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Antibacterial Activity Tests of *Staphylococcus aureus* and Phytochemical Screening in Family Asteraceae, Clusiaceae, Phyllanthaceae

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ABSTRACT

Infection is a type of disease that affects many residents of developing countries, including Indonesia. One pathogenic bacteria that is quite dangerous and causes skin infections both sporadically and endemically is *S aureus*. Several studies intensively report that some of the *Asteraceae*, *Clusiaceae* and *Phyllanthaceae* family plants contain alkaloids, flavonoids, saponins and tannins that have potential as antibacterial *S aureus*. Therefore, this study aims to identify effective plants to deal with infections caused by *S aureus* bacteria. To this end, the ingredients used are the leaves of *G. procumbens*, *E. scaber*, *G. mangostana*, *G atroviridis*, *G. xanthochymus*, leaves and bark of *A. neurocarpum*. Plant material extraction was done by maceration using 90% ethanol solvent. Next, an antibacterial test was conducted, which was begun by testing the Minimum Inhibitory Concentration followed by testing the Obstacle Area Width. The Minimum Inhibitory Concentration test was carried out using the agar dilution method, and the Obstacle Area Width test was carried out with paper diffusion discs. The results showed that all the plants have potential as antibacterials, and the mangosteen leaf extract has the largest Obstacle Area Width of 5.1 mm with a concentration of extract of 30% and a fairly strong inhibitory ability.

Keywords: Antibacterial Activity of *Staphylococcus Aureus, Asteraceae, Clusiaceae, Phyllanthaceae*

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INTRODUCTION

Infectious disease is a type of disease that often affects residents in developing countries, including Indonesia. One pathogenic bacteria that is quite dangerous and causes infection both sporadically and endemically is *S aureus*. *S aureus* bacteria colonize human skin and mucous membranes and are the most common cause of skin infections in the world with varying degrees of infection [1]. Moreover, when bacteria enter the bloodstream, they can spread to other organs and cause infections ranging from poisoning and minor skin infections such as acne and ulcers, to severe infections such as meningitis, osteomyelitis, pneumonia, and mastitis [2]. Infections are generally treated by administering antibiotics. However, according to Schwan [3], some *S. aureus* infections were resistant to various oral or intravenous antibiotics, such as penicillin, methicillin, cephalosporin, erythromycin, linkomycin, vancomycin, and rifampicin. Deyno et al. [4] also reported that *S. aureus* has become resistant to many antibiotics, including penicillin, cephalosporins, tetracyclines, chloramphenicol, methicillin, sulfonamides, and vancomycin. Furthermore, Kong et al. [5] concluded that some *S. aureus* isolates are resistant to methicillin, erythromycin, clindamycin, tetracycline, and ciprofloxacin. Therefore, alternative drugs that can control *S. aureus* bacterial infections must be identified.

Some plants have the potential to be developed as treatments for *S. aureus* bacterial infections. These include plants from the *Asteraceae* family, such as *Gynura procumbens* (Lour) Merr. Connect Lives and *Elephantopus scaber* (Limak Tread); those from the family *Clusiaceae*, such as *Garcinia xmangostana L* (Mangosteen), *Garcinia atroviridis* Griff. ex T.Anderson (Gelugur Acid), and *Garcinia xanthochymus* Hook.f. ex T.Anderson (Kandis Acid); and those from the family *Phyllanthaceae,* such as *Antidesma neurocarpum* Miq (Antidesma). According to Normaharon [38], G. procumbens leaf extract has antimicrobial potential. Rahman and Al-Asad [6] also stated that G. procumbens extract has antimicrobial activity and is cytotoxic. Nawi et al. [7] stated that the highest antimicrobial activity of G. procumbens methanol extract against S. aureus occurred at a concentration of 400 mg/ml with an inhibition zone of 10.5 mm.

Elephantopus scaber Linn, a small plant that grows wild throughout the tropical regions of the world [8], has been used in the treatment system in India as an analgesic, diuretic, astringent, and antiemetic drug. For example, *E scaber* leaves are used for treating bronchitis, smallpox, and diarrhea [9]. Daisy et al. [10] reported that terpenoids isolated from *E. scaber* have *S. aureus* antibacterial activity by inhibiting autolysin. Hiradeve and Rangari [11] also stated that various extracts of *E. scaber* root showed significant antibacterial and antifungal activity. Similarly, Kamalkannan et al. [12] found that the methanol extract of *E. scaber* has antibacterial activity toward *S. polyogenes*. However, the effect of *E. scaber* leaves on *S. aureus* bacteria has not been determined.

