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Toxicity Test, Antibacterial and Antioxidant Activity of Simargaolgaol Plant Stem Extract (Aglaonema modestum Schott ex Engl)

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Abstract
The simargaolgaol plant (*Aglaonema modestum* Schott ex Engl) is one of the plants that grow wild in the Barus forest area of Central Tapanuli, North Sumatra which is used by local people as a traditional medicinal plant. This study aims to determine the toxicity properties by BSLT method, antibacterial activity by disc diffusion method and antioxidant activity by DPPH method. Toxicity test results of simargaolgaol stem extract (Aglaonema modestum Schott ex Engl) against Artemia salina Leach larvae showed that n-hexane extract has toxic properties with an LC50 value of 24.424 ppm (toxic category), ethyl acetate extract with an LC50 value of 40,693 ppm (toxic category) and ethanol extract with an LC50 value of 25,830 ppm (toxic category). The results of antibacterial activity showed that ethyl acetate extract (semipolar) with a concentration of 10% had the greatest antibacterial potential against Escherichia coli bacteria with an inhibition zone of about 14.3 mm (strong category). The results of antioxidant activity of ethanol extract of simargaolgaol stems have an IC50 value of 28.075 ppm and Vitamin C has an IC50 value of 36.867 ppm. Thus, based on the results of the IC50 value, it can be seen that the ethanol extract of simargaolgaol stems has strong antioxidant activity compared to Vitamin C as a comparison.
Keywords: *Simargaolgaol, Toxicity, Antibacterial, Antioxidant*

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INTRODUCTION

Indonesia is a tropical forest country with the second highest biodiversity after Brazil. Natural resources, especially biodiversity, have the potential to be utilized in various fields, especially the medical field. In Indonesia, there are 30,000 types of plants out of 40,000 types of plants in the world, with 940 types of plants being medicinal plants that have long been used as traditional medicine by many tribes in Indonesia (Masyhud, 2010). One of the medicinal plants used in this study is Simargaolgaol which is a wild plant that lives in Central Tapanuli, Barus District, North Sumatra province which has been trusted by the surrounding community as a traditional herbal medicine plant. Based on phytochemical studies, Simargaolgaol (Aglaonema modestum Schott ex Engl) leaves contain secondary metabolites namely flavonoids, alkaloids, steroids, saponins, and tannins. This plant has traditionally been utilized by the local community as a medicinal ingredient to treat inflammation of the heart, kidneys, and wounds including mouth ulcers. Scientific data to reveal the potential of this plant is very limited (Zega, 2021; Silaban *et al.*, 2022).

According to research by Silaban et al. (2022), n-hexane extract is a steroid compound; ethyl acetate extract was identified as an alkaloid, steroid, saponin, and flavonoid derivative, while ethanol extract contains alkaloids, flavonoids, saponins and tannins. Ethanol, ethyl acetate and n-hexane extracts, showed potential toxicity (LC50) that can be developed as antineoplastic, antitumor and antibacterial drugs. In Zega's research (2021), ethanol, ethyl acetate, and n-hexane extracts of Simargaolgaol leaves were toxic with LC50 values of 30.1762 ppm, 65.6553 ppm, and 764.0262 ppm, respectively. For antibacterial testing on ethanol, ethyl acetate and n-hexane extracts of Simargaolgaol leaves potentially inhibit the activity of Escherichia coli (ATCC 25922) which is 13.1 mm. In Pakpahan's research (2022), ethanol extract of simargaolgaol leaves has the potential as an antioxidant that is lower than vitamin C. With the results obtained, the IC50 value is 2.304 ppm, 30.953 ppm, and

109.809 ppm. With the results obtained, the IC50 value is 2.304 ppm, 30.953 ppm, and 109.809 ppm. And the results of LC-MS analysis show that the ethanol extract of simargaolgaol leaves contains Methyl ophiopogonone B, kadsurenin K, and Schizandrin C. However, research on the active compound components of simargaolgaol stem extract in Indonesia is still limited, as well as research on stem extracts from plants of the same genus and family as simargaolgaol is still very limited, testing is limited only to the crude extract on the crude extract.

