

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

Intan Safitri<sup>1</sup>, Ni Putu Eka Leliqia<sup>1\*</sup>

<sup>1</sup>Pharmacy Department, Faculty of Mathematics and Natural Sciences, Udayana University,  
Indonesia

Corresponding author email: eka\_leliqia@unud.ac.id

## 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 macros 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 LD<sub>50</sub> 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<sup>[1,2]</sup>. 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<sup>[3]</sup>. Traditionally *A. bilimbi* is used for the treatment of thrush, cough, acne, tinea versicolor, and digestive dysfunction<sup>[4]</sup>. 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, valeoptrait, 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  $\beta$ -amyryn,  $\beta$ -amyrenone,  $\beta$ -sitosterol, phytol, aurantiamide benzoate, stigmastanol, 4-hydroxycinnamic acid, trans-cinnamic acid, (S)-dehydrovomifoliol, (6S,7aR)-loliolide, phloretic acid, and carambolaflavone. The three new known compounds are 7-O-methylapigenin-6-C-(2"-O- $\alpha$ -L-rhamnopyranosyl)- $\beta$ -L-fucopyranoside, 7-O-methyl-

apigenin 6 – C - (4" – O –  $\alpha$  - L rhamnopyranosyl)- $\beta$ -L-fucopyranoside, and 2'- C- $\beta$ -fucopyranosyl 3',4,4' trihydroxydihydrochalcone- 5' – O -  $\alpha$  arabinofuranosid<sup>[13]</sup>. 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 C<sub>15</sub>H<sub>12</sub>O<sub>8</sub><sup>[14]</sup>. 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-resveratrol, dihydromyricetin, methyl salicylate, boswellic acid, eriocitrin, hydroxy aristolochic acid, benzyl cinnamate, cinnamaldehyde, hydroxy citric acid lactone, benzyl alcohol, phenethylamine, leaf alcohol, caffeolmalic acid, ascorbic acid, tartaric acid, citric acid, xylose, and tyrosine<sup>[15]</sup>.

## 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 dysenteriae*, and *Propionibacterium acnes*<sup>[9, 16, 17, 18]</sup>. 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% ( $\phi$ 11.43 and  $\phi$ 6.32 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% ( $\phi$ 8.8 and  $\phi$ 4.94 mm)<sup>[16]</sup>. 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 ( $\phi$ 13.13 and  $\phi$ 8.63 mm)<sup>[17]</sup>. The 70% ethanol extract also had antibacterial activity against *S. dysenteriae* with the best concentration of 100% ( $\phi$ 14.47 mm)<sup>[18]</sup>. 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% ( $\phi$ 24.7 mm)<sup>[9]</sup>.

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<sup>[8]</sup>. The 95% ethanol extract of *A. bilimbi* leaves with a concentration of 200  $\mu$ g/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. dysenteriae* with an inhibitory diameter of 12.0; 11.0; 10.33; 10.0; and 10.67 respectively<sup>[1]</sup>. The 96% ethanol extract of *A. bilimbi* leaves gave the highest resistance response to *Pseudomonas fluorescens* with a concentration of 85 ppm ( $\phi$ 5.79 mm)<sup>[7]</sup>.

The ethanol extract of *A. bilimbi* fruit was known to have activity against *P. aeruginosa* (ATCC 15692) with an inhibitory diameter of 3 mm<sup>[19]</sup>. 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% ( $\phi$ 30.99 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. acnes* at a concentration of 100% ( $\phi$ 21 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 [2].

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. dysenteriae* 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. dysenteriae* 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<sup>[2]</sup>.

The 80% methanol extract of *A. bilimbi* fruit and leaves had a MIC value of 1.6 mg/mL against *Mycobacterium tuberculosis*<sup>[28]</sup>. In another study, 80% methanol extract of *A. bilimbi* fruit had a MIC value of 5 mg/mL against *B. subtilis*<sup>[21]</sup>. The ethyl acetate extract of *A. bilimbi* fruit and leaves has a MIC value of 60% against *Salmonella thypymurium*<sup>[25]</sup>. The ethanol-water extract mixture (7:3) of *A. bilimbi* leaves gave a MIC value of 100 mg/mL against *P. acnes*<sup>[26]</sup>.

#### 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<sup>[29]</sup>. 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<sup>[8]</sup>. 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<sup>[30]</sup>.

#### 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<sub>50</sub> values above 5,000 mg/kg BW. In this test, there was no death and changes in the test animal's behaviour<sup>[31]</sup>. 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<sub>50</sub> value above 5.400 mg/kg BW<sup>[32]</sup>. Based on the BPOM acute toxicity criteria, an LD<sub>50</sub> above 5.000 mg/kg is defined as practically non-toxic<sup>[33]</sup>. 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<sup>[31]</sup>.

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<sub>50</sub> value of 367.28 µg/mL<sup>[34]</sup>. Meanwhile, the ethanol extract of 95% *A. bilimbi* leaves using the brine shrimp lethality bioassay method in shrimp larvae gave LC<sub>50</sub> and LC<sub>90</sub> values of 5,81 and 10,28 µg/mL<sup>[1]</sup>.

