



COMPARISON OF THE EFFECTIVENESS OF BANDOTAN LEAVES (*Ageratum conyzoides Linnaeus*) AND SINTRONG LEAVES (*Crassocephalum crepidioides*) EXTRACTS ON THE GROWTH OF THE BACTERI *Staphylococcus aureus* ATCC 6538

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ABSTRACT

Staphylococcus aureus is a leading cause of infections and infant mortality in Indonesia, especially pneumonia. According to data from the Indonesian Ministry of Health in 2022, pneumonia accounted for 14.4% of cases in infants and 9.4% in toddlers, with 1,475 cases reported in Cirebon city. Allergic reactions from antibiotics have become a serious issue in treatment, prompting the need for natural alternatives such as bandotan leaf (*Ageratum conyzoides Linnaeus*) and sintrong leaf (*Crassocephalum crepidioides*). The compounds alkaloids, flavonoids, saponins, and tannins present in these leaves are believed to effectively inhibit bacterial growth. The study aims to compare the effectiveness of bandotan leaf extract (*Ageratum conyzoides Linnaeus*) and sintrong leaf extract (*Crassocephalum crepidioides*) on the growth of *Staphylococcus aureus* bacteria. Experimental research with posttest-only control group design from April to July 2023. The study used 8 groups consisting of bandotan leaf extract (*Ageratum conyzoides Linnaeus*) and sintrong leaf extract (*Crassocephalum crepidioides*) at concentrations 50%, 75%, and 100%, as well as chloramphenicol (positive control) and DMSO 10% (negative control). Data were analyzed using One-way ANOVA, Post-hoc Tukey HSD, and Independent T-test. There is a comparison of the effectiveness of bandotan leaf extract (*Ageratum conyzoides Linnaeus*) and sintrong leaf extract (*Crassocephalum crepidioides*) against the growth of *Staphylococcus aureus* bacteria. Sintrong leaf extract (*Crassocephalum crepidioides*) exhibits an average inhibitory zone effectiveness of ± 10.44083 mm (P-value 0.000). The *Crassocephalum crepidioides* extract group was the most effective in inhibiting the growth of *Staphylococcus aureus* bacteria.

Keywords: Bandotan leaf extract, Sintrong leaf extract, *Staphylococcus aureus*

INTRODUCTION

Pneumonia is a major infectious disease that causes death in infants. One of the etiologies of pneumonia is *Staphylococcus aureus* (Gram positive) bacteria that infect tissues by producing enterotoxins (Cheung et al., 2021;

Kemenkes, 2022; Maromon et al., 2020). According to the Ministry of Health of the Republic of Indonesia in 2022, infant mortality due to pneumonia was 14.4% and under-five mortality due to pneumonia was 9.4% (Kemenkes, 2022). Pneumonia cases in West Java based on data from the West Java Provincial Health Office in 2022 were

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169,791 cases with 1,475 of them coming from Cirebon City (Kemenkes, 2022; Sugiarto, 2022).

Antibiotics as a therapy for infectious diseases have side effects on long-term use. Side effects that arise in the form of allergic reactions and antibiotic resistance (Kemenkes, 2021). Alkaloids and terpenoids, secondary metabolite compounds in some plants that are antibacterial (Anggraini et al., 2019). Plants such as sintrong (*Crassocephalum crepidioides*) and bandotan (*Ageratum conyzoides* Linnaeus) contain many chemical compounds consisting of pyrrolizidine alkaloids, terpenoids, sterols, flavanoids, and polymethoxylated flavones (Hasyim, 2020; Kotta et al., 2020).

In previous research conducted by Hasyim (2020) on the inhibition test of ethanol extract of bandotan leaves (*Ageratum conyzoides* Linnaeus) as an antibacterial in inhibiting the growth of *Staphylococcus aureus* that causes boils, the inhibition zone was 26.94 mm with a concentration of 35% (Hasyim, 2020). As in other studies conducted by Maimunah et al (2020) on the antibacterial activity test of sintrong leaf extract (*Crassocephalum crepidioides*) against *Staphylococcus aureus* bacteria, the inhibition zone result was 6.5 mm at 10% extract concentration (Maimunah et al., 2020). Based on these descriptions, it can be seen that the two plants have potential as antibacterials, especially in inhibiting the growth of *Staphylococcus aureus* bacteria.

