

Combined antibacterial activity of ethanol extract of *Artocarpus heterophyllus* Lam. Leaves and *Averrhoa bilimbi* L. Leaves Against *Staphylococcus aureus*.

Aried Eriadi^{1*}, Nurul Kamilah¹, Hendri Satria Kamal Uyun¹, Ully Chairunisa¹,
Dwi Dinni Aulia Bakhtra¹

¹*Sekolah Tinggi Ilmu Farmasi Padang (STIFARM PADANG), Padang, Indonesia*

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ABSTRACT: *Artocarpus heterophyllus* Lam. leaves and leaves of *Averrhoa bilimbi* L. leaves is a plant that has the potential to be antibacterial. This research aims to determine the antibacterial activity of ethanol extract of *Artocarpus heterophyllus* Lam. leaves, *Averrhoa bilimbi* L. leaves and their combinations and find out the effect of combination comparison on antibacterial activity. From the results of the study, it was found that each plant contains alkaloids, flavonoids, phenols, tannins and saponins. On testing the antibacterial activity of ethanol extract *Artocarpus heterophyllus* Lam. leaves With test concentrations of 5%, 10% and 20% obtained average inhibitory diameters of 14.46 mm., 16.24 mm and 18.03 mm. On ethanol extract of *Averrhoa bilimbi* L. leaves. With test concentrations of 5%, 10% and 20% obtained average inhibitory diameters of 16.41 mm., 17.05 mm, and 23.35 mm. On testing the antibacterial activity of a combination of ethanol extracts of *Artocarpus heterophyllus* Lam. leaves and of *Averrhoa bilimbi* L. leaves in ratios of 1:3, 2:2 and 3:1 obtained average inhibition diameters against *Staphylococcus aureus* 23.55 mm., 20.26 mm, and 17.26 mm. It can be concluded that ethanol extract of *Artocarpus heterophyllus* Lam. leaves, leaves of *Averrhoa bilimbi* L., and the combination has antibacterial activity with a strong to very strong category. However, the 1:3 combination produces the best antibacterial activity of 23.55 mm. Based on the one-way ANOVA test, there was a significant effect of combination comparison on antibacterial activity ($p < 0.05$).

KEYWORDS: *Artocarpus heterophyllus* Lam.; *Averrhoa bilimbi* L.; Antibacterial; *Staphylococcus aureus*

I. INTRODUCTION

Infectious diseases are still a major problem that causes the highest mortality rate for developing countries, one of which is Indonesia

(Bakara, 2020). Bacteria are one of the main causes of infectious diseases. Bacteria can cause infection in the individuals they occupy, especially if there are open wounds on the skin. *Staphylococcus aureus* is one of the gram-positive bacteria that causes various types of infections in humans, namely in skin infections, such as boils and furunculosis (Radji, 2010).

Infection by *Staphylococcus aureus* is characterized by tissue damage accompanied by purulent abscesses. Some of the infectious diseases it causes are ulcers, acne, impetigo, and wound infections (Radji, 2010). This infectious disease is overcome using antibiotics. Irrational use of antibiotics such as lack of adherence to society can make pathogenic microorganisms resistant to antibiotics difficult to eliminate in infectious diseases (Rahmawati, 2019), so other drugs are needed as an alternative treatment for infection by utilizing the effectiveness of antibacterial active ingredients from a plant.

One of the herbs used to improve health is used traditionally is jackfruit leaves (*Artocarpus heterophyllus* Lam.). Jackfruit leaves are efficacious for removing dead skin cells, treating acne, fever, ulcers, wounds, and inflammation (Ermawati & Nurmila, 2019). Jackfruit leaves contain compounds of saponins, flavonoids and tannins. These compounds are known to have antibacterial activity (Siahaan et al., 2019). In a study conducted by Siahaan et al., (2019) stated that jackfruit leaf ethanol extract has an antibacterial effect on the growth of *Staphylococcus aureus* bacteria at a concentration of 5%, 10%, 20%, 30%, 40% with an inhibitory power of 8.5 mm., 8.9 mm., 9.1 mm., 9.2 mm., 9.6 mm.

