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Cytotoxic Potential of Compounds Isolated from Non-Polar Fractions of Sungkai Plant Leaves (*Peronema canescens* Jack) Against *Artemia salina* Leach Larvae

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Abstract

The sungkai plant (*Peronema canescens* Jack), belonging to the *Lamiaceae* family, is a plant that is traditionally used as medicine, including toothache, malaria, and fever medicine. In this research, isolation was carried out with vacuum liquid chromatography (VLC), solid and liquid fractions were obtained. The solid fraction was further separated using column chromatography to obtain the isolated compound as a white solid (amorphous) weighing 10 mg (melting point 140˚C-142˚C). The results of the UV spectrum data show that there are no conjugated double bonds. The results of the IR spectrum show the presence of C-H groups at wave numbers 2921,49 cm^{-1} and 2856,94 cm^{-1}, C=O groups at wave numbers, C=C groups at wave numbers 1641,51 cm^{-1}, and dimethyl germinal which is characteristic of triterpenoid compounds at wave numbers 1456.68 cm^{-1} and 1372.41 cm^{-1}. Meanwhile, the isolated oil was analyzed for chemical components using GC-MS. It was discovered that there were 83 chemical compound components contained therein with 4 main compound components, namely pentadecanoic acid (16.65%), 9,12-octadecanoic acid (16.12%), propyl palmitate (7.89%), and hexadecenoic acid, methyl ester (5.59%). A cytotoxic test was carried out on both fractions using the Brine Shrimp Lethality Test (BSLT) method. The results showed that the isolated compound was non-toxic with an LC_{50} value of 190214.2807 mg/L and the isolated oil was very toxic with an LC_{50} value of 34.2452 mg/L.

Keywords: *Peronema canescans Jack, Terpenoid, BSLT, GC-MS.*

Introduction

The sungkai plant (*Peronema canescens* Jack) belongs to the *Lamiaceae* family[1]. This plant is very easy to find in various places such as gardens, yards, roadsides, and forests[1]. Traditionally, the Dayak people in East Kalimantan have used this plant to treat various diseases such as colds, fever, stomach aches, toothaches, malaria, and wounds[2][3][4]. The secondary metabolite content reported from this plant's leaves includes flavonoids, phenolics, tannins, steroids, saponins, and alkaloids[5].

In previous research, various bioactivities from sungkai plant leaves extracts have been reported, the ethanol extract of this plant is also reported to have strong antioxidant activity with an IC_{50} value of 50.838 µg/mL (young sungkai leaves) and 52.835 µg/mL (old sungkai leaves)[6]. Sungkai leaves methanol extract is

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also reported to have various antibacterial activities against *S. mutans, S.thyposa,* and *S.aureus* bacteria with a Minimum Inhibition Concentration (MIC) value of 20%[7].

Various cytotoxic activities of various sungkai plant leaf extracts have been reported, including Ahmad & Ibrahim (2015) have reported that hexane extract of sungkai leaves has cytotoxic activity with an LC_{50} value of 107,399 μg/mL and methanol extract has cytotoxic activity with an LC_{50} value of 387,257 μg /mL against Artemia salina Leach larvae[8]. Suwandi et al. (2018) reported the cytotoxic activity of acetone, ethanol and water extracts on Vero cells with IC_{50} values of 23.37 ± 5.63, 629.46 ± 24.85 and 634.00 ± 144.82 μg/ml, respectively[9]. Ibrahim et al. (2021) also reported that the chloroform fraction from the leaves of this plant also had very strong cytotoxic activity against H-29 colon cancer cells with an IC_{50} value of 14,807 μg/ml[10]. Ibrahim et al. (2023) also reported that ethyl acetate and ethanol extracts from Peronema canescens Jack leaves had a strong cytotoxic activity with IC_{50} values of 28,186 μg/mL and 53,190 μg/mL, respectively[11]. A lot of cytotoxic activity has been reported from various leaf extracts of this plant, so in this study, the cytotoxic potential of compounds isolated from non-polar fractions (solids and oil) of the leaves of the sungkai plant (Peronema canescens Jack) was tested against *Artemia salina* Leach larvae.

In this paper, the isolation of compounds from the nonpolar fraction of ethyl acetate extract of sungkai plant leaves was carried out using the liquid vacuum column chromatography method, obtained as a solid fraction and an oil fraction. The solid fraction was purified by gravity column chromatography and characterized using an ultraviolet (UV) spectrophotometer and Fourier Transform Infrared (FTIR). Gas Chromatography Mass Spectrometry (GC-MS) analyzed the oil fraction for its chemical components. The cytotoxic potential of these two fractions was tested using the Brine Shrimp Lethality Test (BSLT) method using *Artemia salina* L larvae as test animals.

