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Antibacterial Bioactivity Test of Bilimbi Fruit Ethanol Extract (*Averrhoa bilimbi* Linn). Against *Propionibacterium acnes*, *Staphylococcus epidermidis* and *Staphylococcus aureus*

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Abstract

One of the causes of acnes is the bacteria *Propionibacterium acnes*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. An alternative solution to this problem is to develop natural medicines from plants, one of which is the star fruit (*Averrhoa bilimbi* Linn). The purpose of this study was to test the bioactivity of star fruit against the growth of *P. acnes*, *S. epidermidis*, and *S. aureus*. This research method used an experimental research design with the treatment of *A. bilimbi* fruit ethanol extract concentrations of 50%, 60%, 70%, 80%, and 90%, chloramphenicol antibiotics as control (+), and sterile distilled water as control (-). All treatments were given to *P. acnes*, *S. epidermidis, and S. aureus* bacteria using the Kirby Baeur (disc diffusion) method. The results of this study were the effectiveness of the bioactivity compound of the ethanolic extract of *A. bilimbi* fruit seen on *P. acnes* and *S. epidermidis* bacteria with concentrations of 70%, 80%, and 90%. Thus, it can be concluded that *A. bilimbi* fruit has the potential to be developed as a natural medicine for acne caused by *P. acnes* and *S. epidermidis*.

Keywords: *A. bilimbi* fruit; *Propionibacterium acnes*; *Staphylococcus epidermidis*; *Staphylococcus aureus*; *Kirby Bauer*.

INTRODUCTION

One of the causes of acne (acne vulgaris), which triggers the problem of facial skin inflammation, is the bacteria *Propionibacterium acnes, Staphylococcus epidermis*, and *Staphylococcus aureus* (Sari *et al*., 2020). The appearance of acne does not only have an impact on facial skin but also has a psychological impact (Gallitano & Berson, 2018), which results in a decrease in one's self-confidence (Abhinitha *et al*., 2019). This impact is exacerbated by the treatment of acne, which generally uses long-term antibiotics such as tetracycline, erythromycin, clindamycin, doxycycline, and minocycline, which cause bacterial resistance, organ disorders, and immune hypersensitivity (Legiawati *et al*., 2023)**.**

Referring to the problems and impacts caused by acne-causing bacteria, researchers are interested in testing natural sources of plant ingredients that have the potential to inhibit the growth of P. acnes, S. epidermidis, and *S. aureus* bacteria as one of the causes of acne. This research is also an effort to search for bioactive compounds in plants that are expected to be developed into alternative drugs to replace antibiotics that are capable of inhibiting the growth of acne-causing bacteria. The plant species to be tested for the potential of bioactive compounds in inhibiting the growth of *P. acnes, S. epidermidis*, and *S. aureus* is the starfruit (*Averrhoa bilimbi* Linn).

The choice of starfruit as the test sample was based on the results of previous studies that reported the administration of starfruit extract on the growth of S. aureus, showing a larger diameter of the inhibition zone compared to *A. bilimbi* leaf extract. Furthermore, administration of ethanol extract of *A. bilimbi* fruit with concentrations of 10%, 15%, and 20% was able to inhibit the growth of *P. acnes* with inhibition zones respectively of 16.67 mm, 22.70 mm, and 28.10 mm while in *S. aureus* of 18.53 mm, 24.16 mm and 30.40 mm (Mokhtar & Abd Aziz, 2016a) **;** (Pertiwi *et al*., 2020a) while testing the ethanol extract of star fruits with concentrations of 25%, 50%, 75%, and 100% was only able to inhibit *S.epidermidis* by 10 mm, 11 mm, 12 mm, 15 mm (Zarwinda *et al*., 2021).

Another reason for testing *A.bilimbi* fruit as an antibacterial also refers to Sá *et al.* (2019), who reported that the content of oxalic acid in *A.bilimbi* fruit has the potential to have a toxic effect on uremia patients. Hence, it is necessary to carry out another study in the form of its content and its effect as an antibacterial, considering the results of the phytochemical screening shown in the study by (Setyawan et al. (2021) were positive for the presence of alkaloids tannins, flavonoids, saponins, triterpenoids. A literature review study by Garg *et al*. (2022) informed the results of phytochemical screening and chromatograms of *A.bilimbi* fruit extract showing the presence of secondary metabolites such as flavonoids, alkaloids, oxalic acid, polyphenols, essential oils, coumarins, valepotriates, and terpenes. The presence of secondary metabolite compounds was able to produce significant inhibition zones on *S. epidermidis, S.aureus, Bacillus cereus, Citrobacter freundii, Salmonella typhi, Proteus vulgaris, Kocuria rhizophila,* and *Aeromonas hydrophila* bacteria.