Garcinia is a genus of large trees or shrubs that grow in tropical regions, such as Asia. Chew and Lim [13] stated that the fruit of the Garcinia species is mostly edible and has many medicinal properties, including treating fever, urinary disorders, and open wounds [14]. One species of the Garcinia family, *G. mangostana*, has significant antibacterial activity [13]. Hamidon et al. [15] stated that *G. atroviridis*, another species in the Garcinia family, contains many active ingredients that can treat various diseases. Joseph et al. [16] reported that chemical compounds contained in *G. xanthochymus* have various biological activities, such as antioxidant, cytotoxic, antimicrobial, and anti-inflammatory

activity. This finding was supported by Murmu et al. [17], who stated that the fruit of *G. xanthochymus* is rich in various types of bioactive compounds, such as saponins, tannins, alkaloids, terpenoids, and phenolic compounds, that have antibacterial potential. *Neurocarpum antidesma* is a tree or shrub that grows up to 23 m high and 20 cm in diameter [18]. A neurocarpum leaf has efficacy as a wound dressing [19].

The purpose of this study was to determine the antibacterial activity of 96% ethanol extract of leaves of *G. procumbens, E. scaber, G. mangostana, G. atroviridis,* and *G. xanthochymus*, as well as the leaves and stem bark of *A. neurocarpum*. The hypothesis was as follows: 96% ethanol extract of *G. procumbensleaves, E. scaber, G. mangostana, G atroviridis,* and *G. xanthochymus,* as well as *A. neurocarpum* leaves and bark will show antibacterial activity against *S. thypi* bacteria. Antibacterial tests were performed on the six species of plants with potential antibacterial activity toward *S. aureus*. Bacterial testing began by extracting plant material through maceration, and then the extracts were tested for minimum inhibitory concentration (MIC) and obstacle area width (OAW). The MIC test was performed using the dilution method so that the inhibition of antibacterial compounds was marked by the absence of growth of bacterial colonies on the media [20]. The OAW test was performed using the paper disc diffusion method according to Shryock et al. [21]. In the OAW test, a clear zone formed around the disc paper. According to Torar [22], the clear zone indicates the extract's ability to inhibit bacterial growth.

MATERIALS AND METHODS

A. Materials and Tools

For the present study, leaves of *G. mangostana*, *G. procumbens,* and *E. scaber* were obtained from the Spice and Aromatic Plant Research Institute (BALITTRO) Jl. Student Army No. 3 Bogor. Moreover, *G atroviridis, G. xanthochymus*, and *A. neurocarpum* were procured from the Botanical Garden Conservation Center (BGCC), the Indonesian Institute of Sciences in Bogor. All plant materials were determined at the Bogoriense Herbarium, Botany, Biology Research Center-LIPI, Cibinong. In addition, *S aureus* isolate was acquired from the Microbiology Lab, Bogor Agricultural University, while amoxicillin (which served as a positive control) was purchased from the Kimia Farma. Dimethyl sulfoxide 10% (which served as a negative control), acid chloride, ethanol 96%, iron (III) chloride solution, magnesium, Nutrient Agar media, Bouchardat reagent, Dragendorf reagent, and Mayer reagent.

The tools used in this study included: glassware (Pyrex®), autoclaves (All American®), maceration bottles, bunsenes, steam plates, Petri dishes (Pyrex®), grinders (Airlux®), incubators (Memmert®), disc paper (whatman number 40) 6 mm in diameter, crucible, micipipette (Proline plus®), digital balance (Lab pro®), oven (Memmert®), mesh 40 sieve, and furnace (Vulcan A-550®).

B. Methods

1) Simplisia Powder Manufacturing

Fresh leaves and bark utilized in the study were sorted, washed, dried in an oven at 500 °C until dry, then mashed and sieved using a mesh 40 sieve to obtain simplisia powder [23], [24]. Simplisia powder was subsequently characterized by measuring yield, water content, ash content, and organoleptic characters, including color, aroma and taste. Water content and ash content was measured by the gravimetric method, and the following equation was utilized for Powder Yield Calculation (PYC):

$$PYC = \frac{\text{The weight of the powder / extract obtained}}{\text{Simplisia weight / extract}} \text{ x } 100\% \quad (1)$$