Experimental

Materials

The materials used include ethyl acetate extract from the leaves of the sungkai plant (*Peronema canescens* Jack). This sample was obtained from the results of previous research (Rahma Fadila), and it has also been identified with specimen code 393/K-ID/ANDA/IX/2021[12]. Merck silica gel 60 (0.063-0.200 mm), methanol (technical), ethyl acetate (technical), dichloromethane (technical), and hexane (technical) were used for the isolation process. Artemia salina Leach larvae and sea water were used in the toxicity test using the BSLT method.

Instruments

The equipment used are vacuum liquid chromatography (VLC), chromatography column, TLC plate, UV lamp (254 and 365 nm), UV-Vis spectrophotometer, Fourier Transform Infra-red (FTIR) spectrophotometer and Gas Chromatography-Mass Spectrometry (GC-MS) for characterization. glass box for cultivating shrimp larvae, aerator, and micropipette for cytotoxic tests.

Methods

Isolation, purification and characterization of compounds

The ethyl acetate extract of sungkai leaves (70 g) was isolated using a liquid vacuum column using 100% hexane solvent, hexane: ethyl acetate (5:5), 100% ethyl acetate, and 100% methanol, to obtain four fractions, namely hexane fraction (F1), hexane fraction: ethyl acetate (F2), ethyl acetate fraction (F3) and methanol fraction (F4). In the hexane fraction (F1), two phases are formed, namely solid (F1.a) and liquid oil (F1.b). In the F1.b fraction in the form of oil, the chemical components were analyzed using GC-MS because the F1.b fraction is a volatile oil. Meanwhile, the F1.a fraction (87 mg), which had a simpler stain pattern, was further separated using gravity column chromatography using a mixture of hexane and ethyl acetate as eluent (100%:0 – 0:100%), six subfractions were obtained (F1a.1-F1a.6). Subfraction F1a.2 (27 mg) was further

purified using the trituration method to get a pure compound weighing 10 mg. The compounds resulting from the trituration were tested for purity using thin-layer chromatography with elution of various eluent ratios, hexane (100%), hexane: dichloromethane (8: 2), and hexane: ethyl acetate (9.75: 0.25). The isolated pure compound was then subjected to identification of secondary metabolite groups, melting point test, characterization using a UV-VIS and FT-IR spectrophotometer[13].

Analysis of the chemical components of isolated oil using Gas Chromatography Mass Spectroscopy (GC-MS)

The liquid phase (oil) isolated from fraction-1 (F1.b) was analyzed for its chemical components using Gas Chromatography-Mass Spectrometry (GC-MS) series Shimadzu (QP-2010) (Tokyo, Japan) equipped with an AOC-20i autosampler. The column used was an Rxi-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm). Helium gas is used as a carrier gas. The initial column temperature was set at 60 °C for 1 minute and increased to 210 °C every 10 °C/minute. The injector and detector temperatures are 200 °C and 230 °C, respectively. The ionization energy used is 70 eV with a scanning time of 0.3 seconds and a mass range of 45-500 amu. The results of GC-MS analysis were obtained in the form of spectrum data, which was compared with data from the National Institute of Standards and Technologies (NIST) 14[14][15].

Cytotoxicity test of isolated compounds and isolated oil using the Brine Shrimp Lethality Test (BSLT) method of *Artemia salina* Leach Larvae

The test solution was made by weighing 2.5 mg of the isolated compound (solid) and then dissolving it with hexane in a 10 mL volumetric flask. So get main solution with a 250 mg/L concentration. The main solution is made into various concentrations of 125; 62.5; 31.25; 15.625; 7.812; and 3.906 mg/L. Meanwhile, the isolated oil dissolved 50 mg of oil with hexane to obtain a main solution of 1000 mg/L. The main solution was made with varying concentrations of 500; 250; 125; 62.5; 31.25; 15.625 mg/L. To test cytotoxic activity, thirty *Artemia salina* L larvae were added to each test solution at various concentrations. After 24 hours, the number of dead shrimp larvae in each test solution was counted. The number of dead shrimp larvae determines the LC_{50} value through probit analysis and regression equations. The same process was also carried out on the negative control solution[16].