Other herbs using leaves include obtained from the star fruit plant (*Averrhoa bilimbi* L.). So far, people often use the fruit part of this plant for vegetable ingredients. The use of star fruit as a

traditional medicine has been used by the community for a long time to help cure diseases such as acne, thrush, panu, soreness, mumps, rheumatism, high blood pressure and toothache (Aseptianova & Yuliany, 2020). Alkaloids, flavonoids, saponins, tannins, and triterpenoids are the active compounds contained in star fruit leaves (Masduqi & Anggoro, 2017). In the research of Wijayanti & Safitri (2018), ethanol extract of star fruit leaves has antibacterial activity at concentrations of 2.5%, 5%, and 10% with an inhibitory power of 7 mm., 9.67 mm., and 14.67 mm against the growth of *Staphylococcus aureus* bacteria.

However, so far there is no research data that tests the antibacterial activity of the combination of ethanol extract of jackfruit leaves and star fruit leaves against *Staphylococcus aureus* bacteria, so this research needs to be carried out. has never been done. Based on this description, researchers are interested in conducting research on testing the antibacterial activity of a combination of ethanol extract of jackfruit leaves and star fruit leaves against *Staphylococcus aureus* bacteria which are expected to have a synergistic effect to increase the inhibitory power of bacteria from the two plants.

II. METHOD

Tools and Materials

The tools used are rotary evaporators (Hahnvapors), Laminar Air Flow (VL 150 model), incubators (Memmert), micro pipettes (Transferpette), petri dishes (AnormAx), disc paper (Advantec), hotplates, calipers, autoclaves, UV366nm lamps (Lmag). While the materials used; jackfruit leaves (*Artocarpus heterophyllus* Lam.) and star fruit leaves (*Averrhoa bilimbi* L.), Dimethyl sulfoxide (DMSO) (Merck), Nutrient Agar media (Merck), chloramphenicol disk (Mehta), Quercetin (Sigma Aldrich), Silica gel GF₂₅₄, *Staphylococcus aureus* bacteria, and others.

Work Procedure

Sampling

Sample jackfruit leaves (*Artocarpus heterophyllus* Lam.) and star fruit leaves (*Averrhoa bilimbi* L.) fresh obtained in Jalan Kurao Pagang Dalam, West Sumatra as much as 2 kg each.

Extract Creation

A total of 300 grams of each simplician powder was put into a different ma fiber or, then 3 liters of 70% ethanol were added soaked for 24

hours, the first 6 hours were shaken and allowed to stand for 18 hours on each macerator. Macerate is separated by filtration and by repetition 3 times using the same type and amount of solvent. Maserat is collected and evaporated with a rotary evaporator at a temperature below $\pm 50^{\circ}\text{C}$ until a thick extract is obtained (Ministry of Health of the Republic of Indonesia, 2017).

Characterization of Simplicia

Characterization of simplicia includes organoleptic tests, water-soluble juice content, ethanol-soluble juice content, drying shrinkage, total ash content, and acid insoluble ash content (Ministry of Health of the Republic of Indonesia, 2017).

Extract Characterization

Extract characterization includes organoleptic tests, drying shrinkage, total ash content, acid insoluble ash content (Ministry of Health of the Republic of Indonesia, 2000).

Extract Phytochemical Screening Test

Test the content of alkaloid compounds, flavonoids, phenols, tannins, saponins, steroids and terpenoids (Hanani, 2017).

KLT Test

Qualitative analysis of ethanol extracts of jackfruit leaves and star fruit leaves using the thin layer chromatography (KLT) method. The stationary phase used is silica gel and the mobile phase is n-hexane: ethyl acetate (1:1). The appeal used is Quercetin in ethanol.