2) Extraction Process

Leaf and bark extracts were obtained via the maceration method using 96% ethanol (1:10) solvent in stages. Initially, the simplisia powder was added to the bottle with 96% ethanol as much as half the total solvent. The content was shaken at 6-hour intervals for 24 hours, after which it was filtered to separate the pulp and filtrate. The pulp was macerated again using 96% ethanol solvent. Maceration was repeated twice, each time using the remaining quarter of the solvent, and progressing in this manner until no solvent remained. The thus described maceration process required to obtain one type of extract lasted for three days, and the resulting filtrate was further concentrated using a rotary evaporator until a thick extract was obtained [23], [24]. At the end of this stage, there were seven types of 96% ethanol extract, namely *G. procumbens*, *E. scaber*, *G. mangostana*, *G. atroviridis*, *G. xanthochymus*, and *A. neurocarpum* leaf extracts, as well as *A. neurocarpum* bark extract. For all extracts produced, yield, water content, ash content, color and aroma were assessed. As noted previously, water and ash content were calculated by the gravimetric method, while the yield was calculated by applying Equation (1).

3) Phytochemical Screening

Phytochemical screening was carried out qualitatively (using the Tiwari method [25]) for all extracts, aiming to ascertain flavonoid, tannin, saponin and alkaloid content.

4) Inoculum Preparation

a) Bacterial Media Production

For this part of the study, Nutrient Agar (NA) served as the medium, which was prepared by dissolving 28 g of NA powder in 1,000 mL of aquadest, after which the solution was heated and stirred until homogeneous and of clear color. Next, it was sterilized in an autoclave at 121 °C for 15 minutes at a pressure of 1 atm. Finally, 20 mL of the still-warm medium was added into each sterile Petri dish and was left to solidify.

b) Bacterial Regeneration Test

Bacteria derived from primary culture were cultured into NA media in a Petri dish, by taking one ose, after which they were etched into the agar medium, and were subsequently incubated for 24 hours at 37 °C. The growing cultures were stored in a refrigerator at 4 °C.

c) Determination of MIC and OAW

MIC was determined by applying the agar dilution method. For this purpose, 0.2 mL of *S. aureus* bacteria culture at 10^{-6} concentration was placed in 20 mL of NA medium at 400 °C. Next, 1 mL of extract at the test concentration was added, after which the sample was incubated for 24 hours at 37 °C in an incubator. After incubation, bacterial growth was observed. The lowest concentration of extract that is not overgrown with bacteria was interpreted as MIC. The minimum inhibitory concentration was used for determining the next concentration for the OAW test. The concentration of extract test in the determination of OAW is presented in Table 1.

Table 1: Concentration of extract test in MIC designation

Extract type	Extract test concentration (%) to.....				
	1	2	3	4	5
Leaf *G. procumbens*	4	6	8	10	12.5
Leaf *E. scaber*	4	6	8	10	12.5
Leaf *G.mangostana*	4	6	8	10	12.5
Leaf *G atroviridis*	4	6	8	10	12.5
Leaf *G. xanthochymus*	4	6	8	10	12.5
Leaf *A.neurocarpum*	10	20	25	30	40
A.neurocarpum bark	10	20	30	40	50

OAW was determined using the paper disc diffusion method. A total of 0.2 mL of bacteria with a concentration of 10-6 was poured into a petri dish containing NA media at 40°C. After solidifying the paper, the disc containing the extract according to the concentration was placed and then incubated for 24 hours at 37°C. Antibacterial activity is identified by the emergence of inhibitory zones, namely the clear zone around the disc, so inhibition of bacterial growth can be determined from the width of the inhibitory zone around the disc paper [24]. The OAW calculation used the following formula:

$$\text{OAW} = \frac{(IAD) - (PDD)}{2} \qquad (2)$$

where OAW is the obstacle area width, IAD is the inhibition area diameter, and PDD is the paper disc diameter.

The experimental design used in this study was a completely randomized design with four treatments of extract concentration and three replications. If the analysis of variance showed that the treatment had a significantly different effect, then the real difference was determined using Duncan's test.

RESULTS AND DISCUSSION

C. Manufacturing Simplisia Powder

Manufacturing the powdered plant material showed different yields, with woody plants generating higher yields than herbaceous plants. For woody plants, the yield ranged from 32.5% to 60%, whereas for herbal plants, the yield ranged from 14.34% - 16.67%. The results of the water content and ash content measurements for all powder simplicia met the applicable requirements, namely <10% for water content and <7% for ash content [24], [25], [26]. The results of the determination of the ash content of the simplicia powder of the liman leaf met the requirements, i.e., ash content of less than 19.4% [23], [24]. The results of the characterization of powder simplicia are presented in Table 2.