Based on the results of previous studies, there has yet to be any recent data regarding the response of *P. acnes, S. epidermidis,* and *S. aureus* to ethanol extract of *A. bilimbi* fruit with concentrations above 40%. Therefore, researchers are interested in testing the bioactivity of antibacterial compounds contained in the ethanol extract of star fruit with concentrations of 50%, 60%, 70%, 80%, and 90% on the growth of *P. acnes, S. epidermidis* and *S. aureus* bacteria. The results of this study are expected to be able to complete the completeness of the database on the concentration of the inhibition of the *A.bilimbi* fruit, which can be developed as a natural medicine.

MATERIALS AND METHODS

Types and Research Design

This type of research was quantitative research using an experimental research design with treatment of ethanol extract of *A. bilimbi* fruit with concentrations of 50%, 60%, 70%, 80%, 90%, chloramphenicol 30 µg as a positive control, and sterile distilled water as a negative control. All treatments were given to 3 test bacteria, namely *P. acnes, S. epidermidis*, and *S. aureus*, with three repetitions.

Tools and materials

The tools used included automatic autoclaves (Hirayama HG-80, 76L, Japan), P100 micropipette (Socorex, Switzerland), hot plate and Stirer (IKA C-MAG HS7, Germany), incubator (Memmerth IN-30, Germany), Laminar Air Flow (LAF) (ESCO, Singapore), rotary evaporator (IKA-RV -3 V, Germany) 6 Hole water bath (HH, China), digital analytical balance (Acuplus, China), VM 300 micropipette and vortex mixer (Gemmy, Taiwan).

The materials used included *A.bilimbi* fruit taken from Cikarang, Bekasi, Indonesia, pure cultures of *P. acnes* ATCC: 11827, *S. epidermidis* ATCC: 12228, *S. aureus* ATCC: 6538 which were purchased at the microbiology laboratory of the University of Indonesia, chloramphenicol antimicrobial susceptibility discs (oxoid, Germany), Antimicrobial Susceptibility Blank Disks (Oxoid, Germany), sterile cotton swabs (One Med, Indonesia) Media Nutrient Agar (NA) (Merck, Indonesia), Mueller Hinton Agar (MHA) (Merck, Pro Analyst 96% Ethanol (Merck, Indonesia, Pro Analyst 0.9% NaCl (Merck, Indonesia) and Technical Aquades (ROFA, Indonesia).

Identification and sample preparation

The natural product sample in this study was 2.5 kilograms (Kg) of *A.bilimbi* fruit taken from Cikarang, Bekasi, Indonesia. The samples were then observed organoleptically which included shape, smell, color, taste, and other characteristics. Plant identification was carried out by sending samples of *A. bilimbi* fruit to the Research Center for Plant Conservation and Botanical Gardens, Indonesian Institute of Sciences (LIPI), Bogor, West Java, as evidenced by a plant determination certificate. Meanwhile, sample preparation included wet sorting, chopping, drying, and grinding.

Extraction by maceration method

Weighed as much as 100 grams of dried *A. bilimbi* fruit simplicia powder, then put it in an Erlenmeyer containing 700 ml of 96% ethanol and then tightly closed using aluminum foil. Soak for 7 x 24 hours, with stirring carried out every 24 hours until the solvent was completely mixed. The liquid filtrate was filtered with Whatman paper No. 1. The maceration process is repeated for 7 days in the same way to obtain a colorless liquid extract (Oliveira *et al*., 2021).

Evaporation of Extraction Results

The liquid extract obtained from maceration was evaporated using a rotary evaporator with a temperature of 40 ∘C, a pressure of 195 mbar, and a speed of 60 rpm for ± 3 days. After 2 days, heating was carried out on a water bath at 60 ∘C to obtain an optimal thick extract. The results of the thick extract are then calculated by the % yield of the thick extract using the formula:

$$\text{field viscous} = \frac{\text{weight of viscous extract}}{\text{weight of powder}} \text{x } 100\ \%$$

Antibacterial Compound Test

Sterilization of tools and materials

Sterilized tools and materials include Petri dishes, test tubes, 0.9% NaCl, dark glass bottles, Erlenmeyer containing NA and MHA media, tweezers, and distilled water. All tools are washed with detergent, rinsed under running water, and dried. The dried tools were wrapped tightly in paper and then sterilized using an autoclave at 121°C with a pressure of 2 atm for 15 minutes. As for the

Ose Needle, it is sterilized by heating it on a Bunsen flame.