Results and Discussion

Results of isolation, purification, and characterizationof compounds

Isolation of 70 g of sungkai leaves ethyl acetate extract using a liquid vacuum column and column chromatography produced 4 fractions, namely hexane fraction (F1), hexane fraction: ethyl acetate (F2), ethyl acetate fraction (F3) and methanol fraction (F4). In the hexane fraction (F1), two phases are formed, namely solid (F1.a) and liquid in the form of oil (F1.b). In fraction F1.b, the chemical components were analyzed using GC-MS. Meanwhile, the F1.a fraction (87 mg) was further purified, and a white solid (10 mg) was obtained. This white solid was tested for purity using layer chromatography to obtain a single spot, as shown in Figure 1. Purity was also identified using a melting point test, and a melting point of 140˚C - 142˚C was obtained. The results of identifying secondary metabolite content using Liebermann Burchard reagent show that the isolated compound is a terpenoid [17][18].

The results of the characterization of the isolated pure compound using a UV-VIS spectrophotometer show that there is a maximum absorption at a wavelength of 243 nm. At this wavelength, it shows the existence of a π-π* electron transition, a typical UV spectral absorption for triterpenoid compounds with unconjugated C=C double-bond chromophores [19][20][21]. The UV spectrum shown in Figure 2.

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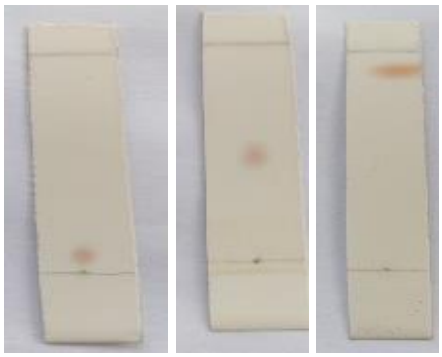

Figure 1. Purity test using thin layer chromatography a. hexane (100%) b hexane: dichloromethane (8: 2), c hexane: ethyl acetate (9.75: 0.25)

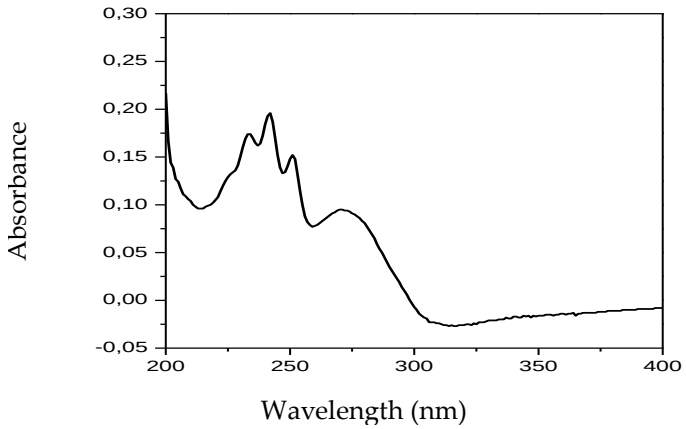

Figure 2. UV spectrum of the isolated compound

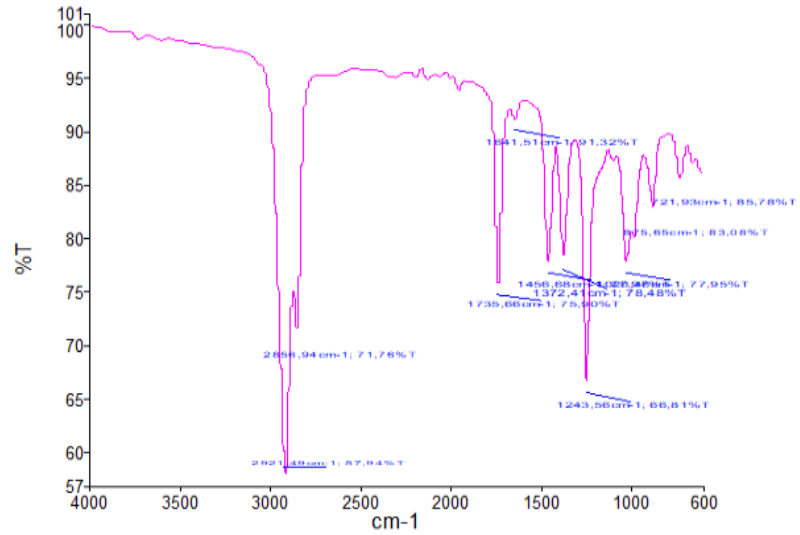

Figure 3. FTIR spectrum of isolated compounds

Table 1. FT-IR spectrum data

Peak Number	X (cm-1)	Y (%T)	Functional groups
1	2921.49	57.94	C-H
2	2856.94	71.76	C-H
3	1735.66	75.90	C=O
4	1641.51	91.32	C=C
5	1456.68	77.97	Geminal dimethyl
6	1372.41	78.48	Geminal dimethyl
7	1243.56	66.81	C-O
8	1020.46	77.95	C-O