METHODS

This research was conducted at the Research and Microbiology Laboratory of Medan State University. The initial process of making extracts is preparation of dry simplisia powder. Samples of stem dried in a room at room temperature. After drying, all of the simargaolgaol stem ispulverized to a powder measuring 60 mesh. The extraction process uses a multistage maceration method. In the initial process, the sample (900 grams) was soaked using 2L of n-Hexan solvent for 2x24 hours. The second was soaked using ethyl acetate solvent as much as 2L for 2x24 hours and the third was soaked using 96% ethanol as much as 2L for 2x24 hours with occasional stirring, then filtered (Ajilye et al., 2015). The resulting filtrate was then evaporated until thick n-hexane, ethyl acetate, ethanol extracts of simargaolgaol stems were obtained. The condensed extracts can be tested for antioxidant, antibacterial, and bioactivity.

Toxicity testing was conducted using the Brine Shrimp Lethality Test (BSLT) method using Artemia salina Leach shrimp larvae as the object of observation. A total of 1 gram of sample was mixed with 10 mL of seawater, then homogenized (2000 ppm sample stock). A total of 10 shrimp larvae were put into a test tube, then given a sample solution with consecutive concentrations of 1000, 500, 100, and 10 ppm that had been made from 2000 ppm sample stock solution, allowed to stand for 24 hours. The bioactivity of the extract is indicated by the number of larvae that die compared to the blank. The bioactivity value is indicated by the term Lethal Concentration (LC50).

Antibacterial testing was carried out by agar diffusion method (Tillah et al. 2017) against E.coli (ATTC 25922) incubated at 37°C for 24 hours in liquid medium. Next, agar medium was added with bacterial inoculum and placed into Petri dishes and then allowed to solidify. Then into the medium that has been solidified, chloramphenicol is inserted for positive control, 4 pieces of paper discs are dabbed with 20 µL of sample solution made with different concentrations of 1%, 2.5%, 5% and 10% and negative control with DMSO.

The antioxidant test method used was DPPH with ascorbic acid as positive control and methanol as blank. A total of 50 µL sample and 2mL DPPH (3mg DPPH in 50 mL methanol) were each added to the test tube using a micropipette. After incubation for 30 minutes, the absorbance was measured at a wavelength of 517 nm and then the inhibitory activity was calculated.

RESULTS AND DISCUSSION

Simargaolgaol Stem Preparation and Extraction

Sample preparation begins with Simargaolgaol stems tested in this study were initially green, separated from the leaves and washed thoroughly. After washing then dried by aerating for 2 days. Then cut into small pieces and dried again until the color changes to brown. The initial weight of the sample before washing was as much as 30kg, when the drying process was obtained as much as 7kg, and then the grinding process was carried out using a grinder to obtain 900gram of simargaolgaol stem simplisia powder.

The first maceration using non-polar solvent, n-Hexan as much as 2L was carried out for 2 days, then filtered to obtain filtrate and dregs. The dregs were macerated again with 2L of n-Hexan for 2 repetitions, getting a filtrate of 1,650 mL. Then the filtrate was evaporated solvent using a rotary evaporator at a temperature of ± 50 ° C, so as to obtain a concentrated extract of n-Hexan as much as 245.007gram. Evaporation is carried out at a temperature of ± 50 ° C to prevent decomposition of the compounds contained therein. The second maceration using a semipolar solvent, namely ethyl acetate as much as 2L, was carried out for 2 days, the pulp was macerated again with 2L ethyl acetate for 2 repetitions and then filtered to get a filtrate of 1,150mL. Then evaporated using a rotary evaporator at a temperature of ± 50 ° C, so that the concentrated ethyl acetate extract was obtained as much as 235.144gram. The third maceration using a polar solvent, namely ethanol as much as 2L, was carried out for 2 days, the pulp was macerated again with 2L ethanol and then filtered to get a filtrate of