Preparation of test bacterial suspension (P.acnes, S. epidermidis, and S. aureus)

Preparation of the test bacterial suspension was carried out by taking several ose of pure cultures of the test bacterial sub-cultures, then putting them in 0.9% NaCl and then vortexing until homogeneous. Then the results were compared for the turbidity with Mc Farland 0.5 solution (equivalent to a bacterial suspension of 1.5 x 10^8 CFU/ml) where if, after being compared with McFarland, it turns out that the bacterial suspension is still too clear, then a few more ose of the test bacteria can be added. In contrast, if it turns out to be too cloudy, 09% NaCl can be added again to obtain a solution of the test bacterial suspension with the same level of turbidity as the standard McFarland 0.5 solution.

Antibacterial compound bioactivity test using Kirby Bauer method

The bioactivity test of antibacterial compounds in *A. bilimbi* fruit was carried out using the Kirby-Bauer method using a 4-quadrant streak plate technique. Then, it was wiped evenly on the surface of the MHA media using a sterile cotton swab and left for ± 5 minutes. Then, prepare a blank disc that has been dripped with ethanol extract of bilimbi fruit with graded concentrations (50%, 60%, 70%, 80%, and 90%) as much as 30 µl using a micropipette and left for ± 15 minutes, positive control for antibiotic chloramphenicol, and negative control for aquadest sterile and blank discs. All discs (discs) were then placed on the agar plate using sterile tweezers. Each petri dish has 3 replicates. All Petri dishes were then incubated for 24 hours at 37 ◦C. The diameter of the inhibition zone was measured after 1x24 hours of incubation by measuring the presence/absence of a clear zone formed around the treatment disc using a ruler. The results of measuring the diameter of the inhibition zone were then compared with CLSI (2020) guidelines to see the sensitivity category of the test bacteria in response to each treatment disc.

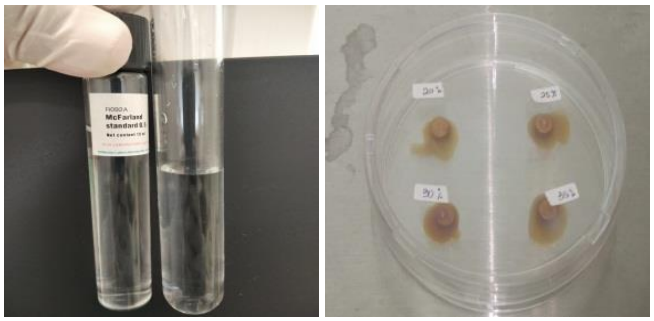

Figure 1. A. Bacterial culture turbidity that complies with the Mc Farland standard of 0.5. B. Blank disk that has been dripped with condensed extract of *A. bilimbi* fruit.

Data analysis

Data analysis in this study was carried out using a quantitative descriptive test by looking at the sensitivity category of the response of the test bacteria in response to treatment discs that had been processed in tabular form. Data in tabular form contains the average size of the diameter of the inhibition zone of the tested bacteria from the three bacteria with various treatments. These results were then interpreted based on the category of bacterial sensitivity response that has been adjusted to the CLSI standard (2020).

RESULT AND DISCUSSION

Identification results in the form of *A. bilimbi* fruit sent to the Bogor Botanical Gardens and Plant Conservation Research Center, LIPI show that the fruit simplicia used in the study has the Indonesian local name Belimbing wuluh with the Latin name *Averrhoa bilimbi* L. and family oxalidaceae. Based on (2013), in general, A. bilimbi plants have green leaves, lancet-shaped, tapered ends, asymmetrical leaf bases, and flat leaf edges. *A. bilimbi* plant stems are brown, cylindrical in shape, and have no distinctive odor. The *A. bilimbi* fruit used as a test sample in this study can be seen in **Figure 2.**

Figure 2. Morphology of *A. bilimbi* fruit.

Figure 2. shows that ripe *A. bilimbi* has an elliptical and oval morphology with shiny flesh and is light green or yellowish. According to Alhassan and Ahmed (2016), starfruit grows on stems with a dark green fruit color

before maturity and has a tough flesh texture, but after ripe, it becomes light green with soft and watery fruit flesh. *A. bilimbi* that has been identified is then carried out wet sorting, chopping, drying, dry sorting, and pollination. The drying results were carried out for 4 days in the sun. The drying results in this study can be seen in **Figure 3.**

Figure 3. Drying process from the first day to the fourth day.

