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adelila antibacterial

by Sri Adelila

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Antibacterial Activity of Sidaguri Plant Extracts (*Sidarhombifolia L.*) against Oral and Dental Bacteria

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2

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Abstract. Dental and oral diseases can be caused by *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus mutans*. This study was aimed to determine the potential of Sidaguri (*Sidarhombifolia L.*) plant extract against some bacteria, i.e., *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus mutans*. The methods used in determining the inhibition zone and MIC were paper disc diffusion and MIC using dilution methods. Phytochemical test results indicated that the Sidaguri plant was contained alkaloids, flavonoids, tannins and saponins. The inhibition zones of antibacterial activity of Sidaguri plant extract against those bacteria were found to be 7.9; 6.8; and 10.3 mm, respectively for *S. aureus*, *E. faecalis* and *S. mutans*. However, the MIC and MBC values for *S. Aureus* were found to be 625 ($\mu\text{g/mL}$) and MIC values for *E. faecalis* were of 625 ($\mu\text{g/mL}$) with MBC values > 5000 ($\mu\text{g/mL}$). The MIC and MBC for *S. Mutans* were up to 1250 ($\mu\text{g/mL}$) and 5000 ($\mu\text{g/mL}$). This study concluded that *S. Rhombifolia* was a bactericidal to *S. aureus* and *S. mutant*. In addition, it was a bacteriostatic to *E. faecalis*.

Keywords: Antibacterial Activity, Sidaguri, (*Sidarhombifolia L.*), Oral and Dental Bacteria.

1 Introduction

The oral cavity is the first gate in the digestive system. The oral cavity also plays a major role in one's health and well-being. One effort to improve health is to maintain oral and dental hygiene (Nonong, 2014). Based National Basic Health Research in 2013 year, the prevalence of dental and oral health problems in Indonesia was of 25.9%. It might due to the lack of awareness and knowledge of the public about the importance of dental and oral health, so that disease in the oral cavity could be happened (Tampubolon, 2016). People in Indonesia have not considered dental and oral health. People were tended to ignore toothache, even though dental disease was the first type of disease complained by the community and children (Nurhidayat et al,

2012). Dental and oral diseases were also could be caused by *Staphylococcus aureus*, *Enterococcus faecalis* and *Streptococcus mutans*.

Plant of Sidaguri was belonged to the genus *Sida Malvaceae* family, which has been used as a traditional medicine, including to treat rheumatism, asthma, influenza, toothache and reduce pain in swelling due to toothache (Kinho et al, 2012; Sari, 2012). Therefore, this study was aimed to report a class of secondary metabolites from Sidaguri (*Sidarhombifolia L.*) plant in the North Sumatra and antibacterial activity that causes oral and dental diseases.

2 Materials And Methods

2.1 Apparatus and Chemicals

Apparatus used in this study were included analytical balance, a set of glassware, blender, vacuum pump, Buchner funnel, rotary evaporator (Heidolph), vortex, incubator (Memmert), autoclave (Tomy), and laminar (B-One). In addition, some chemicals used were acetone (technical), FeCl₃ 10%, acetic acid, amyl alcohol, concentrated HCl, and distilled water. Some reagents such as Mayer, Wagner, and Dragendorff reagents. Other chemicals needed were concentrated H₂SO₄, NaOH 10%, Mg powder, chloroform, Mueller-Hinton Agar (MHA) (Oxoid), Mueller-Hinton Broth (MHB) (Himedia), dimethylsulfoxide (DMSO) p.a (Sigma Aldrich), chloramphenicol disc paper (Oxoid), 0.9% NaCl bacterial culture *Enterococcus faecalis* ATCC 49619, *Staphylococcus aureus* ATCC 25923 and *Streptococcus mutans* ATCC 35668.

2.2 Plant Extraction

Preparation of materials and sample extraction

The dried sample was obtained from the store called Herbal Sempurna Sambu. It was blended into a powder to 200 grams in weight, then macerated with acetone for 3 x 24 hours. The macerated sample was filtered using a Buchner funnel. Furthermore, the filtrate was evaporated using a rotary evaporator to produce an extract.