Antibacterial Activity Check

1. Sterilization

The tools used are cleaned and dried, then sterilized in an autoclave at a temperature of 121°C for 15 minutes. Laminar Air Flow (LAF) is cleaned of dust and then sprayed with 70% ethanol left for 15 minutes and sterilized by turning on the UV lamp for 5 minutes before use. All work is carried out with aseptic techniques (Dwidjoseputro, 1990).

2. Making Nutrient Agar Media (NA)

A total of 5 grams of nutrient to be dissolved with 250 mL of distilled water in Erlenmeyer and heated on a hotplate using a stirring rod until a clear solution is formed. Then sterilized inside the autoclave at a temperature of 121°C pressure of 2 atm for 15 minutes. Nutrient agar is then put into several test tubes with a

predetermined amount, tubes that have been filled with agar placed on a slope of 30-45°. Care must be taken in order not to touch the lid of the tube. Let it cool and hard (Lay, 1994).

3. Bacterial Rejuvenation

One colony of *Staphylococcus aureus* bacteria using sterile ose needles is planted on an oblique medium by scraping after which it is incubated in an incubator at a temperature of 37°C for 24 hours (Muljono et al., 2016).

4. Making Variations in Jackfruit Leaf Ethanol Extract Concentration

The concentration of jackfruit leaf ethanol extract used is 5%, 10% and 20% in 10 mL of DMSO.

5. Making Variations in the Concentration of Star fruit Leaf Ethanol Extract

The concentration of star fruit leaf ethanol extract used is 5%, 10% and 20% in 10 mL DMSO.

6. Making Variations of Jackfruit and Star fruit Leaf Ethanol Extract Combinations

From the results of a single preliminary test of jackfruit leaf ethanol extract and star fruit, the smallest concentration that has a bacterial inhibitory diameter is made into 3 kinds of concentration ratios used in the combination of jackfruit leaf ethanol extract and star fruit leaves, namely 1:3, 2:2, and 3: 1 with a solution volume of 4 mL and each comparison is carried out 3 times (Ningrum et al., 2020). As negative control used DMSO 10µL and positive control used antibiotic disc chloramphenicol 30µL/mL.

7. McFarland Solution Manufacturing

A 9.9 mL solution of H₂SO₄ 0.36 N was mixed with a 0.0mL solution of BaCl₂·2H₂O 1.175% in Erlenmeyer. Then shake until a turbid solution is formed. This turbidity is used as a standard for bacterial suspension turbidity test (Muljono et al., 2016).

8. Bacterial Suspension Manufacturing

A 24-hour-old bacterial culture was taken from an oblique agar 2 ose colony of test bacteria suspended into a sterile 10 mL NaCl in a sterile test tube. Then homogenized with vortex. Turbidity is compared with Mc Farland by measuring transmit values using a UV-vis spectrophotometer at a wavelength of 600 nm (Muljono et al., 2016).

9. Single Extract Antibacterial Activity Test

A total of 15 mL of Nutrient Agar (NA) was put into a sterile petri dish, then a 100 µL bacterial suspension was added. Sterile discs are immersed at various concentrations of the extract then they are affixed to the surface in order. As a negative control used DMSO 10 µL and positive control used antibiotic discs chloramphenicol 30µL/mL. This treatment is repeated 3 times. Then this petri dish was incubated in an incubator for 24 hours at 37°C. Then antibacterial activity was established by measuring the diameter of the inhibition area formed using calipers (Muljono et al., 2016).

10. Test of Antibacterial Activity of Extract Combinations

Nutrient Agar (NA) in a sterile petri dish, a bacterial suspension of 100 µL is added. Then homogenized by shaking the petri dish containing the medium, the medium is then left dense. Sterile discs are soaked in a combination of ethanol extract of jackfruit leaves (*Artocarpus heterophyllus* Lam.) and star fruit leaves (*Averrhoa bilimbi* L.) with a variation in concentration of 1: 3; 2: 2; and 3 : 1, then the disc is attached to the surface in order. Negative control used DMSO, and positive control used 30µL/mL chloramphenicol discs. The treatment is repeated 3 times. Clouds were incubated in an incubator for 24 hours at 37°C. Then antibacterial activity was established by measuring the diameter of the inhibition area formed using calipers (Ilhani & Ismedsyah, 2018).