According to Chau *et al*. (2021) wet sorting is done by washing the simplicia using running water to remove residual soil and dirt attached to *A. bilimbi* samples. The clean sample is then sliced by slicing *A. bilimbi* fruit by 1 cm to help speed up the drying process. The drying process in this study was carried out by covering the simplicia pieces using a black cloth and drying them in the sun. Covering it with a black cloth aims to avoid direct sunlight which has the potential to cause changes in the phytochemical compounds contained in *A. bilimbi* fruit simplicia. The dried simplicia was then extracted using the maceration method and evaporated with a rotary evaporator. The results of the thick extract obtained from 100 grams of *A. bilimbi* fruit simplicia powder can be seen in **Figure 4.**

Figure 4. A. simplicia powder. B. viscous extract.

Figure 4 shows the results of the dried simplicia powder of *A. bilimbi* fruit which is brown with a acid flavour, while the thick extract has a deep black color. The results of the viscous extract were then calculated for the percentage yield of the viscous extract shown in **table 1.**

Table 1. Percentage of viscous extract yield.

Simplicia powder weight	Weight of viscous	Yield percentage
100 gr	24.18 gr	24.18%

Table 1. shows the results of maceration and evaporation extraction from 100 grams of *A. bilimbi* fruit simplicia powder, which obtained viscous extract weight of 24.18 with a yield percentage of 24.18%. The results of this study complement research data Zarwinda *et al*. (2021) which extracted 150 grams of *A.* bilimbi leaf powder in 750 ml of 96% ethanol and produced 210 grams of a viscous extract with a percentage of 28%. Cheong *et al*. (2022) reported that the extraction of 100 grams of *A. bilimbi* fruit powder in 1000 ml of water and ethyl acetate yielded viscous extract weight (% yield) of 16.20 (16.20%) and 2.36 (2.36%).

The choice of the maceration method and 70% ethanol solvent was based on the results of a literature review conducted by Leliqia & Safitri (2021) which stated that qualitative phytochemical screening using the maceration method with 70% ethanol solvent was able to extract the phytochemical compounds found in leaves and fruit *A. bilimbi*, including others are alkaloids, flavonoids, polyphenols, tannins, saponins, steroids, and triterpenoids. According to Garg *et al*. (2022)maceration is extraction method that is carried out at room temperature without involving a heating process so as to prevent damage to secondary metabolites which are thermolabile. Anindita *et al*. (2022) added that 70% ethanol has the same polarity properties as the phytochemical compounds contained in *A. bilimbi* fruit, which are both polar. The same polarity between the solvent and the dissolved substance or according to the principle of like dissolves like causes 70% ethanol to be able to enter and attract the phytochemical compounds contained in the cytoplasm of the star fruit cell membrane. The importance of selecting solvent polarity was shown in Abraham (2016) research which proved that the use of petroleum ether extract of *A. bilimbi* fruit as a non-polar solvent was not able to inhibit *S. aureus* bacteria. This indicates that the use of ethanol is still recommended as a polar solvent for extracting *A. bilimbi* fruit.

The results of the viscous extract of *A. bilimbi* fruit simplicia were then carried out by phytochemical screening to determine the presence or absence of secondary metabolites contained in *A. bilimbi* fruit. Phytochemical screening in this study included tests for

saponins, alkaloids, tannins, phenols, and terpenoids. The results of the phytochemical screening test for *A. bilimbi* fruit viscous extract in this study can be seen in **table 2** and **figure 5.**

Table 2. Results of phytochemical screening of viscous extracts of *A. bilimbi* fruit

Secondary Metabolites	Result	Reagen	Information
Alkaloids	+	Meyer's reagent	White precipitate
	+	Wagner reagent	Brown precipitate
	+	Dragendorf reagent	Red/orange precipitate
Fenol	+	4 drops $FeCl_3$	Blue/dark black
Flavonoids	+	Etanol 5 ml + 10 drops HCL + 0,2 gram magnesium	Yellow/orange
Saponin	+	10 ml hot water + HCL 1 N	Foam 1 cm for 5 minutes
Tannin	+	2 drops $FeCl_3$ 1 %	Blackish blue
Terpenoid	+	2 ml H_2SO_4	Brown-purple

