ethanol extract also had antibacterial activity against S. dysentriae with the best concentration of 100% (ϕ14.47 mm) [18]. The 70% ethanol extract of A. bilimbi leaves formulated as a face toner is known to have antibacterial activity against P. acnes by providing the best inhibitory response at a concentration of 10% (ϕ24.7 mm) [9].

The ethanol extract of A. bilimbi leaves had antibacterial activity against Salmonella typhi by providing a growth inhibition zone at a concentration of 0.3; 0.4; and 0.5 g/mL with an inhibitory diameter of 20.75; 21.88; 22.88 mm [8]. The 95% ethanol extract of A. bilimbi leaves with a concentration of 200 μg/disc gave a moderate inhibitory response to the bacteria Bacillus megaterium, S. typhi, and Vibrio cholerae with an inhibitory diameter of 14.67; 13.33; and 13.67 mm. The extract also gave a low inhibitory response to B. cereus; E. coli; Pseudomonas aeruginosa; Salmonella paratyphi; and S. dysentriae with an inhibitory diameter of 12.0; 11.0; 10.33; 10.0; and 10.67 respectively [11]. The 96% ethanol extract of A. bilimbi leaves gave the highest resistance response to Pseudomonas fluorescens with a concentration of 85 ppm (ϕ5.79 mm) [7].

The ethanol extract of A. bilimbi fruit was known to have activity against P. aeruginosa (ATCC 15692) with an inhibitory diameter of 3 mm [19]. Meanwhile, the ethanol extract of A. bilimbi fruit studied by Dewi et al. (2019) gave the best
antibacterial activity against *Streptococcus pyogenes* at a concentration of 80% (ϕ30.99 mm) \[20\]. Comparatively, the chloroform extract of *A. bilimbi* fruit concentration of 150 mg/disc has a strong antibacterial effect against *B. subtilis; S. aureus;* and *Serratia marcescens* with inhibition diameter above 20 mm, and moderate antibacterial effect against *Klebsiella pneumonia* with inhibition diameter range 11-20 mm \[15\].

The methanol extract of the fruit of *A. bilimbi* with a concentration of 150 mg/disc had a moderate antibacterial effect on *B. subtilis; S. aureus;* and *Klebsiella pneumonia* with an inhibitory diameter range of 11-20 mm, as well as a low antibacterial effect against *Serratia marcescens* with an inhibition zone below 11 mm \[15\]. Methanol extract 80% of *A. bilimbi* fruit gave an inhibitory response to *B. subtilis* by 7 mm \[21\].

The extract of *A. bilimbi* fruit was also known to have the best antibacterial activity against *P. aeruginosa* at a concentration of 100% (ϕ21 mm) \[22\]. Extracts of juice, stew, and fruit infusion of *A. bilimbi* with a concentration of 10% gave an inhibitory response to *P. acnes* with an average diameter of the inhibition zone of each extract, respectively 11; 10; and 10.6 mm \[23\].

Starfruit fruit solution provided the highest antibacterial activity against *E. coli* and *Salmonella* sp. at a concentration of 30% with inhibition zones of 6.8 and 5.07 mm, respectively \[24\]. The water extract of *A. bilimbi* fruit at various levels of maturity, namely young, mature, and ripe fruit had antibacterial activity against *B. cereus; S. aureus;* *P. aeruginosa; E. coli;* and *Salmonella* spp. The best inhibitory response to *B. cereus* and *S. aureus* was given by mature fruit extract with inhibition zones of 9.7 and 12.3 mm, respectively. Meanwhile, the best inhibitory response to *P. aeruginosa; E. coli;* and *Salmonella* spp was given by young fruit extract with 9 inhibition zones; 10.3; and 12 mm \[22\].

The ethyl acetate extract of *A. bilimbi* fruit and leaves could inhibit *Salmonella typhimurium* at the best extract concentration of 10% with an inhibition diameter of 9.5 and 3.33 mm, respectively \[25\]. The extract of the ethanol-water mixture (7: 3) of *A. bilimbi* leaves by giving the higher concentration gives a bigger zone of inhibition. The extract provided the best antibacterial activity against *P. acnes* at a concentration of 225 mg/mL with an inhibition zone of 12.52 mm \[26\].

3. Antibacterial Activities In Vitro With Dilution Method

In vitro studies of antibacterial activity can also be carried out by the dilution method to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). The following are the results of several studies on the antibacterial activity of *A. bilimbi* using this method. The 70% ethanol extract of *A. bilimbi* leaves had antibacterial activity against *B. cereus; S. aureus; Pseudomonas* sp., and *Enterobacter* sp. with MIC/MBC values for each bacteria of 0.25%/1%; 0.75%/2.99%; 0.85%/3.39%; 0.56%/2.24 (v/v) \[16\]. The ethanol extract of 70% *A. bilimbi* leaves also had antibacterial activity against *S. dysentriae* with a MIC value of 25% \[18\]. The MIC value given by the ethanol extract of *A. bilimbi* leaves against *B. megaterium* was 62.50 ppm, while the other bacteria were *B. megaterium; S. typhi; V. cholerae; B. cereus; E. coli; P. aeruginosa; S. paratyphi;* and *S. dysentriae* has a MIC above 100 ppm \[1\].