III. RESULTS AND DISCUSSION

The samples used were obtained in the area of Jalan Kurao Pagang Dalam, West Sumatra. Diidentification at the Herbarium of Andalas University (ANDA) Department of Biology FMIPA UNAND Padang, West Sumatra. Hacyl identification indicates the plant used is jackfruit leaf (*Artocarpus heterophyllus* Lam.) which belongs to the Family Moraceae and star fruit leaves (*Averrhoa bilimbi* L.) which belongs to the Family Oxalidaceae. Fresh samples are wet sorted, washed, chopped, wind-dried, dried, and mashed until simplisia powder is obtained. Jackfruit leaf simplisia powder and star fruit leaves were each extracted using a maceration method using ethanol solvents with multiple shaking at room temperature. This extraction method is used because its implementation is simple, does not require heating so it is good for simplisia to contain

active substances that are not resistant to heating. Masseration is carried out for 3x24 hours. The solvent used 70% ethanol was then followed by 96% ethanol on the second and third repetitions. Ethanol solvent is a universal solvent that can dissolve almost all compounds both polar, semi-polar and non-polar. A 70% ethanol solvent is used because the sample used is a dry sample that has relatively little water content. So, 70% ethanol is used because it contains as much as 30% water. The water content of 30% of this solvent serves to help break the cell wall so that ethanol penetration into cells is faster and optimal (Ministry of Health of the Republic of Indonesia, 2000).

Jackfruit leaf ethanol extract was obtained as much as 73.8459 grams with an amendment value of 24.6153%. Meanwhile, ethanol extract of star fruit leaves obtained as much as 30.6688 grams with an amendment value of 10.2229%. The results of the organoleptic examination of jackfruit leaf simplicia powder are the shape of a powder, green color, characteristic smell, slightly bitter taste, and star fruit leaves, namely powder shape, brownish green color, characteristic smell, slightly bitter taste of astringent. The test results of the water-soluble juice content of jackfruit leaf simplicia powder are $8.2145\% \pm 0.3917$ and star fruit leaf simplicia powder is $19.0842\% \pm 0.1277$. The test results of the soluble juice content of ethanol of jackfruit leaf simplicia powder are $3.9844\% \pm 0.2306$ and simplicia powder and star fruit wuluh which is $13.6633\% \pm 0.6031$. The results of the test for drying jackfruit leaf simplicia powder were $8.6689\% \pm 1.1716$ and star fruit leaf simplicia powder was $6.4468\% \pm 0.8786$. The test results of the total ash content of jackfruit leaf simplicia powder were $3.1388\% \pm 0.1076$ and star fruit leaf simplicia powder was $5.4862\% \pm 0.2534$. The test results of insoluble ash ka dar ash acid of jackfruit leaf simplicia powder are $1.5800\% \pm 0.0114$ and simplicia powder leaf star fruit wuluh which is $0.5841\% \pm 0.4103$.

The results of the organoleptic examination of jackfruit leaf ethanol extract are the form of a thick liquid, brownish-black color, characteristic smell, slightly bitter taste, and star fruit leaves, namely the form of a thick liquid, blackish green color, characteristic smell, slightly bitter taste of astringent. The results of the drying shrinkage test of jackfruit leaf ethanol extract were $7.5118\% \pm 1.2912$ and star fruit leaf ethanol extract was $4.4516\% \pm 0.8237$. The test results of insoluble ash content of jackfruit leaf ethanol

extract are $0.4290\% \pm 0.0397$ and star fruit leaf ethanol extract is $0.0574\% \pm 0.0114$.