2.3 Phytochemical Test

Alkaloid Test

Test with Mayer reagents. 2 mg of the extract was dissolved in acetone. Then 4-5 drops of Mayer reagent were added. The results were called positive if white precipitate was formed. Test with Wagner reagents. Two mg of the extract was dissolved in acetone. Drops of Wagner reagents as the amount of 4-5. If a brown precipitate appeared, it meant positive results. Test with Dragendorff reagent. A two mg of the extract was dissolved in acetone. Then add 4-5 drops of Dragendorff reagent. Positive results are marked as they were red/orange deposits.

Test for Flavonoids

Wastewater test. A twenty mg of acetone extract. *Rhombifolia L.* was added to 1000 mL of hot water, boiled for five minutes and filtered with filter paper. The filtrate was put into a test tube and then added with enough magnesium powder with one ml of concentrated hydrochloric acid and five ml of amyl alcohol. It was strongly shaken, then allowed to separate. The formation of red in the amyl alcohol layer indicated the presence of flavonoid compounds.

Test with NaOH 10%. A two to four mL of 10% NaOH was added to extract *S. rhombifolia L.* Positive results were indicated by a yellow change.

Steroid/Triterpene

Test (Liebermann-Buchard test). A 50-100 mg of *S. rhombifolia L.* extract was placed on a drop plate and acetic acid was added until all the samples were submerged. It was left for 15 minutes, then 6 drops of the solution were transferred into a test tube. After that, a 2-3 drops of concentrated sulphuric acid were added. The colour changes that occur were observed. The

resulting colour intensity was used as a relative measure of the content of triterpenoids and steroids in the sample. The presence of triterpenoids was indicated by the occurrence of orange or purple red, while the presence of steroids was indicated by the formation of blue.

Tanin

Test. A 2gr of extract *S.rhombifolia L.* Was put into a test tube and diluted with acetone. After that, 3 drops of FeCl_3 10% was added. A positive result when a blackish green precipitate was formed.

Saponin

Test. A 2gr of *S.rhombifolia L.* extract was put in a test tube and diluted with 70% ethanol. After that, a 10 mL warm water was added, then shaken for 30 minutes. It was left for 10 minutes and if the foam was not lost, it was added with concentrated HCl. If there was a constant foam, then it showed positive results.

Antibacterial Activity Test (CLSI, 2012).

Sample Preparation. Before the antibacterial test, a test solution (sample) was prepared by weighing 100mg of *S.rhombifolia L* leaf extract and dissolved in DMSO 100%. Did dilution amounted 10 times to obtain a 1% solution in DMSO in 10%. If the sample was not dissolved in 10% DMSO then dilution was carried out with DMSO in 100%, which was equivalent to 1000 $\mu\text{g/mL}$.

Preparation of Inoculum Suspension.

The inoculum was prepared based on the growth method by taking 3-5 bacterial colonies using a sterilized *cotton bud*, then transferred into a tube which contained 4-5mL 0.9% NaCl. Furthermore turbidity was adjusted to 0.5 McFarland turbidity.

Determination of Inhibitory Zones with Paper Disc Diffusion Method (CLSI-M02-A11, 2012).

It was begun by inserting 100 μL of inoculum onto the gel medium, then levelling by using a spider. Let stand for about five minutes. The disc paper which contained the chloramphenicol and empty antibiotics was placed, so that each of the disc was found to be 24mm apart on the plate surface. The paper disc was pressed firmly on the surface. It could be ascertained that the disc paper was in direct contact with the gel plate inoculum. On a blank paper disc, it dropped 20 μL of test solution and another blank disc paper was dripped with 100% and 10% of DMSO solvents. The plate was closed and placed into an incubator at 37°C for 18-24 hours. The emergence of bacterial growth inhibition zones was characterized by the presence of clear areas around the disc paper which was then measured using callipers, so that the diameter of the inhibitory zone of bacterial growth was obtained. The positive control used was a 30 μg chloramphenicol antibiotic disc, while the DMSO solvent used as a negative control.

Determination of MIC by micro dilution method (M02-A11 CLSI, 2012).