RESEARCH METHODS

Type of Research

This research is a true experimental design with a posttest only control group design whose research subject is *Staphylococcus aureus* bacteria. The independent variables were bandotan leaf extract and sintrong leaf extract with concentrations of 50%, 75%, and 100% while the dependent variable was the zone of inhibition of *Staphylococcus aureus* antibacterial activity on MHA media.

Tools and Materials

Tools

Autoclave, stir bar, dilution bottle, bunsen, petri dish, erlenmeyer, measuring cup, beaker, incubator, Laminar Air Flow (LAF), tweezers, test tube rack, horn spoon, syringe, ose, water bath, test tube, analytical balance, vernier, rotary evaporator, and blender.

Materials

Staphylococcus aureus culture with American Type Culture Collection (ATCC 6538) standard collection of Research Laboratory of Faculty of Medicine, Universitas Swadaya Gunung Jati Cirebon, bandotan leaves and sintrong leaves from Raharja village, Wanayasa sub-district, Purwakarta district at an altitude of 667-800 meters above sea level with an air temperature humidity of 17-32 ° C, 70% ethanol, distilled water, Muller Hinton Agar (MHA) media, tissue, 0.9% NaCl solution, chloramphenicol, 10% dimethyl sulfoxide (DMSO), aluminum foil, cotton, label paper,

plastic wrap, paper disk, 1% BaCl₂, and 1% H₂SO₄.

Procedure

1. Sterilization

The equipment was washed with detergent and rinsed with clean water. The equipment was dried and put into an autoclave at a heating temperature of 121°C for 15 minutes at a pressure of 2 atm. Tweezers and ose were heated directly using a flame to make them sterile (Hasyim, 2020).

2. Preparation of sample (extract)

a. Simplisia

Bandotan (*Ageratum conyzoides* Linnaeus) and sintrong (*Crassocephalum crepidioides*) plants of 10 kg each were wet sorted and cleaned of dirt. Bandotan (*Ageratum conyzoides* Linnaeus) and sintrong (*Crassocephalum crepidioides*) leaves were separated from other plant parts, then cut into small pieces and dried in the sun (Hasyim, 2020; Maimunah et al., 2020).

b. Extraction

The dried simplisia was blended into powder. The powdered simplisia was weighed as much as 500 grams and soaked using 70% ethanol solvent in a closed container for 3-5 days at room temperature with occasional stirring. The results of the soak were filtered using filter paper and collected in a container, then, concentrated in a rotary vacuum evaporator at 40°C until it became a semi-viscous extract (Almira et al., 2021). The semi-viscous

extract was thickened with a waterbath at 60°C (Nofita, 2020).

c. Concentration series

The thick extract was diluted using 10% DMSO with the following dilution formula (Trisuci et al., 2020). Dilution formula:

$$M1 \times V1 = M2 \times V2$$

Description:

M1: concentration of the original solution (%)

V1: volume of solution to be diluted (ml)

M2: desired solution concentration (%)

V2: volume of desired solution (ml) (Trisuci et al., 2020).

3. Bacterial growth media and bacterial inoculation media

a. Mueller Hinton Agar (MHA) media

MHA powder was weighed as much as 38 grams and dissolved in 1000 ml distilled water. The MHA solution was homogenized and heated to boiling, then sterilized at 121°C for 15 minutes using an autoclave. The MHA solution was allowed to stand and poured into sterile petri dishes (Nofita, 2020; Wardaniati & Gusmawarni, 2021).

b. Mc Farland's solution 0.5

A 1% H₂SO₄ solution of 9.5 ml and a 1% BaCl₂ solution of 0.5 ml were homogenized in a test tube until turbid. The turbidity of the solution is used as a standard for bacterial suspension (Wardaniati & Gusmawarni, 2021).

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c. Suspension of test bacteria

Staphylococcus aureus bacteria were suspended in 0.9% NaCl solution as much as 10 ml, then homogenized until the suspension turbidity level was the same as the Mc Farland standard (Wardaniati & Gusmawarni, 2021).

d. *Staphylococcus aureus* bacteria inoculum media

A suspension of *Staphylococcus aureus* test bacteria is taken with a sterile cotton stick and applied to the surface of the MHA medium until evenly distributed (Wardaniati & Gusmawarni, 2021).

4. Control solution

a. Negative control

Negative control solution was prepared from 10 ml of liquid 10% DMSO.

b. Positive control

The positive control solution was prepared by opening the shell of a 250 mg chloramphenicol capsule and dissolving it in 50 ml of distilled water to obtain a chloramphenicol solution in the amount of 5 mg/ml (Alouw et al., 2022; Bawondes et al., 2021).