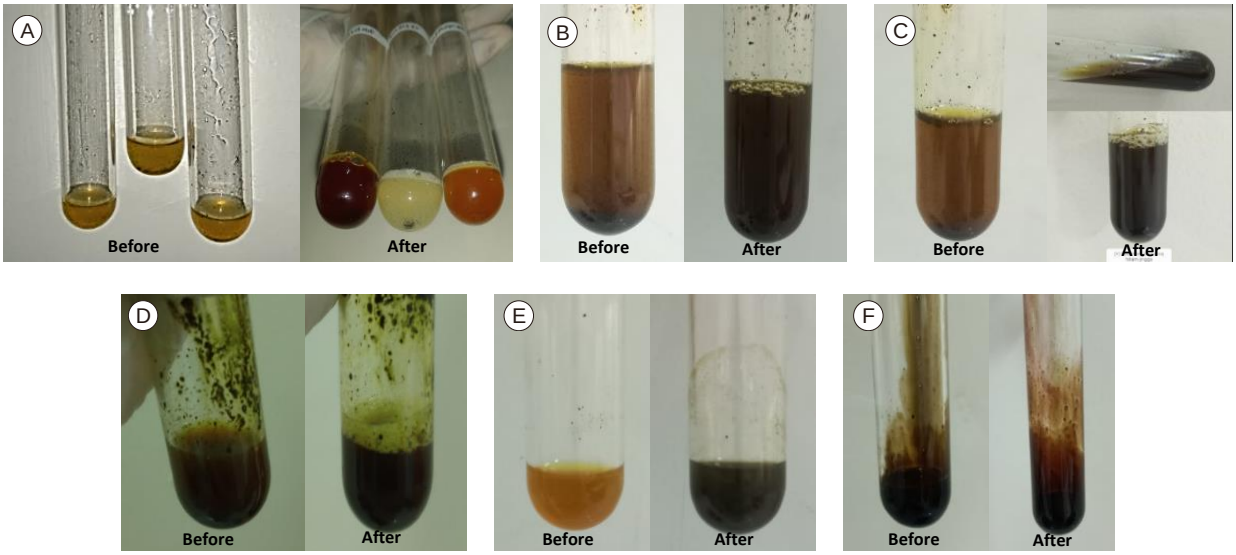

Figure 5. A. Alkaloids test. B. Phenol test. C. Flavonoid test. D. Saponin test. E. Tannin test. F. Terpenoid Test.

The results of the viscous extract of A. bilimbi fruit were then tested for antibacterial effect with concentrations of 50%, 60%, 70%, 80%, and 90% against three acne-causing bacteria, namely P. acnes, S. epidermidis, and *S. aureus*. The positive control in this study was the antibiotic chloramphenicol at a dose of 30 µg, and the negative control was sterile distilled water. All treatments were saturated in discs with a diameter of 6 mm. The results of the inhibition test of ethanol extract of *A. bilimbi* fruit on the growth of *P. acnes* bacteria are shown in **Table 2**

Table 2. The results of giving ethanol extract of *A. bilimbi* fruit to the diameter of the inhibition zone for the growth of *P. Acnes* bacteria.

Treatment	Inhibition zone diameter (mm)			Mean	Category
	Replication 1	Replication 2	Replication 3		
Control (+)	20.5	21.5	21	21	Sensitive
Control (-)	0	0	0	0	Resistant
Concentration 50 %	15.5	15	15.25	15.25	intermediates
Concentration 60 %	15.5	15.5	15.5	15.5	intermediates
Concentration 70 %	22	20	21	21	Sensitive
Concentration 80 %	25	20.5	22.75	22.75	Sensitive
Concentration 90 %	28.5	24	26.25	26.25	Sensitive

Based on **Table 2.** it can be seen that giving of ethanol extract of *A. bilimbi* fruit with a concentration of 50%, 60%, 70%, 80%, and 90% was able to produce the diameter of the inhibition zone for the growth of *P. acnes*

bacteria of 15.25 mm, 15.5 mm, 21 mm, 22.75 mm, 26.25mm. The lowest *P. acnes* inhibition zone diameter was indicated at a concentration of 50%, namely 15.25 in the intermediate category. In comparison, the highest was indicated at a concentration of 90% in the sensitive category. The results of this study are a continuation of previous studies which reported that giving of *A. bilimbi* fruit ethanol extract at doses of 10%, 20%, and 30% was able to produce diameters of inhibition zones of 16.67 mm, 22.7 mm, and 28.1 mm (Pertiwi *et al*., 2020)**.** In addition to *P. acnes* bacteria, this study also examined the inhibition of ethanol extract of *A. bilimbi* fruit against *S. epidermidis*. The test results are shown in **Table 3.**

Table 3. The average results of giving ethanol extract of *A. bilimbi* fruits to the diameter of the inhibition zone for the growth of *S. epidermidis* bacteria.