Klt samples of ethanol extracts of jackfruit leaves and star fruit leaves were used for eluent, namely n-hexane and ethyl acetate, where n-hexane is nonpolar while ethyl acetate is semi-polar. This aims to determine the separation of stains formed based on the degree of polarity of compounds that are retained in the stationary phase or will be attracted by the motion phase. Compounds whose nature is polar will be retained by the silent phase of the KLT plate, while compounds that are semi-polar and non-polar will be separated or interested in forming stain spots that rise to the top of the plate carried by the phase of motion so that a comparison of eluents suitable for ethanol extract of jackfruit leaves and star fruit leaves is obtained, namely ethyl acetate and n-hexane: ethyl acetate (1:1). This KLT observation was seen directly under UV light of 366 nm that is appear stain spots formed.

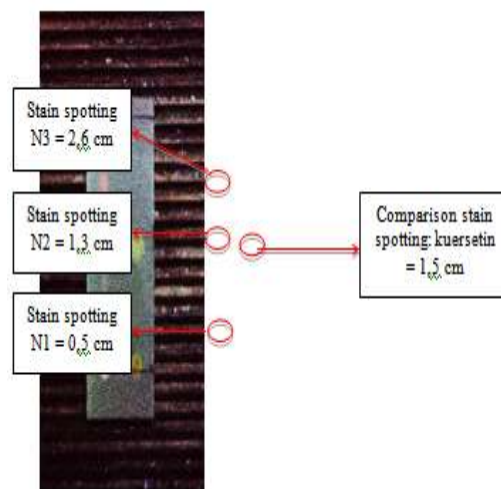


Figure 1. Results of Chromatographic Layer Thinning Ethanol Extract Jackfruit Leaves UV Ray 366 nm

Information:

N1 stain spotting : Stain spotting of jackfruit leaf ethanol extract (1)

N2 stain spotting : Stain spotting of jackfruit leaf ethanol extract (2)

N3 stain spotting : Stain spotting of jackfruit leaf ethanol extract (3)

Checklists : Quercetin

The results showed that in jackfruit leaf ethanol extract there were 3 stain spots with an Rf jackfruit value of 1, namely 0.125; Rf jackfruit 2

i.e. 0.325; Rf jackfruit 3 is 0.65; and the value of Rf of the quercetin comparison is 0.375. While in the ethanol extract of star fruit leaves there are 4 stain spots with an Rf value of star fruit wuluh 1, which is 0.175; Rf star fruit wuluh 2 i.e., 0.3; and Rf star fruit wuluh 3 i.e. 0.425; Rf star fruit wuluh 4 which is 0.825 and the value of Rf comparator quercetin is 0.4.

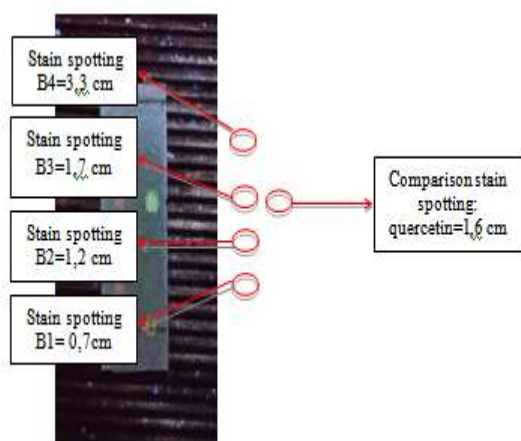


Figure 2. Results of Chromatographic Lapis Tipis Ethanol Extract of Star fruit Leaves wuluh UV Light 366 nm

Information:

- B1 stain spotting : Stain spots of star fruit leaf ethanol extract (1)
- B2 stain spotting : Stain spotting of star fruit leaf ethanol extract (2)
- B3 stain spotting : Stain spotting of star fruit leaf ethanol extract (3)
- B4 stain spotting : Stain spotting of star fruit leaf ethanol extract (3)

