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Toxicity of mangrove plant extract (*Rhizophora stylosa* Griff.) using the brine shrimp lethality test method

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Abstract. *Rhizophora stylosa* is a type of mangrove that is very abundant on the coast of Indonesia and contains many active compounds. So far, research on the toxicity of bark, leaf and flower extracts of *R. stylosa* has not been reported. This research aims to determine the toxic effects of *n*-hexane, dichloromethane and methanol extracts from the bark, leaves and flowers of *R. stylosa* through initial screening using the brine shrimp lethality test (BSLT) method and to determine the components contained in the plant. Toxicity testing using the BSLT method is widely used as the first step in extract screening because it is fast, cheap and easy to do. The results obtained were that all *R. stylosa* plant extracts were classified as non-toxic except for the dichloromethane extract of bark with an LC₅₀ value of 947.76 ppm (toxic). The results of GC-MS analysis show that the compound that has the highest area is lupeol compound.

1. Introduction

The *R. stylosa* plant is a species from the *Rhizophoraceae* family that absorbs water, but can prevent salt from entering through filters in the roots [1]. According to Basyuni et al. [2], triterpenoid compounds play an important role in protecting *R. stylosa* from the effects of salt. Mangrove plants contain several chemical compounds including alkaloids, phenolics, flavonoids, saponins, steroids and tannins, because of these compound mangrove plants can be used as traditional medicines and anticancer drugs [3]. Most plants in the *Rhizophoraceae* family are still little used as medicinal ingredients, but extracts and raw materials from these plants have been used by coastal communities for traditional medicine [4]. Harwoko and Utami [5] found that the *n*-hexane:chloroform fraction from the methanol extract of *R. mucronata* bark was cytotoxic to myeloma cells with an IC₅₀ value of 15 µg/mL. The results of qualitative analysis showed that the fractions tested contained flavonoid and terpenoid compounds. Berawi and Marini [6] reported that phytochemical screening of methanol extract of *R. apiculata* bark contained alkaloid, flavonoid and tannin compounds. Tannin is the main component contained in the *R. apiculata* plant which can counteract the effects of free radical oxidative stress.

The method used for toxicity testing is the Brine Shrimp Lethality Test (BSLT) method. This method is used to determine the ability of a compound as a poison and to determine the level of toxicity of an extract. Toxicity testing using the BSLT method has several advantages, including cheap, fast and easy to carry out [7]. Research that has been carried out on the *Sonneratia alba* plant using the BSLT method includes Suryaningrum and Sasmito [8], they reported that the *n*-hexane extract of *S. alba* leaves has toxicity activity against *Artemia salina* larvae with an LC₅₀ value of 98.37 ppm.

Huong et al. [9] reported that the methanol extract of *R. stylosa* leaves had a cytotoxic effect on human cancer cells KB and LU-1 with IC₅₀ values of 86.7 and 51.0 µg/mL, respectively. Indriaty et al. [10] reported that the methanol extract of *C. decandra* bark, *C. Decandra* leaves, *R. mucronata* bark, and *R. mucronata* fruit had toxicity activity against *A. salina* larvae with LC₅₀ values of 31.5 µg/mL;



32.1 $\mu\text{g/mL}$; 31.8 $\mu\text{g/mL}$ and 35.7 $\mu\text{g/mL}$, respectively. According to McLaughlin and Rogers [11] LC_{50} values > 1000 ppm are included in the non-toxic category, LC_{50} values 30-1000 ppm are included in the toxic category and LC_{50} values < 30 ppm are included in the very toxic category.

So far, research on the toxicity of bark, leaf and flower extracts of *R. stylosa* has not been reported. Based on the background above, it is necessary to carry out research to test the toxicity of *R. stylosa* bark, leaf and flower extracts using the BSLT method. Determination of the chemical components of the active extract was carried out through GC-MS analysis.