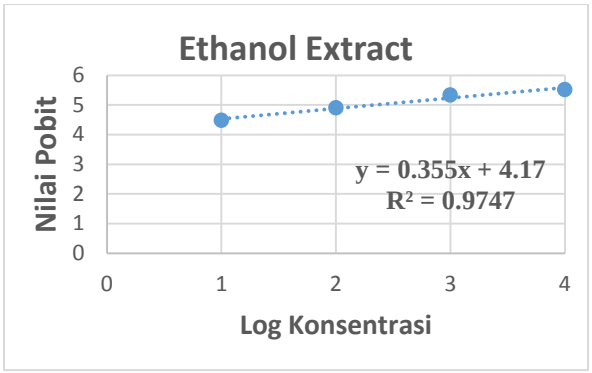
1,675mL. Then evaporated using a rotary evaporator at a temperature of ± 50 ° C, so that the concentrated ethanol extract was obtained as much as 206.063gram. The results of n-Hexan evaporation obtained thick light brown extract, blackish green ethyl acetate and blackish brown ethanol.

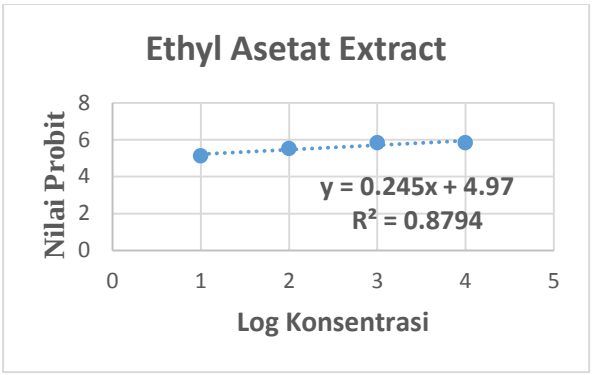
Toxicity Test

Tables 1. Toxicity Test Results of simargaolgaol stem extract with BSLT method

Samples	concentrations	Log of concentration	artemia that died	Mortality (%)	LC_{50} (ppm)
Ethanol	10	1	1	30%	25,830
	100	2	2	46%	
	500	2,69	2,69	63%	
	1000	3	3	70%	
ethyl acetate	10	1	1	55%	40,693
	100	2	2	70%	
	500	2,69	2,69	80%	
	1000	3	3	80%	
n-Hexane	10	1	1	23%	24,424
	100	2	2	36%	
	500	2,69	2,69	53%	
	1000	3	3	63%	

The toxicity test of Simargaolgaol stem extract (Aglaonema modestum Schott ex Engl) was conducted using the Brine Shrimp Lethality Test (BSLT) method. Toxicity testing of a compound must be done to measure the toxicity of the compound and ensure that the estimated dose used can cause toxicity related to safety values or toxicity that can endanger human health (Wolley, 2008). Based on the results in Table 4.1, it shows that the percentage of death of Artemia salina shrimp larvae is mostly found in each extract at a concentration of 1000 ppm and 500 ppm. Where in ethanol extract there is a % mortality of 70% at a concentration of 1000 ppm and 63% at a concentration of 500 ppm, in ethyl acetate extract there is a % mortality of 80% at a concentration of 1000 ppm and 80% at a concentration of 500 ppm, and in n-Hexan extract there is a % mortality of 63% at a concentration of 1000 ppm and 53% at a concentration of 500 ppm. It is proven that the greater the concentration of the extract, the greater the percent mortality of Artemia salina Leach larvae. Therefore, the active substances contained in simargaolgaol stems have a very strong influence on larval mortality. The BSLT test method using Artemia larvae is highly correlated with the cytotoxic activity of anticancer compounds.