Checklists : Quercetin

The phytochemical screening results from ethanol extracts of jackfruit leaves and star fruit leaves were positive containing alkaloid compounds, flavonoids, phenols, tannins and saponins. The results of a study conducted by Kusumawati et al., (2017) found that jackfruit leaf ethanol extract contains flavonoids, saponins and tannins. In research conducted by Harahap et al., (2021) it was found that jackfruit leaf ethanol extract contains alkaloids, flavonoids, phenols, steroids, and triterpenoids. The results of a study conducted by Hasim et al., (2019) found that the ethanol extract of star fruit leaves contains alkaloids, saponins, flavonoids, tannins, steroids, and triterpenoids. In research conducted by (Valsan

& Raphael, 2016) it was found that jackfruit leaf ethanol extract contains flavonoids, phenols, alkaloids, and tannins.

Testing of the antibacterial activity of ethanol extract of jackfruit leaves, star fruit leaves, and their combinations was carried out using the disc diffusion method. According to Pratiwi (2008), the disc diffusion method is a method used to determine the activity of antimicrobial agents. A disk containing antimicrobial agents is placed on Agar media that has been planted with microorganisms that will diffuse in the Agar media. A clear area indicates the presence of an inhibition of the growth of microorganisms by antimicrobial agents on the surface of the Agar medium. The excesses of this method are simple, relatively inexpensive, and easy to interpret the results obtained. Antibacterial activity testing was carried out by making a bacterial suspension solution using two tests bacterial culture ose dissolved with 0.9% NaCl, then the bacterial suspension was compared with Mc. Farland solution by measuring its transmitting value using a UV-vis spectrophotometer at a wavelength of 600 nm.

Ethanol extracts of jackfruit leaves and star fruit were tested for antibacterial activity with a concentration of 5%, 10% and 20% respectively against *Staphylococcus aureus* bacteria. Solution extracts of various concentrations using Dimethyl sulfoxide (DMSO) as solvent and as negative control as well as positive control used antibiotic discs chloramphenicol 30µL/mL. The results of the antibacterial activity test of ethanol extract of jackfruit leaves and star fruit leaves against *Staphylococcus aureus* bacteria can be seen in Table 1.

Extract Concentration (%)	Diameter of Jackfruit Ethanol Inhibition Zone (mm)	Diameter of the Leaf Extract Zone (mm)	Diameter of the Inhibition Zone of Star fruit Leaf Ethanol Extract (mm)
5	14,46		16,41
10	16,24		17,05
20	18,03		23,35

Table.1 Test results of antibacterial activity of jackfruit leaf ethanol extract (*Artocarpus heterophyllus* Lam.) and ethanol extract of star fruit leaves (*Averrhoa bilimbi* L.) against *Staphylococcus aureus* bacteria

From the table. 1 the antibacterial activity of jackfruit leaf ethanol extract obtained on average the inhibitory diameter of jackfruit leaf ethanol

extract at a concentration of 5%, 10% and 20% is 14.46 mm; 16.24 mm and 18.03 mm. Meanwhile, in the ethanol extract of star fruit leaves, the average diameter of the inhibitory against *Staphylococcus aureus* bacteria at a concentration of 5%, 10% and 20% is 16.41 mm; 17.05 mm and 23.35 mm According to Davis & Stout (1971) the classification of inhibitory power strength is grouped into several categories, namely the category of very strong ≥ 20 mm inhibitory diameter, strong 10-20 mm, medium 5-10 mm, and weak ≤ 5 mm. The results showed that jackfruit leaf ethanol extract at a concentration of 5%, 10% and 20% has a strong inhibitory power while in ethanol extract of star fruit leaves at a concentration of 5% and 10% has a strong category of inhibitory power and at a concentration of 20% has a very strong category of inhibitory power against *Staphylococcus aureus* bacteria.