5. Antibacterial effectiveness test

Prescribe disk paper in bandotan (*Ageratum conyzoides* Linnaeus) leaf extract at 50%, 75%, and 100% concentration. Do the same for sintrong (*Crassocephalum crepidioides*) leaf extract at 50%, 75%, and 100% concentration. The positive control solution of chloramphenicol and the

negative control solution of 10% DMSO solution were also treated with disk paper. Prescribing was done by dripping 0.1 ml of solution on each paper disk. The disc paper that has been prescribed, planted on the inoculation medium and incubated for 24 hours in an incubator at 37°C (Wardaniati & Gusmawarni, 2021). The test bacteria that have been incubated, measured the inhibition zone using a caliper and recorded the measurement results in millimeters (mm) (Hasyim, 2020).

6. Interpretation of inhibition zone

The zone of inhibition was obtained from the equation in Figure 1 (Winastri et al., 2020).

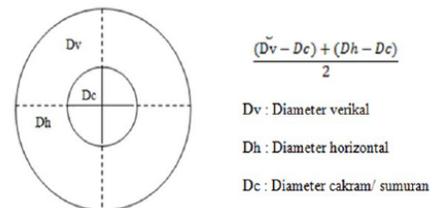


Figure 1. Inhibition Zone Diameter Calculation Formula (Winastri et al., 2020).

The calculation results were interpreted into the antibacterial sensitivity levels in the following table:

Table 1. Antibacterial Sensitivity Categories (Winastri et al., 2020)

Diameter	Inhibition power
≤ 5 mm	No response
6-10 mm	Medium
11-20 mm	Strong
≥ 21 mm	Very strong

Data Analysis

Statistical analysis of the results of the study used the Shapiro-wilk normality test, Levene's homogeneity test, one way ANOVA bivariate test, and Tukey HSD. As well as the Independent T test multivariate test.

The results of this study showed a comparison of the effectiveness of bandotan leaf extract and sintrong leaf extract against the growth of *Staphylococcus aureus* bacteria after incubation in the treatment groups. The mean inhibition of antibacterial activity can be found in Table 2.

RESULTS AND DISCUSSION

Result

Mean Inhibition of Antibacterial Activity

Table 2. Mean Inhibition of Antibacterial Activity

Sample Groups		Inhibition Power (mm)				Mean (mm)	Categorie
		Test 1	Test 2	Test 3	Test 4		
Bandotan Leaves Extract	K (+)	22	24,75	25,9	24,6	24,31250	Very Strong
	K (-)	0	0	0	0	0,00000	No response
	P1	3	2	3,5	4,5	3,25000	No response
	P2	4	6	4	4,5	4,62500	No response
	P3	6,8	7,25	7,5	4,1	6,41250	Medium
Sintrong Leaves Extract	K (+)	22	24,75	25,9	24,6	24,31250	Very strong
	K (-)	0	0	0	0	0,00000	No response
	P4	7,05	8,415	8	7,6	7,78125	Medium
	P5	10,75	10,9	10,35	9,1	10,52500	Medium
	P6	15,575	13,375	10,875	13,3	13,28125	Strong

P1 = Bandotan leaf extract 50% concentration

P2 = Bandotan leaf extract 75% concentration

P3 = Bandotan leaf extract 100% concentration

P4 = Sintrong leaf extract 50% concentration

P5 = Sintrong leaf extract 75% concentration

P6 = Sintrong leaf extract 100% concentration

K (+) = Chloramphenicol

K (-) = 10% DMSO

Based on table 2, the inhibitory activity of sintrong leaf extract (*Crassocephalum crepidioides*) is greater than that of bandotan leaf extract (*Ageratum conyzoides* Linnaeus) by 13.28125 mm for P6 and 6.4125 for P3, by 10.525 mm for P5 and 4.625 mm for P2, as well as 7.78125 mm for P4 and 3.25 mm for P1. The inhibition value formed by the control treatment group has an average of

24.3125 mm in the positive control group of chloramphenicol (K (+)) while for the negative control group of 10% DMSO (K (-)) there is no inhibition zone. The difference in effectiveness between the bandotan and sintrong leaf extract treatment groups against the growth of *Staphylococcus aureus* bacteria in forming the inhibition zone can be seen as in Figure 2 below

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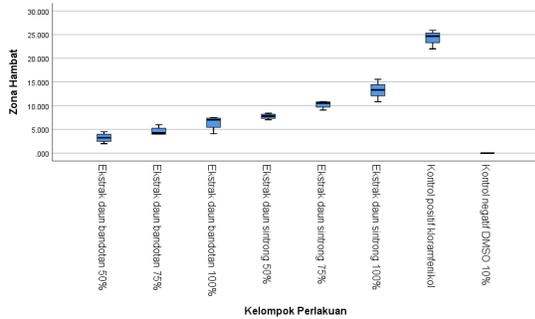


Figure 2. Results of Inhibitory Zone from Bandotan Leaf Extract and Sintrong Leaf Extract Group

Based on Figure 2, it can be concluded that the inhibition zone formed is in direct line with the increase in extract concentration.