#### 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. pneumonia*, *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.

### ACKNOWLEDGMENT

The preparation of this article was supported by all parties from the Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Udayana University.

### REFERENCES

1. Karon B, Ibrahim M, Mahmood A, Huq AKMM, Chowdhury MMU, Hossain MdA, et al. Preliminary Antimicrobial, Cytotoxic and Chemical Investigations of *Averrhoa bilimbi* Linn. and *Zizyphus mauritiana* Lam. Bangladesh Pharmaceutical Journal. 2011; 14(2): 127-131.
2. Mokhtar SI, Aziz NAA. Antimicrobial Properties of *Averrhoa bilimbi* Extracts at Different Maturity Stages. Journal of Medical Microbiology & Diagnosis. 2016; 5(3): 1-3.
3. Depkes RI. *Materia Medika Indonesia*. Jilid V. Jakarta: Departemen Kesehatan Republik Indonesia; 1989. pp. 92.
4. Purwaningsih E. *Multiguna Belimbing Wuluh*. Bekasi: Ganeca Exact; 2007. pp. 26.
5. Slipranata M, Lestari FB, Sandi NA, Salasia SIO. Potensi Ekstrak Daun Sage (*Salvia officinalis*. L) sebagai anti-Streptococcus suis Penyebab Zoonotik Meningitis. Jurnal Sain Veteriner. 2016; 34 (2): 198-202.
6. Karadi RV, Arpan S, Pranav P, Parvez A. Antimicrobial Activities of *Musa paradisiaca* and *Cocos nucifera*. International. Journal of Research in Pharmaceutical and Biomedical Sciences. 2011; 2: 264-267.
7. Andayani S, Suprastyania H, Rahmawati ED. Pengaruh Pemberian Ekstrak Kasar Daun Belimbing Wuluh (*Averrhoa bilimbi* L.) Terhadap Daya Hambat Bakteri *Pseudomonas fluorescens* Secara *In Vitro*. Journal of Fisheries and Marine Research. 2019; 3(3): 301-307.
8. Hasdiana F, Kuswarini S, Koendhari EB. Antibacterial Activity of Belimbing Wuluh (*Averrhoa Bilimbi* Linn.) Extract on *Salmonella Typhi* Growth. Folia Medica Indonesiana. 2012; 48(4): 144-149.
9. Hasanah N, Novian DR. Daya Hambat Ekstrak Daun Belimbing Wuluh (*Averrhoa bilimbi* L.) Terhadap Bakteri Penyebab Jerawat (*Propionibacterium acnes*). Jurnal Ilmiah Farmasi. 2020; 9(1): 46-53.
10. Yan SW, Ramasamy R, Alitheen NBM, Rahmat A. A Comparative Assessment of Nutritional Composition, Total Phenolic, Total Flavonoid, Antioxidant Capacity, and Antioxidant Vitamins of Two Types of Malaysian Underutilized Fruits (*Averrhoa bilimbi* and *Averrhoa carambola*). International Journal of Food Properties. 2013; 16(6): 1231–1244.
11. Patil AG, Koli SP, Patil DA. Pharmacognostical Standardization and HPTLC Fingerprint of *Averrhoa bilimbi* (L.) Fruits. Journal of Pharmacy Research. 2013; 6(2013): 145-150.