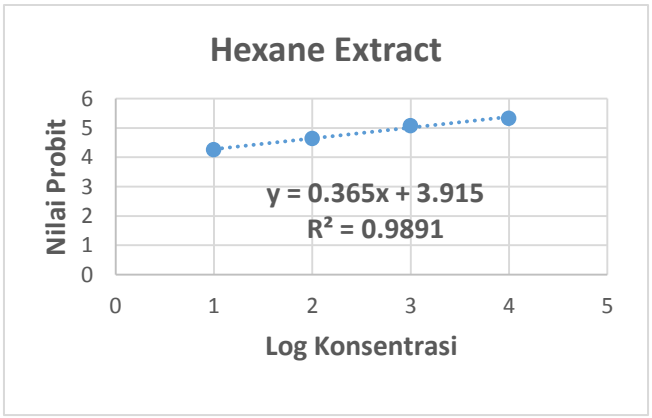


fig.1 Probit Value of ethanol, ethyl acetate, and n-Hexan Extracts of Simargaolgaol Stem

According to research by Meyer et al. (1982), compounds are said to be toxic if the LC50 value is 1000 µgmL LC50 < 200 ppm. This is evidenced from the ethyl acetate extract is toxic and can be expected to have potential as an anticancer against artemia salina larvae. The LC50 value is a value that indicates highly toxic properties. Thus the ethanol, ethyl acetate and n-hexane extracts show toxic properties. The mechanism of death of Artemia salina larvae is related to the function of alkaloid and flavonoid compounds that inhibit the feeding power of artemia larvae (Rafiqah et al., 2019). Flavonoids are plant defense compounds that can inhibit insect growth and are also toxic, and alkaloids that act as poisons in the stomach (Putri et al., 2012). Therefore, if these compounds enter the body of artemia larvae, their digestive organs will be disrupted. In addition, this compound inhibits taste receptors in the larval mouth area. And resulting in larvae failing to get a taste stimulus so that they are unable to recognize their food.

Antibacterial Activity

Tabels 2. Antibacterial activity of simargaolgaol stem extract against E. coli (ATTC 25922) using agar diffusion method

			Clear zone	Diameters	(mm)	
samples	**e.coli**					
	1%	2,5%	5%	10%	K+	K-
Ethyl acetate	10,4	11,3	13,0	14,3	23,3	0

Based on experiments with Escherichia coli bacteria, the inhibition zone is greater at a concentration of 10%. This is according to research by Arum et al. (2012), the antibacterial effect increases with increasing extract concentration, because the higher the concentration, the more

antibacterial active ingredients it contains. Increasing the concentration of antibacterial compounds is also thought to increase the penetration of antibacterial compounds so that they can damage cell metabolism and cause cell death in microbial cells (Ningtyas, 2010).