Treatment	Inhibition zone diameter (mm)			Mean	Category
	Replication 1	Replication 2	Replication 3		
Control (+)	20.1	20.5	20	20.2	Sensitive
Control (-)	0	0	0	0	Resistant
Concentration 50 %	14	16	13.5	14.5	Intermediate
Concentration 60 %	17	15.5	16	16.17	Intermediate
Concentration 70 %	22.5	20	17	19.83	Sensitive
Concentration 80 %	22.5	19.5	20	20.67	Sensitive
Concentration 90 %	22.5	20.5	21	21.33	Sensitive

Superscript description: R = Resistant. I = intermediates. S = Sensitive

The results in **table 3** show that giving of ethanol extract of *A. bilimbi* fruit with concentrations of 50%, 60%, 70%, 80%, and 90% was able to produce diameter of inhibition zone on *S. epidermidis* bacteria of 14.5 mm, 16.7 mm, 19.83 mm, 20.67 mm, and 21.33 mm. The lowest inhibition zone diameter was shown at a 50% concentration of 14.5 mm with the intermediate category. In comparison, the highest inhibition zone diameter was shown at a 90% concentration of 21.33 mm with the sensitive category. The results of this study continued previous research, which reported that giving ethanol extract of *bilimbi fruit* with concentrations of 10%, 20%, 30%, and 40% was able to produce diameters of inhibition zones on *S. epidermidis* bacteria of 28.6 mm, 31.6 mm, 36.3 mm and 39.0 mm (Rahmiati *et al*., 2017). Another acne-causing bacteria whose sensitivity was tested in responding to the antibacterial compounds contained in the ethanol extract of *A. bilimbi* fruits in this study was *S. aureus.* The test results can be seen in **Table 4.**

Table 4. The average results of giving ethanol extract of *A. bilimbi* fruit to the diameter of the inhibition zone for the growth of *S. aureus* bacteria.

Treatment	Inhibition zone diameter (mm)			Mean	Category
	Replication 1	Replication 2	Replication 3		
Control (+)	21.5	22.5	23	22.30	Sensitive
Control (-)	0	0	0	0	Resistant
Concentration 50 %	9.5	10	10	9.80	Resistant
Concentration 60 %	10.1	10.3	10.3	10.23	Resistant
Concentration 70 %	10.5	11	11.5	11.00	Resistant
Concentration 80 %	11.5	12.5	13	12.30	Intermediate
Concentration 90 %	13	13.5	14	13.50	intermediete

Superscript Description: R = Resistant. I = intermediates. S = Sensitive

The test results in **Table 4** show that administration of ethanol extract of *A. bilimbi* fruit with concentrations of 50%, 60%, 70%, 80%, and 90% was able to produce a diameter of inhibition zone on *S. aureus* bacteria of 9.80 mm, 10.23 mm, 11.00 mm, 12.30 mm, and 13.50 mm. The lowest inhibition zone diameter was shown at 50% concentration of 9.80 mm with the resistant category. In comparison, the highest inhibition zone diameter was shown at 90% concentration of 13.50 mm with the sensitive category. The results of this study continued the research of three previous researchers who reported that administration of ethanol extract of *A. bilimbi* fruit to *S. aureus* bacteria with concentrations of 3%, 6%, and 9% was able to produce inhibition zone diameters of 8.50 mm, 9.50 mm, 10.30 mm, whereas in concentrations of 20%, 40% and 15.9 mm, 18 mm and 22.1 mm (Das *et al*., 2011)**;** (Mokhtar & Abd Aziz, 2016); (Lisnawati *et al*., 2019).

Meanwhile, based on **Tables 2, 3**, and **4**, it can be seen that of the three acne-causing bacteria, the inhibitory effect of the antibacterial compounds of bilimbi fruit was best shown on *P. acnes* bacteria. This

can be seen in the difference in the average diameter of the inhibition zone of the three bacteria shown in **Table 5.**

Table 5. Comparison of average inhibition zones between *P. acnes, S. epidermidis*, and *S. aureus* bacteria

Treatment	Inhibition zone diameter (mm)		
	P. acnes	***S. epidermidis***	***S. aureus***
Concentration 50 %	15.25^{I}	14.50^{I}	9.80^{R}
Concentration 60 %	15.50^{I}	16.17^{I}	10.23^{R}
Concentration 70 %	21.00^{S}	19.83^{S}	11.00^{R}
Concentration 80 %	22.75^{S}	20.67^{S}	12.30^{I}
Concentration 90 %	26.25^{S}	21.33^{S}	13.50^{I}
Control (+)	21.00^{S}	20.20^{S}	22.30^{S}
Control (-)	0^{R}	0^{R}	0^{R}