Effectiveness of Bandotan Leaf Extract on the Growth of *Staphylococcus aureus* Bacteria

Effective concentration is the lowest concentration that is sensitive in inhibiting bacterial growth (Dewi et al., 2018). In the results of the study, the average inhibition zone of 50% bandotan leaf extract was 3.25 mm and included in the weak category according to Davis-stout. The average inhibition zone of 75% bandotan leaf extract was 4.625 mm and categorized as antibacterial with weak sensitivity according to Davis-stout while the average inhibition zone of 100% bandotan leaf extract obtained an inhibition zone of 6.4125 mm and included the moderate category according to Davis-stout (Almira et al., 2021; Winastri et al., 2020).

Based on this description, it can be concluded that bandotan leaf extract is effective in inhibiting the growth of

Staphylococcus aureus bacteria. The group with 100% bandotan leaf extract has high effectiveness, while the group with the lowest concentration that is still effective is 50% bandotan leaf extract.

Effectiveness of Sintrong Leaf Extract on the Growth of *Staphylococcus aureus* Bacteria

Effective concentration is the lowest concentration that is sensitive in inhibiting bacterial growth (Dewi et al., 2018). In the results of the study of the effectiveness of sintrong leaf extract, the average inhibition zone of 50% sintrong leaf extract was 7.78125 mm, including the moderate category according to Davis-stout sensitivity calculations. The average inhibition zone produced in 75% sintrong leaf extract was 10.525 mm and was classified as a moderate category. The average inhibition zone formed at 100% sintrong leaf extract was 13.28125 mm and included the strong sensitivity category (Almira et al., 2021; Winastri et al., 2020).

Based on this description, it can be concluded that sintrong leaf extract is effective in inhibiting the growth of *Staphylococcus aureus* bacteria. The lowest sensitive effective concentration is found in 50% sintrong leaf extract, while the best effectiveness extract group is 100% sintrong leaf extract.

Comparative Analysis of Bandotan Leaf Extract and Sintrong Leaf Extract on the Growth of *Staphylococcus aureus* Bacteria

The results of the analysis of differences in the inhibitory power of

bandotan leaf extract and sintrong leaf extract against the growth of *Staphylococcus aureus* bacteria can be seen in Figure 3 below.

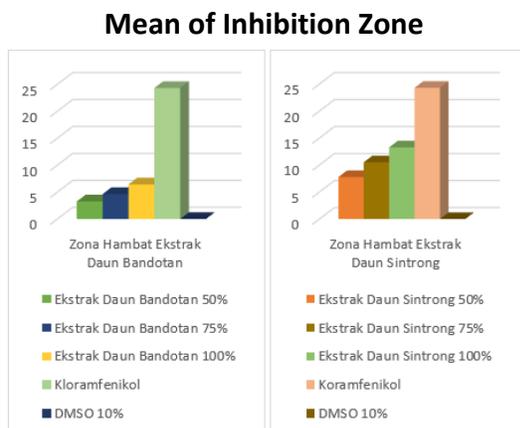


Figure 3. Graph of Average Inhibition Power of Bandotan Leaf Extract and Sintrong Leaf Extract against *Staphylococcus aureus* Bacteria

Figure 3 shows that the average inhibition of the 50%, 75%, and 100% sintrong leaf extract groups is higher than the 50%, 75%, and 100% bandotan leaf extract groups. Comparison of effectiveness for the sintrong leaf extract group obtained an average of ± 4.76250 mm while the effectiveness of the bandotan leaf extract group obtained an average of ± 10.44083 mm. Based on these results, it can be concluded that sintrong leaf extract has better effectiveness than bandotan leaf extract in inhibiting the growth of *Staphylococcus aureus* bacteria.