12. Bhasker B, Shantaram M. Morphological and Biochemical Characteristics of *Averrhoa* fruits. International Journal of Pharmaceutical, Chemical and Biological Sciences. 2013; 3(3): 924-928.
13. Auw L, Subehan, Sukrasno, Kadotaa S, Tezuka Y. Constituents of Indonesian Medicinal Plant *Averrhoa bilimbi* and Their Cytochrome P450 3A4 and 2D6 Inhibitory Activities. Natural Product Communications. 2014; 10 (1): 57-62.
14. Kurian AJ, Geetha G, Thavamani BS. Isolation and Characterisation of an Isolated Flavonoid from *Averrhoa bilimbi*. Asian Journal of Chemical Sciences. 2018; 5(1): 1-8.
15. Abraham CM. Antibacterial Effects of *Averrhoa Bilimbi* L. Fruit Extracts. International Research Journal of Biological Sciences. 2016; 5(8): 72-74.
16. Soedirga LC, Parhusip AJ. Aktivitas Antibakteri Ekstrak Daun Belimbing Wuluh (*Averrhoa bilimbi* L.) Terhadap Bakteri Patogen Pangan. Jurnal Sains dan Teknologi. 2019; 3(2): 27-34.
17. Pendi PACD, Zubaidah E, Sriherfyna FH. Karakteristik Fisik-Kimia dan Aktivitas Antibakteri Ekstrak Daun Belimbing Wuluh (*Averrhoa bilimbi* L.). Jurnal Pangan dan Agroindustri. 2016; 4(1): 400-409.
18. Panjaitan RS, Kadiwijati LR, Seto D, Hengky. Uji Aktivitas Antibakteri Ekstrak Etanol 70% dari Daun Belimbing Wuluh (*Averrhoa bilimbi* L.) Terhadap Bakteri *Shigella dysenteriae*. Indonesia Natural Research Pharmaceutical Journal. 2017; 2(1): 81-90.
19. Alva PP, Suresha S, Gururaj MP, Premanath R. Evaluation of anti-quorum sensing activity of indigenous dietary plants against *Pseudomonas aeruginosa*. 2019; 30(2019): 1-7.
20. Dewi P, Ratih GA, Burhannuddin, Sudarmanto IG. In vitro Inhibitory Activity of Ethanolic Fruit Extract from *Averrhoa bilimbi* L. Against *Streptococcus pyogenes* Bacteria. Health Nations. 2019; 3(1): 13-17.
21. Sundis MA, Baharuddin S. Inhibitory Activity of Plant Extracts Against Microbes Isolated from Sick Building. Health and the Environment Journal. 2012; 3(2): 61-73.
22. Rusdian. Uji Daya Hambat Perasan Buah Belimbing Wuluh (*Averrhoa bilimbi* L) Terhadap Pertumbuhan *Propionibacterium acnes*. Media Farmasi. 2018; XIV(1): 153-157.
23. Arisanty, Dewi RP. Efektivitas Ekstrak Air Buah Belimbing Wuluh (*Averrhoa bilimbi*) Terhadap Pertumbuhan *Propionibacterium acnes*. Media Farmasi. 2018; XV(2): 66-71.
24. Wijayanti DA, Sjojfan O, dan Djunaidi IH. Pengaruh Variasi Konsentrasi Larutan Belimbing Wuluh (*Averrhoa bilimbi*) Terhadap Uji Aktivitas Antimikroba Secara *In Vitro*. Jurnal Ilmu-Ilmu Peternakan. 2019; 29(1): 9 – 14.
25. Suliani A, Latief M, dan Rahmi SL. Aktivitas Antimikroba Ekstrak Etil Asetat Buah dan Daun Belimbing Wuluh (*Averrhoa bilimbi* Linn) Terhadap Mikroba *Salmonella typhimurium* dan *Aspergillus flavus*. Chempublish Journal. 2016; 1(2): 32-41.
26. Afifi R, Erlin E, Rachmawati J. Uji Anti Bakteri Ekstrak Daun Belimbing Wuluh (*Averrhoa bilimbi* L.) Terhadap Zona Hambat Bakteri Jerawat *Propionibacterium acnes* Secara *In Vitro*. Jurnal Pendidikan dan Biologi. 2018; 10(1): 10-17.
27. Azizah F. Pengaruh Perasan Daun Belimbing Wuluh (*Averrhoa bilimbi*) Terhadap Pertumbuhan Bakteri *Escherichia coli* Patogen. The Journal of Muhammadiyah Medical Laboratory Technologist. 2017; 1(2): 47-54.
28. Mohamad S, Zin NM, Wahab HA, Ibrahim P, Sulaiman SF, Zahariluddin

- ASM, et al. Antituberculosis Potential of Some Ethnobotanically Selected Malaysian Plants. *Journal of Ethnopharmacology*. 2011; 133(2011): 1021–1026.
29. Do Nascimento PGG, Lemos TLG, Bizerra AMC, Arriaga AMC, Ferreira DA, Santiago GMP, et al. Antibacterial and Antioxidant Activities of Ursolic Acid and Derivates. *Molecule*. 2014; 19(1):1317 - 1327
  30. Xiao X-N, Wang F, Yuan Y-T, Liu J, Liu Y-Z, and Yi X. Antibacterial Activity and Mode of Action of Dihydromyricetin from *Ampelopsis grossedentata* Leaves against Food-Borne Bacteria. *Molecules*. 2019; 24(15): 2831
  31. Othman FA, Hashim N, Abdullah N, Hamid AA, Abdullah MFF, Noor ZM, et al. Toxicity Evaluation of *A. Bilimbi* L. Fruit Extract on Haematological and Histopathological Analysis in Animal Model. *International Journal of Pharmaceutical Sciences Review and Research*. 2014; 26(2): 39-43.
  32. Wulandari WP. Uji Toksisitas Ekstrak Daun Belimbing Wuluh (*Averrhoa bilimbi* L.) Terstandar Kuersetin pada Mencit Putih (*Mus msuculus*). Thesis. Universitas Airlangga; 2017.
  33. BPOM. *Peraturan Kepala Badan Pengawas Obat dan Makanan Republik Indonesia Nomor 7 Tahun 2014 Tentang Pedoman Uji Toksisitas Nonklinik Secara In Vivo*. Jakarta: Badan Pengawas Obat dan Makanan; 2014.
  34. Rahimah S, Maryam FBA, Limbong BA. The Toxicity Test of Ethanol Extract of Leaves *Averrhoa bilimbi* L. Using Brine Shrimp Lethality Test (BSLT). *Journal of Pharmaceutical and Medicinal Sciences*. 2019; 4(1): 10-14.