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ANTIBACTERIAL ACTIVITIES TEST AND BRINE SHRIMP LETHALITY TEST OF *Simargaolgaol* (*Aglaonema modestum* Schott ex Engl.) LEAVES FROM NORTH SUMATERA, INDONESIA

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ABSTRACT

The research based on empirical information from the community that used the leaves of Simargaolgaol (*Aglaonema modestum* Schott ex Engl.) as traditional treatments. This study aims to determine the class of phytochemical compounds contained in the extracted leaves samples based on the polarity of the solvent. Screening of phytochemical compounds was carried out using standard methods. Toxicity testing was carried out using the Brine Shrimp Lethality Test (BSLT) method for observing the mortality of *A. salina* Leach larvae and testing for biological activity as antibacterial conducted using the paper disc diffusion method. The study showed that the *n*-hexane extract had a class of steroid compounds; ethyl acetate extract contains alkaloids, flavonoids, saponins, and steroids while the ethanol extract contains alkaloids, flavonoids, saponins, and tannins. LC_{50} for *n*-hexane, ethyl acetate, and ethanol extract was 780.297, 33.083, and 20.548 respectively. The n-hexane and ethyl acetate extract had moderate inhibitory ability on *S. aureus*, *S. mutans*, *B. cereus*, and *S. viridans*. The ethanol extract had strong activity as antibacterial against *S. mutans* and *B. cereus*, and showed potential toxicity (LC_{50}) to be developed as natural antibacterial drugs.

Keywords: *Aglaonema modestum*, Toxicity, BSLT, *A. salina*, Antibacterial activities

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INTRODUCTION

Natural resources, especially biodiversity, are very abundant and have potential that can be utilized in various fields, especially in the health sector. This is because, in addition to having good nutrients as food ingredients[1], it also has other components that can be used for various pharmacological activities.[2,3] This is supported by the content of secondary metabolites. One of the plants that need to be tested for toxicity and activity is Simargaolgaol (*Aglaonema modestum* Schott ex Engl.). This Simargaolgaol plant belongs to the genus *Aglaonema* with synonyms *Aglaonema acutispathum* N.E. Brown., *Aglaonema laoticum* Gangnepain.[4] This plant has been used traditionally by the community as an ingredient in medicine to cure inflammatory diseases, heart, kidney and wounds especially rotting wounds, and scientific data was still very limited in revealing the potential of this plant.

Previous studies reported that the secondary metabolite content of *Aglaonema hookerianum* ethanol extract showed the presence of alkaloids, glycosides, tannins, and saponins. The study also reported that the ethanol extract of *A. hookerianum* leaves had good inhibitory activity as antibacterial.[5] This study aims to determine the class of phytochemical compounds contained in the extracted leaves samples Simargaolgaol based on the polarity of the solvent, starting with *n*-hexane, ethyl acetate and followed by ethanol.

EXPERIMENTAL

Materials

The tools used are 60 mesh filter, blender, analytical balance, watch glass, vacuum pump, branch erlenmeyer (Pyrex), vacuum rotary evaporator (Heidolph), refrigerator, autoclave (TOMY ES-315), petri

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dish (Iwaky), incubator (Memmert), laminar flow (B-ONE V 915 S), micropipette, micro tube, micro tip, spreader, cotton bud, vortex (SBS), caliper, incandescent lamp. The materials used were Staphylococcus aureus, Streptococ mutans, Bacillus cereus, Streptococcus viridans, n-hexane (Merck), ethyl acetate (Merck), ethanol (Merck), Mg powder (Merck), 2% HCl (Merck), FeCl3 (Merck), 2N HCl (Merck), Dragendorf reagent (Merck), H2SO4 (Merck), Anhydrous CH3COOH (Merck), Mueller Hinton Agar (MHA) (Oxoid CM0337), Dimethyl Sulfoxide (DMSO) (Merck), 0.9% physiological NaCl, MC Farland 0.5%, Chloramphenicol, blank disc paper (Oxoid), whatman filter paper No. 1, NaCl, and shrimp larvae *Artemia salina* Leach.

Preparation and Process of the Extract Plant

We prepared 7 kgs sample of fresh of Simargaolgaol leaves that were taken from the Barus area of North Sumatra Province, Indonesia. Barus area has abundant potential of it's plant and used by communities as traditional treatments. Determination was carried out by a Botanist at the Herbarium Medanense FMIPA North Sumatera University.[3] Samples were cleaned using running water and then drained. Samples were dried in the open air and protected from direct sunlight. The dry sample was powdered with a size of 60 mesh and then stored in the pharmacognosy laboratory for use in the next stage.