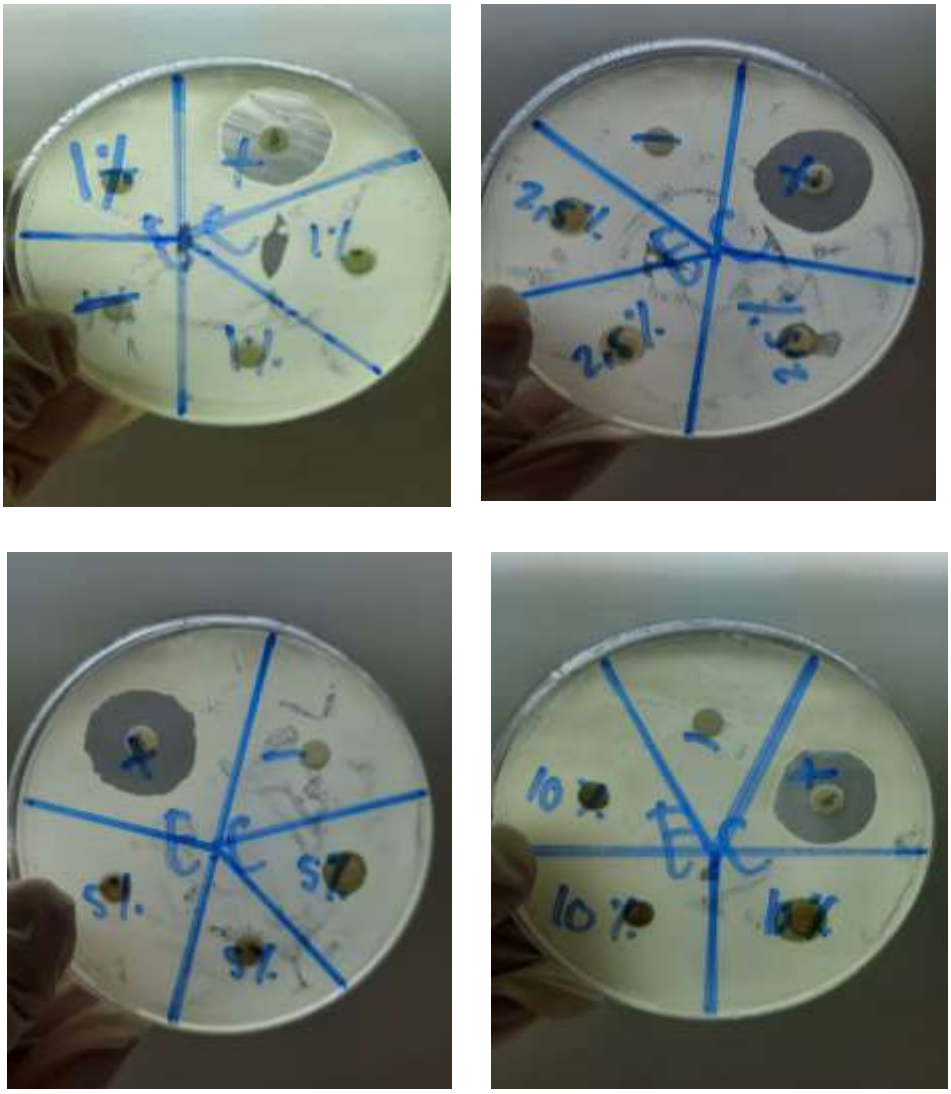


fig.2 Test Results of Simargaolgaol Stem Ethyl Acetate Extract against Escherichia coli Bacteria

Escherichia coli bacteria at different concentrations (1%, 2.5%, 5%, and 10%) in the ethyl acetate extract of simargaolgaol stems showed that the greater the concentration variation, the greater the side effects produced and the greater the inhibition. This is because the potential of simargaolgaol stem extract appears stronger. The highest inhibition of simargaolgaol stem extract against Escherichia coli bacteria was found at a concentration of 10% which inhibited Escherichia coli bacteria with a diameter of 14.3 mm. The class of bacterial inhibition according to Davis and Stout (1971) states that if the diameter of the bacterial inhibition zone is 5 mm or less, it is classified as weak, if the diameter of the inhibition zone is 5-10 mm, then it is classified as moderate.

Antioxidant Activity

Tabels 3. Antioxidant Activity Test Results Data

Type of Solution	Concentration	Absorbance	% Inhibition	IC_{50} (ppm)	Antioxidant Categories
	20	1,3	60,293		
Extract	40	0,92	71,899		

Ethanol	80	0,855	73,885	28,075	extremely
Stem	160	0,793	75,778		strong
Simargaolgaol	320	0,721	77,978		
	2	0,468	84,911		
Vitamin C	4	0,346	88,637		extremely
	8	0,139	94,960	36,867	strong
	16	0,069	97,098		
	32	0,02	98,595		

Antioxidant activity testing of ethanol extract of simargaolgaol (Aglaonema modestum Schott ex Engl) using DPPH (2,2-diphenyl-1-picrylhydrazyl) method and vitamin C as positive control. DPPH method is a method to determine the antioxidant activity of a sample by looking at its ability to capture free radicals 2,2-diphenyl-1-picrylhydrazyl expressed by IC50 value. Testing antioxidant activity with the DPPH method is a simple procedure to determine whether a compound acts as an antioxidant. The IC50 value is defined as the concentration of the test compound that is able to reduce free radicals by 50%. The lower the IC50 value, the greater the free radical scavenging activity (Molyneux, 2004).