Superscript Description: R = Resistant. I = intermediates. S = Sensitive

Based on Table 5, it can be seen that the same sensitivity in response to antibacterial bioactivity compounds of ethanol extract of *A. bilimbi* fruit was shown in *P. acnes* and *S. epidermidis* bacteria with intermediate categories at concentrations of 50% and 60% with sensitive categories at concentrations of 70%, 80%, and 90 %. The sensitivity of *S. aureus* still showed the resistant category at concentrations of 50%, 60%, and 70%, while concentrations of 80% and 90% showed an intermediate response.

In this study, the sensitivity response categories of the three test bacteria to antibacterial compounds in *A. bilimbi* fruit were determined based on the CLSI standard (2020) according to the effect of the antibiotic chloramphenicol as a positive control on the sensitivity of *S. aureus* bacteria, where if the diameter of the inhibition zone ≥ 18 mm is classified as sensitive, 13-17 mm is in the intermediate category, and ≤ 12 mm is a resistant category . The sensitive response category indicates that the ethanol extract of *A. bilimbi* fruit is effective in inhibiting bacterial growth, the intermediate category has moderate effectiveness, and the resistant category illustrates that ethanol extract is not effective in inhibiting bacterial growth (Hombach *et al.*, 2013)

In general, the results of this study prove that the ethanol extract of *A. bilimbi* fruit contains bioactive compounds that can inhibit bacterial growth. This evidence is shown in **Table 5,** which shows that all bacteria *P. acnes, S. epidermidis*, and *S. aureus* produce varying diameters of inhibition zones. The ability to inhibit bacterial growth is suspected because *A. bilimbi* fruit extract contains secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, and terpenoids as antibacterial bioactive compounds that can inhibit the growth of *P. acnes, S. epidermidis*, and *S. aureus* bacteria (Masud Rana *et al.*, 2014) ; (Seebaluck-Sandoram *et al.*, 2019) ; (Setyawan *et al.*, 2021). According to Yan *et al*. (2021), the principle of alkaloids as antibacterial by inhibiting protein synthesis, transcription, and replication of microorganisms, disrupting the integrity of peptidoglycan component bacterial cell wall and disrupting cell membrane transport, causing cytoplasmic leakage in gram-positive bacteria. This was evident in the study of Mabhiza *et al.* (2016), which reveal that the administration of alkaloids isolated from *Callistemon citrinus* and *Vernonia adoensis* was able to inhibit the efflux pump of *S. aureus* and *P. aeruginosa* bacteria as indicated by percentage of Rhodamine 6G accumulation of 114% and 121% with potency the largest in *S. aureus* which produced MIC values of 0.0025 mg/mL and MBC of 0.835 mg/mL.

On saponins, Nurzaman *et al*. (2018) stated that saponins were able to inhibit the growth of pathogenic bacteria by increasing the permeability of bacterial cell membranes. This is because saponins have the same hydrophilic and lipophilic properties as surfactants. This property causes saponins to bind to bacterial cell membranes. As a result, there is a decrease in voltage and disruption of the surface stability cell membrane, which results in cytoplasmic leakage and bacterial cell death. For tannins, Kaczmarek (2020) suggested that tannins as antibacterial occur through a reaction mechanism with cell membranes, protein precipitation, enzyme inactivation, and functional genetic material of bacterial cells. Tannins diffuse more easily in gram-positive bacterial cells than in gram-negative bacterial cells. This is because gram-positive bacterial cells only have one layer of cell membrane, while gram-negative bacterial cells have two layers of cell membrane. Tannins that enter bacterial cells will bind to iron ions, which are needed as cofactors for several bacterial enzymes such as reverse transcriptase and DNA topoisomerase. The result is the inhibition of enzymes needed for the formation of protein, RNA, and bacterial DNA. In addition, because tannins function as protein precipitation, the entry of tannins into bacterial cells can inactivate the adhesin protein, which acts as a virulence factor for pathogenic bacteria.