Table 2: The results of the simplicia characterization of plant material powder in the study

Simplisia	Rendemen (%)	Water content (%)		Ash levels (%)		Taste	Smell	Color
		Measured	Requirement	Measured	Requirements			
Leaf *G. procumbens*	14.34	2.21	< 10	6.33	< 7	It's a bit bitter	Typical	Brownish
Leaf *E. scaber*	16.67	3.50	< 10	3.44	< 7	Tasteless	Typical	Green
Leaf *G.mangostana*	60.00	3.20	< 10	3.44	< 7	Tasteless	Typical	Green
Leaf *G atroviridis*	40.00	5.10	< 10	3.69	< 7	Tasteless	Typical	Dark green
Leaf *G. xanthochymus*	32.50	5.43	< 10	3.07	< 7	Tasteless	Typical	Brown
Leaf *A.neurocarpum*	42.00	6.13	< 10	3.29	< 7	Tasteless	Typical	Reddish
A.neurocarpum bark	52.33	6.13	< 10	4.79	< 7	Tasteless	Typical	Dark green

Determination of water content is done to determine the amount of water contained in the powder and extracts simplicia. High water content can enable microbial growth and thus cause damage to the compounds contained within. Determination of ash content was carried out to determine the mineral content contained in the simplicia powder. The water content and ash content of all the simplicia powders from the study results met the requirements of Kartini et al. [24] & Paisey et al. [26], which is a water content of less than 10% and an ash content of less than 7%.

D. Extraction Results

The maceration extraction method was used in this study. This method was chosen because it uses simpler equipment and natural materials are not easily decomposed by heating. We used 96% ethanol in our extraction because this solvent can dissolve almost all organic compounds, both polar and non-polar, and has a low boiling point, so it is easily evaporated [24]. The results of all extracted material in the form of a thick extract with characterization are presented in Table 3.

Table 3: Results of extract characterization

Extract type	Simplisia (g)	Extract (g)	Rendemen (%)	Water content (%)		Ash levels (%)	
				Measured	Requirements	Measured	Requirements
Leaf *G. procumbens*	400	40.1	10.03	18.70	5-30	3.46	< 5
Leaf *E. scaber*	400	47.0	11.75	11.50	5-30	4.23	< 5
Leaf *G.mangostana*	300	37.7	12.57	9.72	5-30	4.49	< 5
Leaf *G atroviridis*	500	88.35	17.67	9.66	5-30	4.50	< 5
Leaf *G. xanthochymus*	200	55.74	27.87	9.80	5-30	4.55	< 5
Leaf *A.neurocarpum*	500	160.1	32.02	6.442	5-30	1.58	< 5
A.neurocarpum bark	300	83.56	27.85	11.25	5-30	2.63	< 5

Extraction results show that the yield produced for all ingredients ranged from 10% to 32.02%. In general, the yield of woody plants was higher than herbal plants. Woody plants range from 12% to 32%, while herbaceous plants range from 10.03% to 11.75%. Extraction results showed all extracts had water content and ash content that met the requirements of Kartini et al. [24] & Paisey et al. [26], namely for extract water content between 5-30% and for extract ash content not more than 5%, as shown in Table 3.

The yield of *G. procumbens* leaf extract obtained is not much different from the results of the research by Yusoff et al. [27], which is 10.07%. *E. scaber* leaves produce a greater yield than the research conducted by Aldi et al. [28], which is 9.13%. This can be understood because the solvent used is 70% ethanol. The yield of *G.mangostana* leaf extract yielded was lower in the Sinaga and Siregar [36] research, which reached 17%. The yield of *A. neurocarpum* leaf extraction was 32.02%, which is different from what Elya et al. [37] reported that the percentage of yield obtained was 36.54%.

E. Phytochemical Screening Results

Phytochemical tests are carried out to determine the compounds contained in a plant. The results of phytochmia testing from all three families are presented in Table 4.