In this study, 550 g of simplicia powder of Simargaolgaol leaves was extracted by maceration method. The extraction process is carried out by increasing the polarity of the solvent. Sample extraction was started h 2 L of *n*-hexane solvent soaked for 72 hours at room temperature and stirring occasionally. The extract was filtered using Whatman No.1 paper. Macerate was separated and the residue was macerated with the same solvent under new conditions and repeated 3 times. Further extraction was carried out with ethyl acetate and ethanol solvents with the same treatment in the next step. The obtained macerate was evaporated using a rotary evaporator at a temperature of 50 °C. The viscous extract obtained was stored in a jar and continued at the next stage of phytochemical screening, toxicity test and antibacterial activity test.[6]

Phytochemical Screening

Phytochemical screening was performed on each extract of n-hexane, ethyl acetate and ethanol using standard methods. Phytochemical screening carried out included alkaloids, flavonoids, steroids, triterpenoids, saponins, and tannins.[7]

Brine Shrimp Lethality Test

Toxicity test using *A. salina* Leach larvae with the Brine Shrimp Lethality Test (BLST) method. 0.4 g of *Artemia salina* Leach eggs were immersed in 500 mL of seawater in an aquarium and illuminated with a 40-60 watt incandescent lamp for 48 hours. The extract f each sample was dissolved in seawater with a volume of 10 mL each. 4,5 concentration variations of 1000 ppm, 500 ppm, 100 ppm ,10 ppm and 0 ppm as controls. Each concentration was placed in a vial, added 10 larvae, observations were made after 24 hours by counting the number of deaths. The BLST test was repeated 4 times so that the percentage of death was obtained, then the linear regression equation was calculated to obtain the lethal concentration 50 (LC_{50}).[8,9]

ntibacterial Tests

The antibacterial test method was carried out by the disc diffusion method.[10] Antibacterial activity tests were carried out on S. *aureus*, *S. mutans*, *B.s cereus*, and *S. viridans*. The test begins with the sterilization of the tools and materials used in the autoclave at a pressure of 1.5 atm at 121°C for 15 minutes, then placed in laminar flow. Rejuvenated bacteria were cultured in 20 mL sterile petridic dishes and incubated for 24 hours at 37°C. Bacterial culture was carried out by taking one ose and inoculated into a test tube which was diluted with 0.9% physiological NaCl solution and then vortexed until the turbidity was obtained according to the McFarland standard of 0.5. Antibacterial activity testing was carried out on each extract (*n*-hexane, ethyl acetate and ethanol). The concentration of each extract was dissolved with DMSO with a concentration variation of 1.25%, 2.5%, 5%, and 10%.[11] Negative control of DMSO solution without sample and 30 g of chloramphenicol as positive control. The bacterial suspension was spread evenly with a spreader over the media. The paper discs that have been dipped in each concentration variation of the ethanol extract, negative control and chloramphenicol discs that have been dipped in DMSO were added to

the surface of the media. Incubation was carried out for 24 hours, then the clear zone was observed and measured using a caliper. The experiment was carried out with 3 repetitions.[10,12]

RESULTS AND DISCUSSION

Phytochemical Screening

The results of screening on the content of chemical compounds contained in each extract of Simargaolgaol leaves. Not all of the extracts of *A. modestum* Schott ex Engl. leaves contain a class of phytochemical compounds as shown in Table-1.

Table-1: Table-1: Phytochemical Screening of *Aglaonema modestum* Schott ex Engl Leaves Extract

Phytochemical screening	Reagent	Extract		
		n-hexane	Ethylacetate	Ethanol
Alkaloids	Dragendorf	+	+	-
Flavonoids	$FeCl_3$ 5%	+	+++	-
Saponins	Aquadest	+++	++	-
Terpenoids	Acetic anhydride and $H_2SO_{4\,(p)}$	-	-	-
Steroids	Acetic anhydride and $H_2SO_{4\,(p)}$	-	+++	+++
Tannins	$FeCl_3$ 1%	++	-	-

The most important groups of phytochemical compounds contained in the n-hexane extract are alkaloids, flavonoids, tannins, and saponins. The most important group of phytochemical compounds contained in ethyl acetate extracts include alkaloids, flavonoids, saponins, and steroids. The ethanol extract contains the most important phytochemical compounds, namely steroids. The group of alkaloid compounds has an important role as antimicrobial, analgesic, and antispasmodic, flavonoids as antioxidants, saponins and tannins have potential antimicrobial activity (both bacteria and fungi).[13] The steroid compound group has anti-inflammatory activity.[14,15]

Toxicity Test Brine Shrimp Lethality Test

The pharmacological activity of each extract was tested by looking at the mortality rate of *A. salina* Leach larvae. The mortality of *A. salina* Leach larvae shows pharmacological activity that deserves consideration in its development as a potential drug. In line with information from the community that the leaves of Simargaolgaol were using for various traditional ailments. The toxicity of each extract was evaluated by the BLST method. Toxicity test data of each extract were shown in Fig.-1 and Table-2.