Discussion

Effectiveness of bandotan (*Ageratum conyzoides* Linnaeus) leaf extract on the growth of *Staphylococcus aureus* bacteria

Based on the results of the study, bandotan leaf extract (*Ageratum conyzoides*

Linnaeus) has the ability to inhibit the growth of *Staphylococcus aureus* bacteria. This is evidenced in bandotan leaf extract concentrations of 50%, 75%, and 100% forming a clear zone that has potential as an antibacterial. The results of this study are in accordance with research conducted by Dewi, et al (2018) on the difference in the inhibition zone of *Staphylococcus aureus* growth at various concentrations of biduri leaf ethanol extract in vitro which states that the effective concentration is the lowest concentration that has sensitivity in inhibiting the growth of *Staphylococcus aureus* bacteria. 50% bandotan leaf extract is the lowest concentration that is sensitive to the growth of *Staphylococcus aureus* bacteria with an average inhibition of 3.25 mm (Dewi et al., 2018).

In the observation of the antibacterial test of bandotan leaf extract (*Ageratum conyzoides* Linnaeus), an increase in antibacterial inhibition was obtained at each increase in extract concentration. This is in line with research conducted by Jungjunan, et al (2023) on the test of activity and antibacterial effectiveness of ethanol extract of bandotan leaves (*Ageratum conyzoides* Linnaeus) against *Staphylococcus aureus* bacteria which shows that the increase in antibacterial inhibition is proportional to the increase in extract concentration (Jungjunan, 2022).

Effectiveness of sintrong (*Crassocephalum crepidioides*) leaf extract on the growth of *Staphylococcus aureus* bacteria

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Based on the results of the research that has been done, sintrong leaf extract (*Crassocephalum crepidioides*) has the ability to inhibit the growth of *Staphylococcus aureus* bacteria. This is evidenced in the 50%, 75%, and 100% concentrations of sintrong leaf extract, a clear zone is formed which has the potential to inhibit the growth activity of *Staphylococcus aureus* bacteria. The results of this study are in line with research conducted by Dewi, et al (2018) on differences in the inhibition zone of *Staphylococcus aureus* growth at various concentrations of biduri leaf ethanol extract in vitro which states that the effective concentration is the lowest concentration that is sensitive in inhibiting the growth of *Staphylococcus aureus* bacteria (Jungjunan, 2022). 50% sintrong leaf extract is a group of sintrong leaf extracts with the lowest concentration in this study and is sensitive in inhibiting the growth of *Staphylococcus aureus* bacteria by forming an average inhibition of 7.78125 mm.

The results of the inhibition test of sintrong leaf extract in this study are in accordance with the results of previous research conducted by Malik, et al (2022) on the analysis of secondary metabolites and antibacterial sintrong leaves (*Crassocephalum crepidioides* (Benth.) S. Moore) against *Escherichia coli* which shows sintrong leaf extract has antibacterial activity at concentrations of 5%, 10%, 20%, and 30% by forming clear zones of 12.20 mm, 12.67 mm, 19.15 mm, and 20.85 mm respectively (Malik, 2022).

Comparative analysis of the effectiveness of bandotan (*Ageratum conyzoides* Linnaeus) and sintrong (*Crassocephalum crepidioides*) leaf extracts on the growth of *Staphylococcus aureus* bacteria.

Sintrong leaf extract is a group of extracts that have greater effectiveness than the effectiveness of bandotan leaf extract at the same type of concentration. This can occur because bandotan leaf extract (*Ageratum conyzoides* Linnaeus) and sintrong leaf extract (*Crassocephalum crepidioides*) have different amounts of secondary metabolite components. The content of secondary metabolite compounds in bandotan (*Ageratum conyzoides* Linnaeus) leaf extract quantitatively in the phytochemical screening results showed the composition of alkaloids (0.31 mg/g), saponins (0.13 mg/g), total phenol compounds (8.46 mg TAE g⁻¹), tannins (3.86 mg TAE g⁻¹), and flavonoids (5.80 mg QE g⁻¹) (Adeniji, 2021; Domithesa et al., 2021). As for the composition of secondary metabolite compounds in sintrong (*Crassocephalum crepidioides*) leaf extract quantitatively consists of tannins (1.05 mg TAE g⁻¹), flavonoids (7.59 mg QE g⁻¹), phenolic compounds (8.68 mg TAE g⁻¹), steroids (6.39 mg/100 g), and oxalic acid (7.8 mg/100 g) (Adeniji, 2021; Domithesa et al., 2021). Based on the difference in secondary metabolite content, it can be seen that sintrong leaf extract (*Crassocephalum crepidioides*) quantitatively has a greater antibacterial content compared to

bandotan leaf extract (*Ageratum conyzoides* Linnaeus).