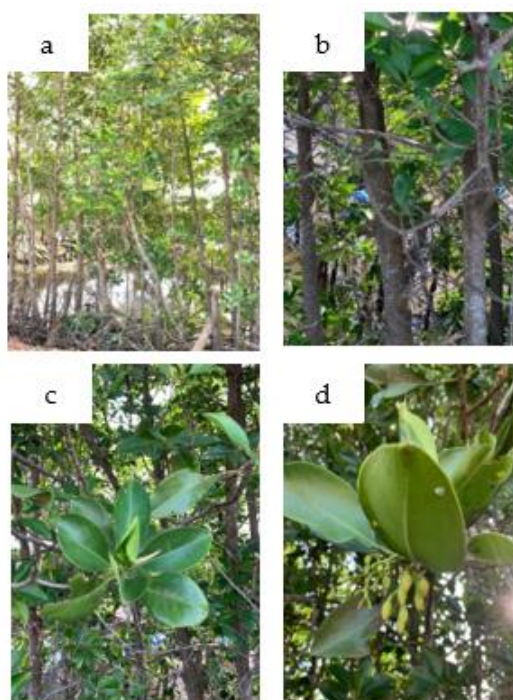


Figure 1. Morphology of the *R. stylosa* plant: (a) tree, (b) bark, (c) leaves and (d) flowers

2. Materials and methods

2.1. *R. stylosa* samples

The samples used in this research were the bark, leaves and flowers of *R. stylosa* obtained from the coast of Alue Naga, Syiah Kuala, Banda Aceh. Sampling location coordinates: 5°35'26.7"N 95°20'59.8"E. Sampling was carried out in January 2023. Bark, leaves and flowers were taken respectively; 3 Kg, 1 Kg and 500g. Fresh samples are cleaned, dried in the air and pureed using a blender. Dried sample of bark (1,95 Kg), leaves (628 g) and flowers (308 g) were macerated with *n*-hexane, dichloromethane and methanol respectively.

2.2. Herbarium specimens and chemicals

Identification of the specimen (574/UN11.1.8.4/TA.00.01/2022) was carried out at the Biosystematics Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Syiah Kuala University and confirmed as a *R. stylosa* Griff plant.

The chemicals used in this research are *n*-hexane, dichloromethane, methanol, ammonia, FeCl_3 , vanillin sulfate, Dragendroff, dimethyl sulfoxide (DMSO), *A. salina* eggs and TLC plate.

2.3 Extraction of *R. stylosa*

The sample was macerated using *n*-hexane solvent first to remove non-polar compounds, then continued using dichloromethane and methanol solvents. Before soaking with dichloromethane solvent, 10% ammonia was added to the dregs and then left overnight. After completion of maceration using dichloromethane, the dregs were then macerated again using methanol solvent to remove the polar compounds contained in the sample. The filtrate resulting from maceration was concentrated using a rotary evaporator to produce concentrated *n*-hexane extract, concentrated dichloromethane extract and concentrated methanol extract. The extract that has been obtained is then calculated using the formula:

$$\% \text{ yield} = \frac{\text{Extract weight}}{\text{Sample weight}} \times 100\% \quad (1)$$

2.4 Phytochemical Screening

Phytochemical tests on each *R. stylosa* extract were carried out using TLC. Phytochemical screening was carried out on secondary metabolite compounds in the alkaloids, phenolics, flavonoids, tannins, saponins, terpenoids and steroids.

2.5 Toxicity Assay of *R. stylosa* Extract Using BSLT Method

The toxicity test of the bark, leaf and flower extracts of *R. stylosa* was carried out using the BSLT method. The test animals used in this method are *A. salina* larvae.

2.5.1 Preparation of *Artemia salina* larvae

Preparation of *A. salina* larvae is done by preparing a glass jar into which 500 mL of sea water and 50-100 mg of *A. salina* larvae have been added. A lamp was placed next to the glass jar, and an aerator was inserted into the jar as a source of oxygen for the larvae.

2.5.2 Preparation of test solution concentration

Each extract of the bark, leaves and flowers of *R. stylosa* was weighed at 50 mg and dissolved using 25 mL of sea water, to obtain a stock solution concentration of 2000 ppm. Specifically for *n*-hexane and dichloromethane extracts, DMSO is added first to help dissolve the extract in seawater, then sonicated so that it dissolves completely. The test solution was made with varying concentrations of 1000 ppm, 500 ppm, 250 ppm, 100 ppm and 50 ppm from the stock solution of 2000 ppm. A negative control was made by adding 5 mL of sea water and 3 drops of DMSO without plant extract.