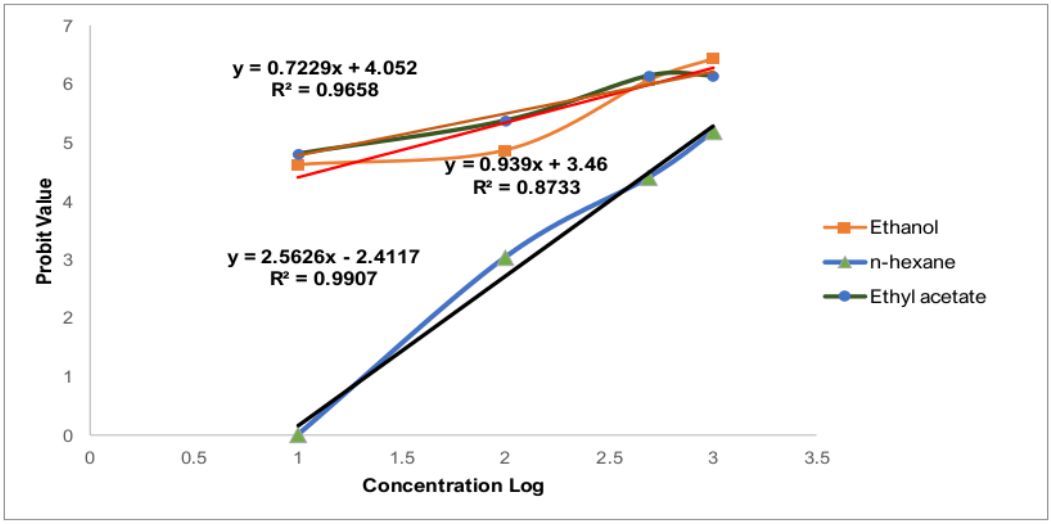

Fig.-1: Linear Regression Equation of *Aglaonema modestum* Schott ex Engl. Leaves in Determining LC_{50} Between Probit Value and Log Concentration.

Table-2: LC_{50} *Aglaonema modestum* Schott ex Engl. Leaves Extract with BLST Method

Sample	Concetration [ppm]	Treatment				Acumulation of death/ total cell	% mortality cell	LC_{50}
		1	2	3	4			
n-hexane	1000	6	6	5	6	23/40	57.5	780.297
	500	4	2	4	1	11/40	27.5	
	100	0	0	1	0	1/40	2.5	
	10	0	0	0	0	0/40	0	
	0	0	0	0	0	0/40	0	
Ethylacetate	1000	10	10	8	9	37/40	92.5	33.083
	500	9	8	9	8	34/40	85	
	100	6	2	5	5	18/40	45	
	10	3	8	2	1	14/40	35	
	0	0	0	0	0	0/40	0	
Ethanol	500	10	9	8	8	35/40	87.5	20.548
	100	8	5	2	8	23/40	57.5	
	10	8	3	4	2	17/40	42.5	
	0	0	0	0	0	0/40	0	

The LC_{50} value of the three extracts showed that the ethanol extract had a lower value, followed by the value of the ethyl acetate extract and finally the *n*-hexane extract. The smaller the LC_{50} value indicates the stronger (very toxic) the toxicity of the active compounds contained in each extract.[16,17,18,19] Toxicity test data were reported to have a correlation on potential anticancer and antitumor activity.[20,21]

Antibacterial Tests

Antibacterial activity test of various extracts of Simargaolgaol leaves against *S. aureus*, *S. mutans*, *B. cereus*, and *S. viridans* (Fig.-2 and Table-3). The selection of chloramphenicol as a positive control was based on its broad spectrum properties as an antibacterial both Gram-negative and Gram-positive, as well as being the most commonly used antibacterial drug.[22,23]

Table-3: Zona of Inhibition of Extracts Simargaolgaol (*Aglaonema modestum* Schott ex Engl.) Leaves