Table 4: Phytochemical screening results

Simplisia	Chemical compounds			
	Alkaloid	Flavonoid	Saponin	Tanin
Asteraceae				
Leaf *G. procumbens*	+	+	+	+
Leaf *E. scaber*	+	+	+	+
Clusiaceae				
Leaf *G.mangostana*	+	+	+	+
Leaf *G atroviridis*	+	+	+	+
Leaf *G. xanthochymus*	+	+	+	+
Phyllanthaceae				
Leaf *A.neurocarpum*	+	+	-	+
A.neurocarpum bark	+	+	-	+

Phytochemical test results on leaf extracts of the family Asteraceae and Clusiaceae, *G. procumbens, E. scaber, G. mangostana, G atroviridis,* and *G. xanthochymus* positively contain alkaloids, flavonoids, saponins, and tannins. In the family Phyllanthaceae, both leaf extracts and *A.neurocarpum* bark contain alkaloids, flavonoids, and tannins, but do not contain saponins. Thus the 7 extracts above have the potential to be antibacterial. According to Rahman et al. [29] & Xie et al. [30], although not specifically described, flavonoids can be used as antibacterial through 3 mechanisms, namely inhibiting nucleic acid synthesis, inhibiting cell membrane function and inhibiting energy

metabolism. The same thing was stated by Wahyuningtyas [31], flavonoid compounds can inhibit microorganisms because of their ability to form complex compounds with proteins. Medium Ozolua [32] states that flavonoids can cause coagulation in membrane proteins so that the cell wall is damaged and results in bacterial cell lysis.

Alkaloids can otherwise disrupt the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed intact [33]. While Tiwari et al. [25] states that the mechanism of action of alkaloids as an antibacterial is to interconnect bacterial cell walls. The mechanism of tannin as an antibacterial is by deactivating bacterial adhesin, inhibiting the action of enzymes and inhibiting protein transport in the envelope of bacterial cells [34], [35]. According to Hayati et al. [20], tannin compounds are secondary metabolites in plants that are as antibacterial by forming stable bonds with proteins so that bacterial protoplasm coagulation occurs. Saponin compounds also function as antibacterial. Saponins will interfere with cell wall surface tension, when surface tension is disturbed, antibacterial compounds will easily enter the cell and will disrupt metabolism until bacterial death occurs [33].

F. Minimum Inhibitory Concentration (MIC) Test Results

The MIC test was carried out using the dilution method so that the MIC test results showed different plant parts in the same plant could be different. This shows that the content of active compounds that have the potential to be antibacterial can only be produced or stored in different organs in plants. The results of the MIC extract test are presented in Table 5.

Table 5: Test results for minimum inhibition concentrations

Extract type	Minimum inhibition concentrations (%)
Leaf *G. procumbens*	10
Leaf *E. scaber*	10
Leaf *G.mangostana*	10
Leaf *G atroviridis*	10
Leaf *G. xanthochymus*	10
Leaf *A.neurocarpum*	30
A.neurocarpum bark	40

Table 5 shows that the 96% ethanol extract of the leaves of *G. procumbens, E. scaber, G. mangostana, G. atroviridis* and *G. xanthochymus*, had a stronger MIC when compared to other extracts, at a concentration of 10%. Extracts that had the weakest MIC were ethanol extract 96% of leaves and *A.neurocarpum* bark, at a concentration of 30%. The Minimum Inhibition Concentration is determined by the content of the active substance present in the extract, while the content of the active ingredient in the extract is influenced by internal and external factors. Internal factors are influenced by plant genes, while external factors are more influenced by plant ecophysiology.

G. Obstacle Area Width (OAW) Test Results

The results of the analysis of variance showed that the extract of *G. procumbens* leaves gave a significantly different effect on OAW of *S. typhi* bacteria. Inhibitory width for extracts of 10%, 20% and 30% had OAW that were not significantly different, but all three were significantly different from positive control and included in the weak categories. Analysis of variance for *E scaber* leaf extracts showed a significantly different effect on OAW of *S. typhi* bacteria. OAW for extracts of 10%, 20% and 30% had LDH that were not significantly different, but all three were significantly different from positive controls and were included in the weak category.

The results of the analysis of variance showed that *G. mangostana* leaf extract had a significantly different effect on OAW of *S. typhi* bacteria. Extract 10%, have significantly different OAW extract 20%, and 30%, also extract 20% significantly different from extract 30%. Extracts of 10%, 20% and 30% were significantly different from positive controls. The highest OAW extract value of *G. mangostana* leaves is at a concentration of 30% which is 5.1 mm, and classified as quite strong in katori. The overall OAW test results table is presented in Table 6.