2.5.3 Toxicity test procedure using the BSLT method

The toxicity test was carried out by inserting 10 48-hour-old *A. salina* larvae into a tube filled with the test solution and sea water. Each concentration was carried out three times (triplo). The test tube was kept under lamp lighting for 24 hours. Live larvae were counted in each test tube. The standard criterion for measuring larvae death is if the larvae do not show movement during observation. The number of dead shrimp larvae for each concentration was counted and recorded, then the results were compared with the control and the LC₅₀ value was looked for.

2.5.4 LC₅₀ calculation

The LC₅₀ value is determined using probit analysis. The LC₅₀ value is the concentration of the test substance that can kill 50% of shrimp larvae. Meyer et al. [12] Calculate the percent death of larvae using the following equation:

$$\% \text{ death} = \frac{\text{number of larval deaths} - \text{number of larval deaths in the control}}{\text{number of test larvae}} \times 100\% \quad (2)$$

3. Results and discussion

3.1. Extraction

The yield data from each plant part extract is tabulated in Table 1.

Table 1. Results of the *R. stylosa* plant extract

Extract	Sample	Sample Weight (g)	Extract Weight (g)	Yield (%)
<i>n</i> -hexane	RSB	1,951.4	2.6	0.86
	RSL	628.4	5.9	0.93
	RSF	308.8	7.8	0.40
Dichloromethane	RSB	1,951.4	1.6	0.53
	RSL	628.4	11.1	1.77
	RSF	308.8	5.5	0.28
Methanol	RSB	1,951.4	5.6	1.82
	RSL	628.4	32.8	5.21
	RSF	308.8	25.8	1.32

Information:

RSB : *R. stylosa* bark, RSL : *R. stylosa* leaves, RSF : *R. stylosa* flowers

Table 1 shows that methanol extracts from all plant parts have the highest percent yield compared to other extracts. This means that all parts of the plant contain more bioactive compounds that are polar in nature. According to Dewastisari [13], the yield value is related to the amount of bioactive content contained in plants. Budiyanto [14] stated that the higher the extract yield, the higher the content of substances that can be withdrawn by the solvent. The results obtained are in line with the results of research conducted by Hanapi et al. [1] who reported that *R. stylosa* leaf extract had the highest percent yield in the polar solvent methanol, namely 18.60%, followed by the semi-polar solvent ethyl acetate (7.09%) and followed by the non-polar solvent *n*-hexane (4.53%). Manuhuttu and Saimima [15] reported that the largest part of the active compounds in *S. alba* leaves was soluble in the polar solvent, namely methanol, followed by the semi-polar solvent ethyl acetate and followed by the non-polar solvent *n*-hexane. Research conducted by Burhanuddin et al. [16] reported that the highest yield of *A. alba* fruit extract was obtained in the polar solvent methanol at 8.61%, followed by the semi-polar solvent chloroform at 1.04% and the non-polar solvent *n*-hexane at 0.64%.

3.2. Phytochemical Screening

Phytochemical screening was carried out using TLC. The results of the phytochemical screening of *R. stylosa* plants can be seen in Table 2.

Table 2. Phytochemical Screening of *R. stylosa* Extract

Extract	Sample	Alkaloids	Terpenoids	Steroids	Flavonoids	Phenolic	Saponin	Tannin
<i>n</i> -hexane	RSB	+	+	+	-	-	-	-
	RSL	+	+	+	-	-	-	-
	RSF	+	+	+	-	-	-	-
Dichloromethane	RSB	+	+	+	-	+	+	-
	RSL	+	+	+	-	+	+	-
	RSF	+	+	+	-	+	+	-
Methanol	RSB	+	+	+	+	+	+	+
	RSL	+	+	+	+	+	+	+
	RSF	+	+	+	+	+	+	+

Information:

+: contains secondary metabolite compounds

-: does not contain secondary metabolite compounds

The results of phytochemical screening showed that *n*-hexane extracts from all parts of the plant contained alkaloids, terpenoids and steroids, but did not contain flavonoids, phenolics, saponins and tannins. Terpenoids and steroids are non-polar compounds, so they can be attracted by the *n*-hexane solvent [17]. Hanapi et al. [1] reported that *n*-hexane extract of *R. stylosa* leaves contains terpenoid and steroid compounds. Mustafa and Fahmi [18] reported that *n*-hexane extract of *R. mucronata* bark contains steroid compounds. Prasetyo et al. [19] reported that the quarternary alkaloid group can be slightly soluble in non-polar solvents, so it is possible that this alkaloid group is soluble in the *n*-hexane solvent. Phenolics are easily soluble in polar and semi-polar solvents such as water, ethanol, methanol and acetone [17] so it cannot dissolve in non-polar solvents. Saponin is a triterpenoid/steroid that has a sugar group so it is polar [20].

Dichloromethane extracts from all parts of the plant contain alkaloids, terpenoids, steroids, phenolics and saponins, but do not contain flavonoids and tannins. Usman et al. [21] reported that the dichloromethane extract of *R. mucronata* leaves contains alkaloid and phenolic compounds, and does not contain tannin compounds. Tannin is a polyphenolic compound that has a hydroxyl group so it can dissolve in polar solvents such as methanol and ethanol [17]. Flavonoids are a class of polar phenolic compounds that are found in almost every plant. Flavonoids will generally be dissolved by solvents with the same polarity, for example ethanol or methanol [22].

Methanol extracts of all plant parts contain all the secondary metabolite compounds tested, namely alkaloids, terpenoids, steroids, phenolics, flavonoids, saponins and tannins. Hanapi et al. [1] reported that the methanol extract of *R. stylosa* leaves contains alkaloids, flavonoids, tannins, saponins, steroids and terpenoids. Paputungan et al. [23] reported that the methanol extract of *S. alba* fruit contains alkaloids, flavonoids, phenolics, tannins, saponins and steroids. Results of research conducted by Mustofa and Fahmi [24] reported that methanol extract of *R. apiculata* bark contains saponin, terpenoid, alkaloid and flavonoid compounds.

3.3. Toxicity Assay of *R. stylosa* Extract Using BSLT Method

Testing the toxicity of *R. stylosa* plant extracts was carried out using the BSLT method. The *n*-hexane extract, dichloromethane extract and methanol extract from each part of the plant were tested for toxicity against *A. salina* larvae. The test was carried out by varying the solution concentration by 50 ppm, 100 ppm, 250 ppm, 500 ppm and 1000 ppm from the stock solution of 2000 ppm. The toxicity of an extract can be seen from the LC₅₀ value, where the LC₅₀ value indicates the concentration of the extract that can kill 50% of the test animals. The results of the toxicity test of extracts from each part of the plant can be seen in Table 3.

Table 3. Results of toxicity test of *R. stylosa* plant extract

E	C test (ppm)	Log C	Number of test larvae (tails)	Number of dead larvae				Percent of deaths (%)	Probit value	Regression Equations	LC ₅₀ (ppm)
				1	2	3	Average				
RSBH	1000	3	10	4	5	4	4.3	43.3	4.82	$y = 1.104x + 1.5382$	1366.74
	500	2.7	10	3	3	3	3	30	4.47		
	250	2.4	10	3	2	2	2.3	23.3	4.27		
	100	2	10	1	1	1	1	10	3.71		
	50	1.7	10	0	1	0	0.3	3.3	3.16		
RSBD	1000	3	10	5	5	5	5	50	5	$y = 0.5236x + 3.4414$	947.76
	500	2.7	10	4	4	5	4.3	43.3	4.83		
	250	2.4	10	4	4	4	4	40	4.74		
	100	2	10	3	3	3	3	30	4.47		
	50	1.7	10	2	1	1	1.3	13.3	3.88		