Extracts	Bacteria	Treatment					
		Control		Concentration variation			
		CHL (+)	DMSO 10% (-)	1%	2.5%	5%	10%
n-hexane	*S. aureus*	16.1 ± 1.39	0	6.07 ± 0.06	7.07 ± 0.75	7.93 ± 0.12	8.2 ± 0.10
	S. mutans	20.53 ± 1.15	0	6.93 ± 0.50	7.70 ± 0.52	8.17 ± 0.40	8.30 ± 0.35
	B. cereus	19.67 ± 1.10	0	6.27 ± 0.23	6.40 ± 0.10	7.07 ± 0.58	7.27 ± 0.51
	S. viridans	15.27 ± 1.4	0	6.70 ± 0.10	7.10 ± 0.35	7.30 ± 0.10	9.03 ± 0.06
Ethylacetate	*S. aureus*	21.5 ± 0.69	0	7.63 ± 1.15	8.00 ± 1.04	8.40 ± 0.69	8.87 ± 0.40
	S. mutans	19.87 ± 1.10	0	7.10 ± 0,52	7.97 ± 0.58	8.63 ± 0.81	8.87 ± 0.75
	B. cereus	19.50 ± 1.17	0	6.87 ± 0.40	6.77 ± 0.23	7.87 ± 1.27	8.93 ± 0.81
	S. viridans	14.57 ± 1.27	0	7.17 ± 0.23	6.97 ± 0.06	7.33 ± 0.29	7.57 ± 0.40
Ethanol	*S. aureus*	19.73 ± 0.81	0	7.00 ± 0.17	7.37 ± 0.23	8.13 ± 0,12	9.60 ± 0.52
	S. mutans	15.50 ± 0.69	0	7.17 ± 0.40	8.27 ± 0.06	8937 ± 0.12	10.30 ± 0.17
	B. cereus	20.33 ± 0.46	0	7.00 ± 0.87	8.37 ± 1.10	9.77 ± 0.40	10.07 ± 0.58
	S. viridans	15.27 ± 1.44	0	7.03 ± 0.12	8.13 ± 0.06	8.43 ± 0.06	8.77 ± 1.10

Based on the inhibitory ability of each bacterium, in general, the higher the concentration of each extract, the higher the inhibitory ability of the bacteria. The *n*-hexane extract had moderate inhibitory ability on *S. aureus*, *S. mutans*, *B. cereus*, and *S. viridans* bacteria. The ethyl acetate extract also had moderate activity as antibacterial against *S. aureus*, *S. mutans*, *B. cereus*, and *S. viridans* bacteria. The ethanol extract has moderate activity as antibacterial against *S. aureus* and *S. viridans* bacteria and is in the strong category as

antibacterial against *S. mutans* and *B. cereus* bacteria. The existence of different classes of phytochemical compounds from each leaf extract of Simargaolgaol indicates biological activity that can be used as a valuable therapeutic agent.[24,25]

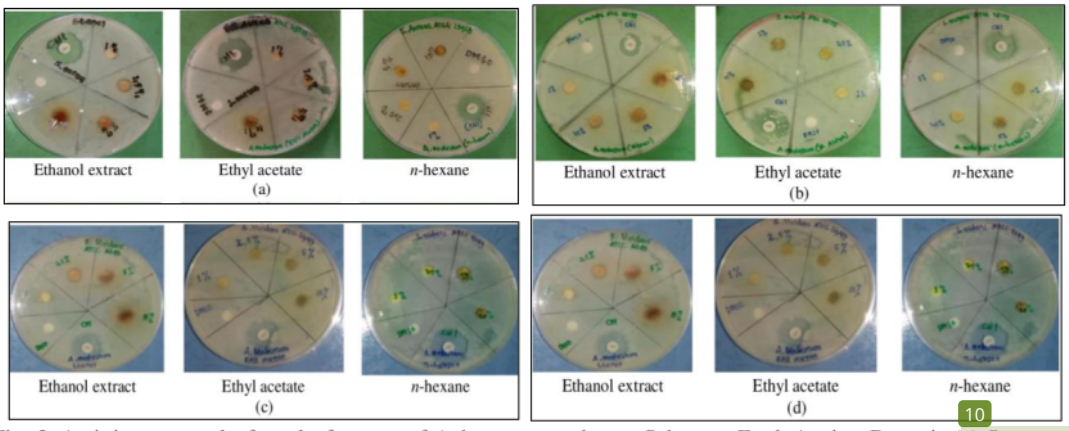

Fig.-2: Activity test results from leaf extract of *Aglaonema modestum* Schott ex Engl. Against Bacteria (a) *S. aureus*; (b) *S. mutans*; (c) *B. cereus* and (d) *S. viridans*

CONCLUSION

Siamargaolgaol (*Aglaonema modestum* Schott ex Engl.) extract leaves were extracted in stages starting from *n*-hexane, ethyl acetate, and ethanol as solvents. The Simargaolgaol extract leaves had a class of alkaloids, flavonoids, saponins, steroids, and tannins compounds. The ethanol extract had strong activity as antibacterial against *S. mutans* and *B. cereus*, and showed potential toxicity (LC_{50}) to be developed as natural antibacterial drugs.

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