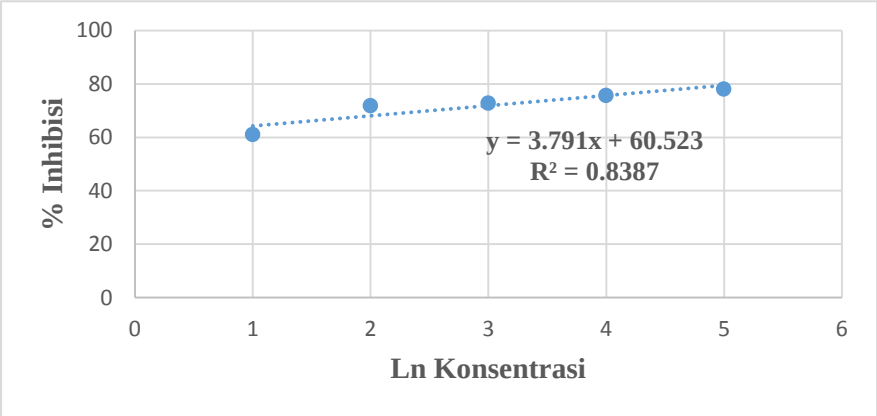


fig.3 Antioxidant Activity of Simargaolgaol Stem Extract

In this study, antioxidant testing was carried out by dissolving DPPH in methanol. According to Molyneux (2004), the presence of antioxidant activity causes a change in the color of DPPH methanol solution in the sample which was initially dark purple to pale yellow. The concentration of each test extract was then measured using UV-Vis spectrophotometer with vitamin C (positive control). That vitamin C has very strong antioxidant activity with an IC50 value of 36.867 ppm. Vitamin C is used as a comparison compound in the antioxidant activity test because of its different properties. Among them are secondary antioxidants that are able to absorb free radicals and have very high antioxidant activity, are easily available, can prevent chain reactions and are polar compared to other vitamins. Vitamin C contains free hydroxyl groups that act as free radical antidotes, so it can increase antioxidant activity (Damanis et al., 2020). Meanwhile, ethanol extract of simargaolgaol stems also has very strong antioxidant activity with an IC50 value of 28.075 ppm. The results obtained in this study indicate that the ethanol extract solution of simargaolgaol stem has very strong antioxidant activity, while vitamin C also has very strong antioxidant activity. Based on the IC50 value obtained, it can be seen that the ethanol extract has a very strong antioxidant effect compared to vitamin C as a comparison.

CONCLUSIONS

1. Toxicity test results of simargaolgaol stem extracts (Aglaonema modestum Schott ex Engl) on Artemia salina Leach larvae showed all extracts were highly toxic. Ethanol extract has an LC50

value of 12,111 ppm, ethyl acetate extract has an LC50 value of 2,594 ppm, and n-Hexan extract has an LC50 value of 24,900 ppm.

2. The results of the antibacterial activity test of simargaolgaol stem extract (Aglaonema modestum Schott ex Engl) obtained that the ethyl acetate extract with a concentration of 10% has the greatest atibacterial potential against Escherichia coli bacteria with an inhibition zone of 14.3 mm.
3. Antioxidant activity test results of ethanol extract of simargaolgaol stem (Aglaonema modestum Schott ex Engl) has an IC50 value of 28.075 ppm, while vitamin C has an IC50 value of 36.867 ppm. The ethanol extract of simargaolgaol stem has very strong antioxidant activity compared to Vitamin C as a comparison.

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