RSBM	1000	3	10	5	5	4	4.7	46.7	4.92	$y = 0.402x + 3.5891$	3233.71
	500	2.7	10	4	4	3	3.7	36.7	4.67		
	250	2.4	10	4	3	3	3.3	33.3	4.56		
	100	2	10	3	2	3	2.7	26.7	4.39		
	50	1.7	10	2	1	1	1.3	13.3	3.87		
RSLH	1000	3	10	2	2	1	1.7	16.7	4.05	$y = 1.5447x - 0.5391$	3853.66
	500	2.7	10	1	1	1	1.0	10.0	3.72		
	250	2.4	10	1	0	0	0.3	3.3	3.12		
	100	2	10	0	0	0	0	0	0		
	50	1.7	10	0	0	0	0	0	0		
RSLD	1000	3	10	4	4	3	3.7	36.7	4.67	$y = 1.5447x - 0.5391$	3853.66
	500	2.7	10	3	2	3	2.7	26.7	4.39		
	250	2.4	10	2	1	1	1.3	13.3	3.87		
	100	2	10	1	0	0	0.3	3.3	3.12		
	50	1.7	10	0	0	0	0	0	0		
RSLM	1000	3	10	3	3	4	3.3	33.3	4.56	$y = 1.1461x + 1.1801$	2152.56
	500	2.7	10	2	3	3	2.7	26.7	4.39		
	250	2.4	10	1	1	2	1.3	13.3	3.87		
	100	2	10	1	0	0	0.3	3.3	3.12		
	50	1.7	10	0	0	0	0	0	0		
RSFH	1000	3	10	2	3	3	2.7	26.7	4.39	$y = 1.445 + 0.0866$	2513.48
	500	2.7	10	2	1	2	1.7	16.7	4.05		
	250	2.4	10	0	1	1	0.7	6.7	3.52		
	100	2	10	0	0	1	0.3	3.3	3.12		
	50	1.7	10	0	0	0	0	0	0		
RSFD	1000	3	10	3	4	4	3.7	36.7	4.67	$y = 1.0298x + 1.5906$	2045.22
	500	2.7	10	3	2	3	2.7	26.7	4.39		
	250	2.4	10	1	2	2	1.7	16.7	4.05		
	100	2	10	1	1	1	1	10	3.72		
	50	1.7	10	1	0	0	0.3	3.3	3.12		
RSFM	1000	3	10	4	6	5	5	50	5	$y = 1.8603x - 0.6742$	1122.41
	500	2.7	10	2	2	2	2	20	4.16		
	250	2.4	10	2	1	1	1.3	13.3	3.88		
	100	2	10	1	1	1	1	10	3.71		
	50	1.7	10	1	0	0	0.3	3.3	3.16		

Information:

E : Extract
 C test : Concentration test
 Log C : Concentration log
 RSBH : *R. stylosa bark n*-hexane
 RSBD : *R. stylosa bark* dichloromethane
 RSBM : *R. stylosa bark* methanol
 RSLH : *R. stylosa leaf n*-hexane
 RSLD : *R. stylosa leaf* dichloromethane
 RSLM : *R. stylosa leaf* methanol

RSFH : *R. stylosa* flower n-hexane
 RSFD : *R. stylosa* flower dichloromethane
 RSFM : *R. stylosa* flower methanol

Table 3 shows the mortality of *A. salina* larvae to plant part extracts and the LC₅₀ value of each extract. These data indicate that the higher the extract concentration, the higher the death rate of *A. salina* larvae. Larval death is caused by the presence of toxic compounds in the extract. Several studies show that *A. salina* has a positive correlation with bioactive extracts. If the extract tested is toxic to shrimp larvae, then the extract is an initial indication of pharmacological effects [12].

Toxicity is the ability of a substance to be toxic so that it can cause damage to an organism's organs [25]. Testing the toxicity of *R. stylosa* plant extracts uses the BSLT method because this method is fast, easy, cheap and has a confidence level of 95%. Tests were carried out on *A. salina* larvae with the aim of finding out whether the extract was toxic to *A. salina* larvae. Extracts that are toxic to *A. salina* larvae can be the basis for selecting doses or concentrations in determining other biological activities such as anti-inflammatory and anti-diabetic activities [26]. *A. salina* larvae can grow and survive in a temperature range of 15–55°C and salinity of 3–300 g/L, this makes *A. salina* larvae easy to breed and research [27]. Test solutions from various extracts were carried out in triplicate to determine the average death rate of *A. salina* larvae on a scale. After the test solution and negative control were made, 10 of *A. salina* larvae were added to which 5 mL of sea water was added. The number of larvae that died at each concentration was counted and recorded and then the LC₅₀ value was found.