Determination of MIC was carried out by inserting MHB liquid media that has been suspended with bacteria into each microplate hole from the second to the twelfth column as much as 100 μL . The first column of microplate was filled with 100 μL of liquid medium (negative control), while for the second column was filled with 100 μL of liquid medium containing bacterial suspension (positive control). A 1000 $\mu\text{g/mL}$ test solution was entered starting from column twelve. The concentration of the test solution was carried out by

transferring 100 μ L of solution from the twelfth hole to the eleven hole. From the eleven holes, it was taken as much as 100 μ L and put into the ten hole. The same thing was done to the third hole. The amount of solution in each hole was of 100 μ L. Micro plate was then incubated at 37 $^{\circ}$ C for 24 hours. Positive control used in this study was chloramphenicol with a concentration of 500mg/mL.

Determination of MBC. MBC determination was carried out by inoculating all test solutions as much as 10 μ L from each hole from the micro plate, then it was grown on gel MHA media and incubated at 37 $^{\circ}$ C for 24 hours.

3 Results And Discussion

Extraction. The extraction of samples (200g) of Sidaguri plants were carried out by maceration technique with acetone for 3 x 24 hours. It has been produced 3.3 gr of green crude extract. Results of phytochemical test were *Srhombifolia L.* plant was contained alkaloids, flavonoids, tannins and saponins.

The results of inhibitory zone test can be seen in Table 1. The method used in this determination was the paper disk diffusion method. This method was done by placing paper discs that have been dripped with a test solution on solid media those has been inoculated with bacteria. Bacterial growth was observed to see clear zones around the disc. The choice of this method was due to easy and simple in determining the antibacterial activity of the sample being tested. The disc paper used was 0.6 cm in diameter. According to Davis and Stout (1971), that the inhibitory area of >20mm or more, meant having very strong antibacterial activity. In addition, the resistance area was of 10-20mm (strong antibacterial activity), 5-10mm (moderate activity), and below 5mm (weak activity).

Table 1. Inhibitory Zone Results of *S. Hombifolia L.* Extract.

	<i>S.aureus</i>	<i>E.faecalis</i>	<i>S.mutans</i>
	Inhibitory Zone (mm)		
DMSO 10%	0	0	0
Kloramfenik	33,8	9,3	26
<i>S. rhombifolia L.</i> extract	7,9	6,8	10,3
DMSO 10%	0	0	0

Determination of the Inhibitory Zone. Fig.1 (a) shows the inhibitory zone, whereas in the medium category to inhibit the growth of *S. Aureus* bacteria. Likewise for *E.faecalis* bacteria which have an inhibition zone of 6.8 mm as Fig.1 (b). While in *S. mutans* bacteria, the inhibitory zone was formed in 10.3mm (Fig.1c) indicated that it has strong potential to inhibit bacterial growth. A positive control was chloramphenicol. Inhibitory zone against the bacteria were *S. aureus*, and *S. Mutans E.faecalis* of 33.8mm (Fig.1a); 9.3mm (Fig.1b), and 26mm (Fig.1c), respectively. The difference in diameter of the inhibitory zone between extracts with chloramphenicol standard used was likely because the extract was still a crude extract, so affected to its ability to inhibit bacterial growth.

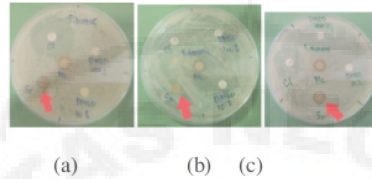


Fig. 1. The Results of inhibition zone test on:

(a) *S. aureus*, (b) *E. faecalis*, and (c) *S. mutans*

Determination of MIC and MBC. Based on Table 3, acetone extract in all parts of the sidaguri plant with a concentration of 10,000 $\mu\text{g/mL}$ has an inhibitory zone of 7.8 mm. An extract was categorized as active, if the MIC value was $<100 \mu\text{g/mL}$, medium ($100 < \text{MIC} < 625 \mu\text{g/mL}$), and was not active if the MIC value was $>625 \mu\text{g/mL}$ (Dzoyemet al, 2012). Based on Table 3, the MIC value of *S. rhombifolia L* extract was of 625 $\mu\text{g/mL}$ in the medium category against *S. Aureus* and *E. faecalis*. However, it has been found that 1250 bacteria against *S. Mutans* with inactive category.