The 96% ethanol extract of *A. bilimbi* leaves had a MIC value of 5 ppm against *Pseudomonas fluorescens* \[7\]. The extract of *A. bilimbi* leaves tested by Azizah (2017) had a MIC value of 80% against *E. coli* \[27\]. Studies on the antibacterial activity of the water extract of young and mature *A. bilimbi* fruit showed that the extract from the young fruit was more effective against *E. coli* with a MIC value of 0.125 g/mL,
while the extract from the mature fruit was more effective against *S. aureus* with a MIC value of 0.125 g/mL \[2\].

The 80% methanol extract of *A. bilimbi* fruit and leaves had a MIC value of 1.6 mg/mL against *Mycobacterium tuberculosis* \[26\]. In another study, 80% methanol extract of *A. bilimbi* fruit had a MIC value of 5 mg/mL against *B. subtilis* \[21\]. The ethyl acetate extract of *A. bilimbi* fruit and leaves has a MIC value of 60% against *Salmonella typhymurium* \[25\]. The ethanol-water extract mixture (7:3) of *A. bilimbi* leaves gave a MIC value of 100 mg/mL against *P. acnes* \[26\].

4. Antibacterial Activity Mechanism

*Averrhoa bilimbi* has been shown to have antibacterial activity in vitro. The antibacterial activity is thought to be obtained from several chemical compounds contained in *A. bilimbi*. Several chemical compounds that have been identified and have antibacterial activity include flavonoids, alkaloids, glycosides, saponins, tannins, triterpenoids, and steroids. The chemical compounds that have been identified can work as antibacterial agents through different mechanisms. Flavonoids and tannins are thought to interact nonspecifically and affect on cell wall biosynthesis \[29\]. Alkaloids and glycosides in their role as antibacterial work by disrupting the integrity of the peptidoglycan components of bacterial cells; saponins increase the permeability of bacterial cell membranes; tannins interfere with bacterial cell metabolism with complex formation; phenolic denatures bacterial cell proteins; triterpenoids reduce the permeability of the bacterial cell wall so that the bacteria are deprived of nutrients; and steroids by mechanisms that cause bacterial liposomes to leak\[8\]. Dihydromyricetin caused morphological changes and damages the membrane of bacterial cells. This may be due to lysis of the cell wall, the release of intracellular components, and inhibits the tricarboxylic acid cycle which is one of the stage of cellular respiration in bacteria \[30\].

5. Toxicity Study

Toxicity testing is very important to do in ensuring the safety of the extract to be used as medicine. So that the risk due to exposure to the extract in humans can be avoided. Based on the acute and sub-chronic toxicity tests conducted by Othman et al. (2014), 80% ethanol extract of *Averrhoa bilimbi* fruit given orally to female Sprague Dawley Rats gave LD\(_{50}\) values above 5,000 mg/kg BW. In this test, there was no death and changes in the test animal’s behaviour\[31\]. The results of a study conducted by Wulandari (2017) also showed that the standardized extract of *A. bilimbi* leaves containing 5.24% (w/w) quercetin had an LD\(_{50}\) value above 5,400 mg/kg BW \[32\]. Based on the BPOM acute toxicity criteria, an LD\(_{50}\) above 5,000 mg/kg is defined as practically non-toxic \[33\]. The results of a short sub-chronic toxicity test of 80% ethanol extract of *A. bilimbi* fruit given orally for 28 days on male and female Sprague Dawley rats showed that there were no deaths, toxic symptoms, and changes in body weight at all test doses, namely 125 mg/kg BW, 250 mg/kg BW, and 500 mg/kg BW\[31\].

The toxicity test of 70% ethanol extract of *A. bilimbi* leaves using the brine shrimp lethality test (BSLT) method on shrimp larvae (*Artemia Salina* L.) had a moderate toxic effect with an LC\(_{50}\) value of 367.28 µg/mL \[34\]. Meanwhile, the ethanol extract of 95% *A. bilimbi* leaves using the brine shrimp lethality bioassay method in shrimp larvae gave LC\(_{50}\) and LC\(_{90}\) values of 5,81 and 10,28 µg/mL\[1\].

CONCLUSION

*Averrhoa bilimbi*, both the fruit and the leaves, are known to have antibacterial activities against several Gram-positive bacteria and Gram-negative bacteria. Based on the testing of several *A. bilimbi* extracts, Gram-positive bacteria that can be inhibited
include *B. cereus*, *B. megaterium*, *B. subtilis*, *M. tuberculosis*, *P. acnes*, *S. aureus*, and *S. pyogenes*. Meanwhile, Gram-negative bacteria that can be inhibited by *A. bilimbi* extract include *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *Pseudomonas fluorescens*, *Salmonella* spp., *S. typhi*, *Salmonella paratyphi*, *S. typhimurium*, *Serratia marcescens*, *S. dysenteriae*, and *V. cholerae*. The antibacterial activity is associated with the chemical content of *A. bilimbi*. Based on the results of acute and sub-chronic toxicity tests, it is known that *A. bilimbi* extract is safe to use.

**CONFLICT OF INTEREST**

There was no conflict of interest in the preparation of this article. This article was written independently without any third party involvement.

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