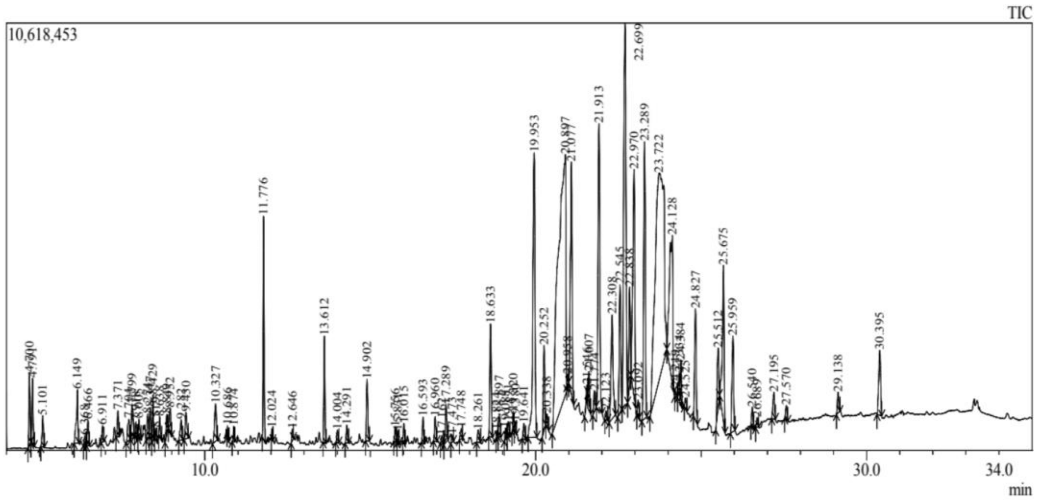

Figure 4. GC-MS chromatogram of isolated oil chemical components

The characterization results with an FT-IR spectrophotometer show the absorption of several functional groups, including aliphatic C-H groups at wave numbers 2921.49 cm^{-1} and 2856.94 cm^{-1}. The C=O (carbonyl) group appears at the wave number 1735.66 cm^{-1}. The C-O (ketone) group appears at wave numbers 1243.56 cm^{-1} and 1020.93 cm^{-1}, and the C=C alkene (steching) group appears at wave number 1641.51 cm^{-1}. Other absorptions also appear at wave numbers 1456.68 cm^{-1} and 1372.41 cm^{-1}, indicating the presence of geminal dimethyl groups characteristic of terpenoid compounds[22]. The FT-IR spectrum of the isolated compound is shown in Figure 3 and Table 1.

Results of chemical component analysis using Gas Chromatography-Mass Spectrometry (GC-MS) from oil fractions

The chromatogram of chemical component analysis of the oil fraction using GC-MS showed that there were 83 peaks (Figure 4) which suggested the presence of 83 chemical components contained in the isolated oil. The chemical components of the isolated oil are shown in Table 2.

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Table 2. Chemical components of oil fractions