Table 3 shows that all plant extracts are classified as non-toxic except for the dichloromethane extract of bark. The dichloromethane extract of bark has an LC₅₀ value of 947.76 ppm, so the extract is included in the toxic category. Puspitasari et al. [28] reported that the methanol extract of *R. mucronata* bark had an LC₅₀ value of 1711.42 µg/mL which was included in the non-toxic category. The methanol extract of *A. marina* and *S. alba* bark is also included in the non-toxic category, with LC₅₀ values of 6900.54 µg/mL and 4501.02 µg/mL respectively. All of the extracts are classified as non-toxic except for the dichloromethane extract of bark, it is suspected that these extracts do not contain bioactive compounds that are toxic. According to Puspitasari et al. [28], an extract that does not contain bioactive compound activity does not mean that the extract is inactive, but it is possible that this bioactive compound was not detected at the concentration tested or that the concentration used has not been reached.

3.4. GC-MS Analysis

Table 3 shows that bark dichloromethane extract (RSBD) has the lowest LC₅₀ value, namely 947.76 ppm which is classified as toxic. Therefore, dichloromethane extracts from all parts of the *R. stylosa* plant were analyzed using GC-MS to determine the compound components contained in the extract. Chromatograms of bark, leaf and flower extracts of *R. stylosa* can be seen in Figures 2, 3 and 4, respectively.

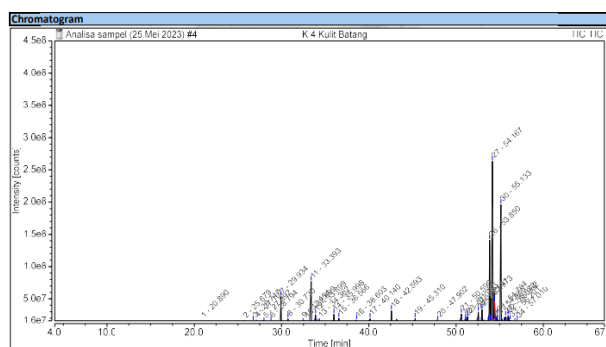
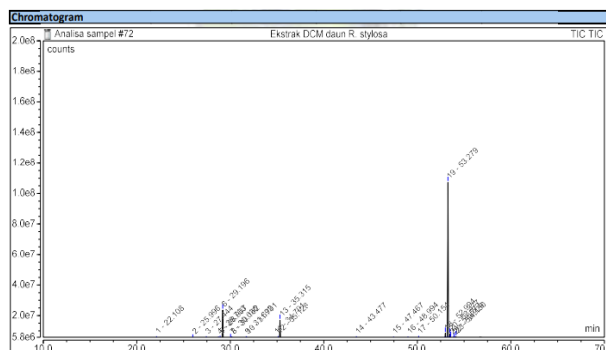


Figure 2. Chromatogram of dichloromethane extract of *R. stylosa* bark



10	<i>Cyclopropaneoctanoic acid, 2-[[2- [(2ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester</i>	32.69	1,2	97.3
11	<i>n-Hexadecanoic acid</i>	33.39	9.3	98.9
12	<i>trans-Sinapyl alcohol</i>	33.89	2,2	94
13	<i>Estra-1,3,5(10)-trien-17β-ol</i>	34.36	0.2	96.5
14	<i>Phytol</i>	35.99	1	95.5
15	<i>trans-13-Octadecenoic acid</i>	36.56	3,4	97.9
16	<i>3-Oxo-10(14)-epoxyguai-11(13)-en-6,12-olide</i>	38.60	0.7	98.4
17	<i>4,8,12,16-Tetramethylheptadecan-4-olide</i>	40.14	0.7	91.6
18	<i>Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl)ethyl ester</i>	42.59	2	96
19	<i>9-Octadecenoic acid (Z)-, 2-hydroxy-1- (hydroxymethyl)ethyl ester</i>	45.31	1.7	98.4
20	<i>α-Tocospiro A</i>	47.90	0.9	85.4
21	<i>9,10-Secocholesta-5,7,10(19)-triene-3,24,25- triol, (3β,5Z,7E)-</i>	50.59	2.1	91.7
22	<i>1-Heptatriacotanol</i>	51.07	0.7	96.2
23	<i>α-Tocopheryl acetate</i>	51.29	1	94.1
24	<i>Campesterol</i>	52.54	1.4	97.9
25	<i>Stigmasterol</i>	52.97	1.9	95.2
26	<i>γ-Sitosterol</i>	53.85	8.9	99.1
27	<i>Lupeol</i>	54.16	24.9	99.7
28	<i>Betulinaldehyde</i>	54.46	0.9	94.6
29	<i>α-Amyrin</i>	54.70	1	93.7
30	<i>α-Amyrin</i>	55.13	18	98.1
31	<i>Lup-20(29)-en-3-ol, acetate, (3β)</i>	55.67	1.6	97.5
32	<i>1,25-Dihydroxyvitamin D3, TMS derivative</i>	55.96	1.1	89.8
33	<i>3-Isopropyl-6a,10b-dimethyl-8-(2-oxo-2- phenyl-ethyl)-dodecahydrobenzo[f]chromen-7-one</i>	56.20	0.8	95
34	<i>Spirost-8-en-11-one, 3-hydroxy-, (3β,5α,14β,20β,22β,25R)</i>	57.01	1.1	96.7