Table.2 Test results of antibacterial activity of a combination of jackfruit leaf ethanol extract (*Artocarpus heterophyllus* Lam.) and star fruit leaves (*Averrhoa bilimbi* L.) against *Staphylococcus aureus* bacteria

Comparison	Diameter of the <i>Staphylococcus aureus</i> Inhibitory Zone
1 : 3	23,55
2 : 2	20,26
3 : 1	17,26
Control (+)	35,15
Control (-)	0

Information:

Control (+): Disk Kloramfenikol

Control (-): DMSO

From the table. 2 the antibacterial activity of the combination of ethanol extract of jackfruit leaves and star fruit leaves obtained on average the inhibitory diameter of the combination of ethanol extract of jackfruit leaves and star fruit leaves against *Staphylococcus aureus* bacteria at a ratio of 1:3 is 23.55 mm and at a ratio of 2:2 is 20.26 mm is included in the category of very strong inhibitory power. While in a ratio of 3:1 the average inhibition diameter is 17.26 mm is included in the category of strong inhibitory power against *Staphylococcus aureus* bacteria. The normality test results showed normally distributed data ($p > 0.05$), then analysis was carried out using a one-way anova resulting in a sig value of 0.000. This shows that the sig < 0.05 value indicates a significant

influence between treatments on *Staphylococcus aureus* bacteria. Each treatment also had a noticeable difference, except for a 5%-star fruit sample with a ratio of 3:1 not significantly different based on the Duncan test results. The combination test of jackfruit leaf ethanol extract and star fruit leaf ethanol extract at a ratio of 1:3 resulted in the largest average inhibitory zone compared to other combinations and a single extract due to synergism as an antibacterial chovies against *Staphylococcus aureus* bacteria. These results are in line with research (Rifda & Lisdiana, 2022), where the results of antibacterial activity in the combination test provide a synergistic effect and produce greater antibacterial activity than the single extract.

The positive control showed a clear zone with greater inhibitory power compared to the extract at each concentration. Chloramphenicol is basically bacteriostatic, broad-spectrum, its dosage and levels in the blood are the same as tetracyclines (Jawetz & Adelberg, 2007). While the negative control does not produce inhibitory power so it can be said that DMSO does not affect the inhibitory power of the extract.

The inhibitory activity is obtained due to the content of secondary metabolites in the ethanol extract of jackfruit leaves and star fruit leaves. In each ethanol extract jackfruit leaves and star fruit leaves have secondary metabolite compounds of alkaloids, flavonoids, phenols, tannins and saponins. The mechanism of action of alkaloids as antibacterial by disrupting the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed intact and causes cell death (Amalia et al., 2017). The mechanism of action of flavonoids as antibacterial is to form complex compounds with extracellular and dissolved proteins so that they can damage bacterial cell membranes followed by the exit of intracellular compounds (Amalia et al., 2017). Tannin compounds as antibacterial can shrink cell walls or cell membranes to interfere with the permeability of the cells themselves. Due to impaired permeability, cells cannot carry out life activities so that their growth is inhibited or even dies (Kusumawati et al., 2017). The mechanism of action of saponins as antibacterial is to reduce surface tension to result in increased permeability or cell leakage and cause intracellular compounds to come out (Kumalasari et al., 2020). The mechanism of action of phenol compounds in killing bacterial cells, namely by denatured bacterial cell proteins. As a result of the denatured protein of bacterial cells, all metabolic activities of

bacterial cells are stopped because all metabolic activities of bacterial cells are catalyzed by enzymes which are proteins (Marfuah et al., 2018).



Figure 3. Best Combination Antibacterial Activity Test on Ethanol Extract of Jackfruit Leaves and Star fruit Leaves in a ratio of 1:3

IV. CONCLUSION

From the research it can be concluded that the ethanol extract of *Artocarpus heterophyllus* Lam., the leaves of *Averrhoa bilimbi* L., and its combination have antibacterial activity with a strong to very strong category. However, the 1:3 combination produces the best antibacterial activity of 23.55 mm. Based on the one-way ANOVA test, there was a significant effect of combination comparison on antibacterial activity ($p < 0.05$).

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