Table 6: Test results of extract inhibitory width

Extract type	Concentration (%)	OAW (mm)
Asteraceace		
Leaf *G. procumbens*	10	$3.6^{a} \pm 0.58$
	20	$4.1^{a} \pm 0.58$
	30	$4.5^{a} \pm 0.58$
Amoxicillin 10 ppm	10 ppm	$8.6^{b} \pm 0.58$
Leaf *E. scaber*	10	$4.1^{a} \pm 0.29$
	20	$4.1^{a} \pm 0.29$
	30	$4.1^{a} \pm 0.29$
Amoxicillin	10 ppm	$8.0^{b} \pm 0.29$
Clusiaceae		
Leaf *G.mangostana*	10	$4.1^{a} \pm 0.29$
	20	$4.6^{b} \pm 0.29$

	30	$5.1^{c} \pm 0.29$
Amoxicillin 10 ppm	10 ppm	$9.5^{d} \pm 0.00$
Leaf *G atroviridis*	10	$1.6^{a} \pm 0.29$
	20	$2.1^{b} \pm 0.29$
	30	$2.6^{b} \pm 0.29$
Amoxicillin	10 ppm	$9.5^{c} \pm 0.00$
Leaf *G. xanthochymus*	10	$2.5^{a} \pm 0.50$
	20	$3.0^{b} \pm 0.50$
	30	$3.5^{c} \pm 0.50$
Amoxicillin	10 ppm	$9.5^{d} \pm 0.00$
Leaf *A.neurocarpum*	30	$3.50^{a} \pm 0.00$
	40	$3.83^{b} \pm 0.29$
	50	$4.00^{b} \pm 0.00$
Amoxicillin 10 ppm	10 ppm	$11.5^{c} \pm 0.29$
A.neurocarpum Bark	30	$4.33^{a} \pm 0.29$
	40	$4.50^{a} \pm 0.00$
	50	$4.50^{a} \pm 0.00$
Amoxicillin	10 ppm	$8.00^{b} \pm 0.00$

Numbers followed by the same letter show no significant difference according to the Duncans test at $\alpha = 5\%$

Analysis of variance for *G atroviridis* leaf extract, *G. xanthochymus*, showed a significantly different effect on OAW of *S. typhi* bacteria. Inhibitory width for extracts of 10%, 20% and 30% have lower OAW compared to positive controls and are still classified in the weak category [38].

Analysis of variance for *A. neurocarpum* leaf extract and A.neurocarpum bark extract showed a significantly different effect on OAW of *S. typhi* bacteria. Inhibitory width for extracts of 30%, 40% and 50% have lower OAW with positive control and are still classified in the weak category [38].

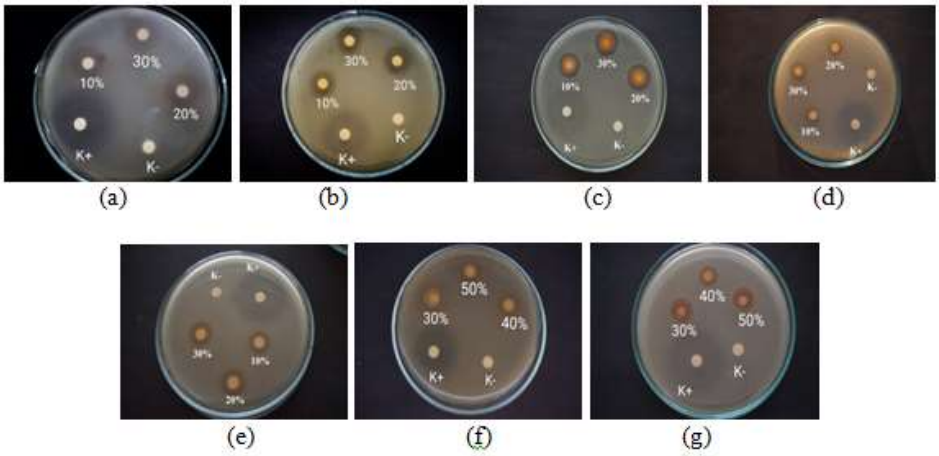

Figure 1: AOW results of leaf extract: (a) *G atroviridis,* (b) *E. scaber,* (c) *G. mangostana,* (d) *G atroviridis,* (e) *G. xanthochymus,* (f) *A.neurocarpum,* (g) and bark skin *A.neurocarpum* against *S. Aureus* bacteria

CONCLUSION

This study aimed to find effective plants to treat infections caused by *S. aureus* bacteria. Based on the results of the study, seven plant extracts from three families had potential as an antibacterial, but only *G. mangostana* leaf extract was a strong enough antibacterial at the extract concentration of 30% with 30% OAW of 5.1 mm.

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