Table 5. GC-MS results of compounds making up dichloromethane extract of *R. stylosa* leaves

No	Compound Name	Retention Time (minutes)	Area (%)	SI (%)
1	1,3,7,7-Tetramethyl-9-oxo-2-oxabicyclo[4.4.0]dec-5-ene	22.10	0.6	82.6
2	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl	25.99	1.8	100
3	1H-Indene, 1-ethylideneoctahydro-7amethyl-, cis	27.44	0.9	100
4	1H-Indene, 1-ethylideneoctahydro-7amethyl-, cis	28.73	1,2	97
5	cis-Za-Bisabolene epoxide	28.90	0.9	96.8
6	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-enone	29.19	13.1	95.6
7	Neophytadiene	30.03	1.3	92
8	9-Undecenol, 2,10-dimethyl-	30.16	0.6	99.7
9	2-Piperidinone, N-[4-bromo-n-butyl]- 3,5,5-trimethyl	31.69	0.7	98
10	Tetradecanoic acid, 12-methyl-, methyl ester, (S)-	31.79	0.5	93.6
11	(2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	34.79	0.3	100
12	2-Hydroxy-1,1,10-trimethyl-6,9-epidioxydecalin	35.12	0.9	100
13	Phytol	35.31	7.3	98.8
14	9,10-Secocholesta-5,7,10(19)-triene-3,25,26-triol, (3 β ,5Z,7E)--, methyl ester	43.47	0.4	91.1
15	2-Piperidinone, N-[4-bromo-n-butyl]-	47.46	0.3	90.4
16	β -Acorenol	48.99	0.3	93.6
17	1-Heptatriacotanol	50.15	0.4	95.1
18	β -Sitosterol	52.99	3.1	98.5
19	Lupeol	53.27	57.9	98.9

20	<i>β</i> -Amyrin	53.47	2.5	97.5
21	<i>Lupeol</i>	53.57	1.6	98.5
22	<i>Androst-5-en-3-ol, 4,4-dimethyl-, (3β)-</i>	53.92	1.1	94.3
23	<i>Lupeol</i>	54.13	2,2	98.5

Table 6. GC-MS results of compounds making up the dichloromethane extract of *R. stylosa* flowers

No	Compound Name	Retention Time (minutes)	Area (%)	SI (%)
1	<i>2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl</i>	26.74	0.7	98.4
2	<i>2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl</i>	28.03	0.9	93.6
3	<i>(E)-4-(3-Hydroxyprop-1-en-1-yl)-2-methoxyphenol</i>	28.79	2.1	95
4	<i>6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one 7a-methyl-, cis</i>	29.43	0.8	96.5
5	<i>(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-enone</i>	29.93	2.7	95.1
6	<i>8-Hexadecenal, 14-methyl-, (Z)-</i>	32.39	1	95.2
7	<i>n-Hexadecanoic acid</i>	33.41	8.4	99.3
8	<i>trans-Sinapyl alcohol</i>	33.90	2,2	95.3
9	<i>Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester trimethyl</i>	42.60	2,3	96.9
10	<i>trans-13-Octadecenoic acid</i>	45.31	1.3	96.7
11	<i>Erucic acid</i>	45.96	0.7	96.4
12	<i>Ethyl iso-allocholate</i>	47.83	0.7	99.7
13	<i>7-Methyl-Z-tetradecen-1-ol acetate</i>	48.11	0.7	96.4
14	<i>Cholesta-4,6-dien-3-ol, (3β)-</i>	50.60	2.7	85.5
15	<i>17-acetyl-10,13-dimethyl-2,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl 2,2,2-trifluoroacetate</i>	51.32	1.4	92
16	<i>Campesterol</i>	52.55	1	98