According to Pankey and Sabbath (2004), the ways of antibacterial working were bacteriostatic and bactericidal. The results of the determination of MIC and MBC of sidaguri plants can be seen in Table 2.

Table 2. MIC and MBC values of *S. rhombifolia L.* extract against test bacteria

	S. aureus		E. faecalis		S. mutans	
	MIC	MBC	MIC	MBC	MIC	MBC
	$\mu\text{g/mL}$					
Chloramphenicol	0,48	7,8	7,8	250	0,97	125
<i>S. rhombifolia L.</i>	625	625	625	>5000	1250	5000

Value of MBC extract of *S. rhombifolia L.* was amounted to 625 $\mu\text{g/mL}$ against *S. aureus* bacteria which meant at 625 $\mu\text{g/mL}$ could not only inhibit but also could be killed (bactericidal). As for the *E. faecalis* bacteria, the MBC value was extracted *S. rhombifolia L.* to be $> 5000 \mu\text{g/mL}$ meant sidaguri was only inhibitory (bacteriostatic).

The values of MBC extract of *S. rhombifolia L.* was found to be 5000 $\mu\text{g/mL}$ against *S. Mutans* bacteria, which means *rhombifolia S. L.* extract was a bactericidal with two fold-killing ability of the ability to block it. Based on the phytochemical screening results, all parts of the *S. rhombifolia L.* Plant contained alkaloids, flavonoids, tannins and saponins. Based on literature studies, Sidaguri plants contain alkaloid alkaloid indoquinolin compounds that were isolated from the plant Sidaguri, namely quindolinone; 11-methoxy-quindoline; quindoline [10], so that the possibility of these compounds acting as antibacterials. The alkaloid mechanism that was suspected by disrupting the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer was not formed intact and causes the cell death (Ajizah, 2004). However, *S. rhombifolia L.* also contained flavonoid compounds namely flavonol-3-O-Galactose (Jubahar et al, 2013). Kaempferol; kaempferol-3-O- β -D-

glycosyl-6"- α -D-rhamnose (Ajizah, 2004), were likely to act as an antibacterial. The mechanism of action of flavonoids in inhibiting bacterial growth, namely flavonoids, causes damage to the permeability of bacterial cell walls (Kurniawan, 2014).

S. rhombifolia L. al⁵ contained tannins and saponins (Wake et al, 2013; Heinichen et al, 2017; Azad et al, 2017). The mechanism of action of saponins was included in the antibacterial group which disrupts the permeability of bacterial cell membranes, which results in damage to cell membranes. It could be caused the release of various important components in bacterial cells name¹ proteins, nucleic acids and nucleotides Based on research (Nurhidayat et al, 2012). The mechanism of the action of tannin as an antibacterial by causing the cell would become lysis (Ngajow et al, 2012).

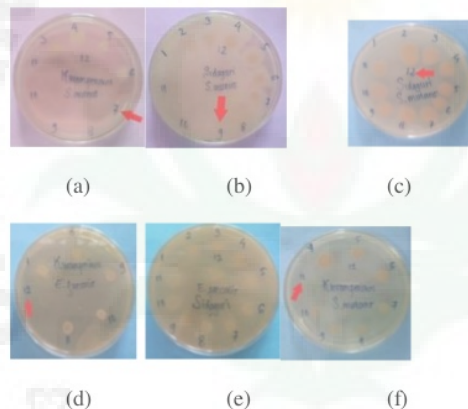


Fig. 2. MBC test results: (a) Chloramphenicol; (b) Sidaguri extract against *S. aureus* bacteria; (c) Chloramphenicol; (d) Sidaguri extract against *E. faecalis*; (e) Chloramphenicol; (f) Sidaguri extract against *S. mutans*.

4 Conclusions

Based on phytochemical tests that have been carried out, the secondary metabolites found in plants of *S. rhombifolia* L. was an alkaloid, flavonoid, tannin and saponin. Extracts of *S. rhombifolia* L. have the potential as an antibacterial agents. The best activity against *S. aureus* and *E. faecalis* MIC values were found to be 625 μ g/mL and 625 μ g/mL. Extract of *S. rhombifolia* L. was a bactericidal action against *S. aureus* and bacteriostatic to *E. faecalis*.

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