Associated with flavonoids as antibacterial, Shamsudin et al. (2022) stated that one of the important characteristics of flavonoids is amphophilic, which easily enters the bacterial cell membrane. The presence of flavonoids in bacterial cells can inhibit nucleic acid synthesis, especially inhibition of the DNA gyrase enzyme, which is more effective in *S. aureus* and *S. epidermidis* bacteria. Plaper *et al*. (2003) suggested that the inactivation of DNA gyrase would further inhibit the activity of the ATPase enzyme, as evidenced by the absence of DNA gyrase in *E. colli* isolation. Farhadi *et al*. (2019) added that the use of radioactive markers shows that flavonoids can inhibit the synthesis of DNA, RNA, peptidoglycan, and protein in bacteria. In addition, flavonoids can damage the integrity of the bacterial cell membrane. This was proven by the results of a study by Stapleton et al. (2004) with an electron microscope, which showed that catechin administration resulted in the formation of pseudomulticellular aggregates and

cytoplasmic leakage in *S.aureus*. Evidence of cytoplasmic leakage is shown by a decrease in the number of bacterial colonies and 20% potassium (Cushnie & Lamb, 2005). Other evidence is shown in the research of Aisyah *et al*. (2019); Scania & Chasani (2021) showed that the administration of phenol isolated from Red Zingiber and three microalgae was able to inhibit *S. aureus* and *E. coli* bacteria in the strong category. All of these secondary metabolites interact with each other to produce a high inhibitory effect (Nathania *et al.*, 2023).

In this study, the ability of ethanol extract of *A. bilimbi* fruits with concentrations of 70%, 80%, and 90% results in the same inhibition zone sensitivity response as the antibiotic chloramphenicol on *P. acne* and *S. epidermidis* bacteria in the sensitive category. According to Drago (2019), Chloramphenicol is a broad-spectrum antibiotic that can inhibit the growth of gram-positive and negative bacteria. This antibiotic is bacteriostatic (inhibits the growth of bacteria), but at high doses, it can be bactericidal (kills bacteria). The working principle of chloramphenicol is to inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit, thereby preventing protein formation in bacteria. Other antibiotics that work, like chloramphenicol, include clindamycin (lincosamide) and macrolides such as erythromycin and clarithromycin (Dinos *et al*., 2016).

The selection of the fruit part as test sample was based on several reviews of the results of previous studies which reported that *A. bilimbi* fruit extract treatment was more effective in inhibiting the growth of *P. acnes, S. epidermidis* and *S. aureus* bacteria compared to using *A.bilimbi* leaf extract (Seebaluck-Sandoram et al., 2019); (Zarwinda *et al*., 2021) ; (Afifi et al., 2018)**;** (Kusuma *et al*., 2020)**.** The results of Mokhtar & Abd Aziz's (2016) research report proved that *A. bilimbi* fruit juice purchased from local farmers in the Machang and Jeli districts of Kelantan, Malaysia, with good conditions (color, size, shape, not deformed, and rotten) in three stages of maturity (young, mature, ripe) showing *A. bilimbi* juice Young is more effective in inhibiting the growth of gram-positive bacteria. The high content of secondary metabolites such as oxalic acid has the potential to act as a strong acid that can inhibit the growth of gram-positive bacteria.

In contrast to previous studies, the important point of this study lies in the aspect of originality in the form of giving higher graded concentrations than previous studies. In addition, this study used three test bacteria that cause acne, namely *P. acnes, S. epidermidis*, and *S. aureus*, so that the difference in the sensitivity of the three bacteria in responding to the antibacterial compounds contained in the ethanol extract of *A.bilimbi* fruit can be seen clearly. Still, the research this has limitations, namely data on the diameter of the bacterial inhibition zone resulting from the Kirby Bauer method (disc diffusion) has not been able to be used by clinical practitioners in determining an effective antibacterial dose for the treatment of acne caused by *P. acnes, S. epidermidis*, and *S. aureus* bacteria. Therefore, further research is needed using molecular tests, the MIC (Minimum Inhibitory Concentration) method and the MBC (*Minimum* bactericidal concentration), as well as equipped with bioautographic tests to see which bioactive compounds in the ethanol extract of *A. bilimbi* fruit have the most potential to inhibit *P. acnes, S. epidermidis* and *S. aureus*.

CONCLUSION

Antibacterial test of ethanol extract of *A. bilimbi* fruit taken from Cikarang showed the effectiveness of the inhibition zone on *P. acnes* and *S. epidermidis* bacteria at concentrations of 70%, 80%, and 90% with the sensitive category. As for *S. aureus*, it shows the intermediate category. Based on this, the ethanol extract of *A. bilimbi* fruit taken from Cikarang, Bekasi, Indonesia, has the potential to be used as a raw material candidate for the manufacture of pharmaceutical products that function to prevent acne.

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