17	<i>Stigmasterol</i>	52.98	1	96
18	<i>γ-Sitosterol</i>	53.90	8.8	98.9
19	<i>Lupeol</i>	54.24	42.2	99.7
20	<i>(E)-Labda-8(17),12-diene-15,16-dial</i>	54.90	1.8	95
21	<i>α-Amyrin</i>	55.11	5	96.6
22	<i>9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol, (3β,5Z,7E)-</i>	55.69	1.3	90.1
23	<i>Betulinaldehyde</i>	55.97	1.7	96.3
24	<i>9,19-Cyclocholestan-3-ol-7-one, 4β,14βdimethyl- (20R)</i>	57.06	1.1	99.2
25	<i>13,14-Epoxyoleanan-3-ol, acetate</i>	57.96	1.4	96
26	<i>Stigmastane-3,6-dione, (5a)-</i>	59.24	1.4	89.7
27	<i>4-Norlanosta-17(20),24-diene-11,16-diol-21-oic acid, 3-oxo-16,21-lactone</i>	60.59	4.2	93.3
28	<i>8,14-Seco-3,19-epoxyandrostane-8,14-dione,17-acetoxy-3β-methoxy-4,4-dimethyl</i>	61.01	0.8	97.3

The results of GC-MS analysis showed that the highest peak of the bark, leaf and flower extract of *R. stylosa* was the lupeol compound with an area of 24.93%, 57.85% and 42.16% respectively. The compound that is thought to play a role in toxicity is a triterpene group compound, namely lupeol, which has the highest area percentage. The structure of the lupeol compound can be seen in Figure 5.

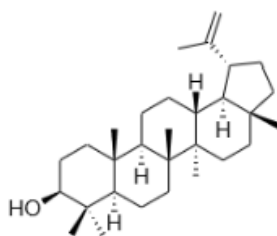


Figure 5. Structure of the lupeol compound

Table 4 shows that the dichloromethane extract of *R. stylosa* bark is toxic compared to other dichloromethane extracts because it is suspected that there are several compounds other than lupeol that

also have a toxic effect on shrimp larvae *A. salina*, such as *n*-hexadecanoic acid, γ -sitosterol and α -amyrin, all three of which have the most dominant area compared to other compounds. Panggua et al. [29] reported that fatty acid compounds such as *n*-hexadecanoic acid have toxicity activity against *A. salina* shrimp larvae. Agustini [30] reported that terpenoid compounds can function as poison in the digestive organs of *A. salina* larvae and can inhibit taste receptors in the mouth area of *A. salina* larvae so that they fail to stimulate taste and are unable to recognize food, resulting in *A. salina* larvae will starve to death. The results of phytochemical screening of the dichloromethane extract of bark (Table 2) proved that the dichloromethane bark extract contains secondary metabolite compounds that are toxic to *A. salina* larvae, namely terpenoids. The bioactivity of lupeol compounds includes research reported by Lalthanpuui et al. [31] reported that molecular predictions of the compound lupeol could provide clues as an antiviral drug because of its ability to interact with important cell surface proteins of the corona virus. Apart from being an antiviral, lupeol also has potential as an antifungal, antioxidant, antidiabetic and antimicrobial drug [32].

4. Conclusions

The results of the toxicity test showed that all plant part extracts were classified as non-toxic except for the dichloromethane extract of *R. stylosa* bark which had an LC₅₀ value of 947.76 ppm (toxic). The results of GC-MS analysis show that the compound that has the highest area is lupeol. It is hoped that this compound can be further isolated and antiviral tested.

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