Alkaloid compounds have an antibacterial mechanism of action by disrupting the peptidoglycan constituent components of bacterial cells. The disrupted peptidoglycan constituent components result in the death of bacterial cells because they cannot form a cell wall layer (Jungjunan, 2022).

Flavonoids are polar compounds that can penetrate the peptidoglycan layer of bacteria. These compounds work by forming complexes that bind to proteins and cause the bacterial cell membrane to be damaged by releasing intracellular compounds. Flavonoid compounds also inhibit cell membrane function by damaging membrane permeability and inhibiting the binding process of *ATPase* and *Phospholipase enzymes* (Yevani et al., 2023).

Phenolic compounds (saponins) have antibacterial ability by disrupting the stability of the cell membrane through the content of glycosyl groups (polar) and steroid groups (non-polar). The content of glycosyl groups and steroid groups in saponins can cause intracellular fluid to escape and lyse bacterial cells (Sogandi, 2018).

Tannin compounds have the ability to destroy the formation of bacteria through a system of inhibiting bacterial metabolic enzymes. This compound, will precipitate a protein that interferes with *reverse transcriptase* and *DNA topoisomerase enzymes* which ultimately results in the

inactivation of microbial cell adhesins and disrupts protein transport (Hasanah & Novian, 2020).

The antibacterial mechanism of triterpenoids works by forming polymeric bonds to transmembrane proteins of the outer cell wall which results in damage to the porin and reduces the permeability of the cell wall so that, bacteria become deprived of nutrients and there is a decrease in the number of bacteria. Steroid phytochemical compounds in the extract can react with lipids in bacteria which results in leakage of lysosomes in bacteria (Herrialfian et al., 2020).

The structure and composition of *Staphylococcus aureus* bacterial cells also play an important role in the mechanism of antibacterial inhibition. *Staphylococcus aureus* bacteria have thick peptidoglycan walls and contain teichoic acid which functions in the entry and exit of ions into bacterial cells (Hamida et al., 2023; Magvirah et al., 2020). This is in line with the research of Hamida, et al (2018) on the antibacterial activity of 96% ethanol extract of kecapi seeds (*Sandoricum koetjape* (Burm. F.) Merr.) against *Propionibacterium acnes* and *Escherichia coli* stating that differences in peptidoglycan components in bacterial cells can affect the effectiveness of the tannin mechanism of action. Tannins have stronger effectiveness against Gram-positive bacteria compared to Gram-negative bacteria.

Statistical analysis in this study showed that the inhibition zone data from bandotan and sintrong leaf extracts were

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normally distributed and homogeneous, with a $p > 0.05$ value. The One-Way ANOVA test resulted in a p value < 0.05 , indicating a significant difference in treatment against *Staphylococcus aureus* bacteria. In the Post-hoc Tukey HSD test, a significant difference was found in the treatment of 100% concentration of sintrong leaf extract against the growth of *Staphylococcus aureus* bacteria. However, bandotan leaf extract treatment showed no significant difference in the inhibition of *Staphylococcus aureus* bacterial growth. In an effort to compare the effectiveness of bandotan leaf extract and sintrong leaf extract at the same concentration, an Independent T-test was conducted and resulted in a p value = 0.000 ($p < 0.05$). This indicates a statistically significant difference between the ability of the two extracts to inhibit the growth of *Staphylococcus aureus* bacteria.

CONCLUSION

Based on results of study, it can be concluded that; (1) Bandotan leaf extract (*Ageratum conyzoides* Linnaeus) has the effectiveness in inhibiting the growth of *Staphylococcus aureus* bacteria with an average inhibition zone of 50% concentration of 3.25 mm, 75% concentration averaging 4.625 mm, and 100% concentration averaging 6.4125 mm (P value = 0.000), (2) Sintrong leaf extract (*Crassocephalum crepidioides*) has effectiveness in inhibiting the growth of *Staphylococcus aureus* bacteria with an average inhibition zone of 50%

concentration of 7.78125 mm, 75% concentration averaged 10.525 mm, and 100% concentration averaged 13.28125 mm (P value = 0.000), and (3) Sintrong leaf extract (*Crassocephalum crepidioides*) has a higher average comparison of ± 10.44083 mm, while bandotan leaf extract (*Ageratum conyzoides* Linnaeus) with an average of ± 4.76250 mm (P value = 0.000).

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First publication right:

Asian Journal of Engineering, Social and Health (AJESH)

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