No	Retention time (minute)	Compound	Formula Molecule	Area (%)	Index Similarity (%)	m/z (gram/ mol)	Functional group
1	4.700	Ethyl benzene	C_8H_{10}	0.70	98	106	C=C
2	5.101	1,2 dimethyl benzene	C_8H_{10}	0.36	96	106	C=C
3	4.791	1,4 dimethyl benzene	C_8H_{10}	0.68	96	106	C=C
4	6.149	Octamethyl cyclotetrasiloxane	$C_8H_{24}O_4Si_4$	0.77	68	296	-
5	6.368	Butanoic acid	$C_8H_{12}O_2$	0.09	80	116	C-O C=O R-COOR
6	6.466	1,2,4 Trimethyl Benzene	C_9H_{12}	0.27	92	120	C=C
7	6.911	1,2,4 Trimethyl Benzene	C_9H_{12}	0.17	96	120	C=C
8	7.371	1,3-Cyclopentadiene	$C_{10}H_{14}$	0.25	94	134	C=C
9	7.704	1-methyl-2-(1-methyl ethyl) Benzene	$C_{10}H_{14}$	0.34	89	134	C=C
10	7.799	1-ethyl-2,3-dimethyl Benzene	$C_{10}H_{14}$	0.50	87	134	C=C
11	7.918	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl acetate	$C_{12}H_{18}O_2$	0.16	78	194	C=C, C-O, C=O
12	8.005	2,3-dihydro-2-methyl -1H-Inden-2-ol	$C_{10}H_{12}O$	0.23	89	148	C=C, R-OH
13	8.274	1,2,3,4-tetramethyl-5-methylene 1,3-Cyclopentadiene	$C_{10}H_{14}$	0.26	89	134	C=C
14	8.347	1,2,3,4-tetramethyl-5-methylene 1,3-Cyclopentadiene	$C_{10}H_{14}$	0.28	96	134	C=C
15	8.429	Decamethyl-Cyclopentasiloxane,	$C_{10}H_{30}O_5Si_5$	0.35	88	370	-
16	8.519	3-isopropenyl-2,5-dimethyl-3,4-Hexadien-2-ol,	$C_{11}H_{18}O$	0.11	77	166	C=C, C-OH
17	8.638	Diethylmethyl-Benzene,	$C_{11}H_{16}$	0.20	91	148	C=C
18	8.849	1,2,3,4-tetramethyl-Benzene	$C_{10}H_{14}$	0.20	87	134	C=C
19	8.952	Octanoic acid	$C_8H_{16}O_2$	0.30	97	144	R-COOH

20	9.282	Dodecane	$C_{12}H_{26}$	0.27	88	170	-
21	9.430	Azulene	$C_{10}H_{8}$	0.41	93	128	C=C
22	10.327	Nonanoic Acid	$C_{9}H_{18}O_{2}$	0.61	92	158	R-COOH
23	10.686	Hexadecane	$C_{16}H_{34}$	0.06	94	184	-
24	10.874	Dodecamethyl Cyclohexasiloxane	$C_{12}H_{36}O_{6}Si_{6}$	0.13	88	444	-
25	11.776	Didehydro-7-[(trimethylsilyl)oxy]-9-ketoabietic acid - methyl ester	$C_{24}H_{36}O_{4}Si$	1.97	67	416	R-COOR, C=O, C=O
26	12.024	Hexadecane	$C_{16}H_{34}$	0.06	95	226	-
27	12.646	1-methyl-4-(1-methylethyl)-2,3-Dioxabicyclo[2.2.2]oct-5-ene	$C_{10}H_{16}O_{2}$	0.11	77	168	-
28	13.612	Dis2,2,4,4,6-Pentamethyl-6-((1,1,3,3-Tetramethyl-3-[(2,4,4,6,6-Pentamethyl-1,3,5,2,4,6-Trioxatrisilinan-2-Yl)Oxy]	$C_{14}H_{42}O_{9}Si_{8}$	1.01	80	578	-
29	14.004	Hexahydro-8a-methyl-, cis-1,8(2H,5H)-Naphthalenedione	$C_{11}H_{16}O_{2}$	0.15	76	180	C=O
30	14.291	Dodecanoic acid	$C_{12}H_{24}O_{2}$	0.22	88	200	R-COOH
31	14.902	Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1,3-propanediyl ester	$C_{16}H_{30}O_{4}$	0.73	92	286	R-COOR
32	15.756	Silicate Anion Tetramer	$C_{24}H_{72}O_{12}Si_{12}$	0.17	67	888	-
33	15.831	Trisiloxanyl)Oxy)-2,4,2-((1,1,3,3,5,5-Hexamethyl-5-[(2,4,4,6,6-Pentamethyl-1,3,5,2,4,6-Trioxatrisilinan-2-Yl)Oxy]	$C_{16}H_{48}O_{10}Si_{9}$	0.10	74	652	-
34	16.015	Trisiloxanyl)Oxy)-2,4,2-((1,1,3,3,5,5-Hexamethyl-5-[(2,4,4,6,6-Pentamethyl-1,3,5,2,4,6-Trioxatrisilinan-2-Yl)Oxy]	$C_{16}H_{48}O_{10}Si_{9}$	0.17	76	652	-
35	16.593	2,4,4,6,6,8,8,10,10-Nonamethyl-1,3,5,7,2-[(2	$C_{16}H_{48}O_{10}Si_{9}$	0.27	81	652	-

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		,4,4,6,6,8,8-Heptamethyl-1,3,5,7,2,4,6,8-Tetraoxatetrasilocan-2-Yl)Oxy]					
36	16.690	2,4,4,6,6,8,8,10,10-Nonamethyl-1,3,5,7,-[(2,4,4,6,6,8,8-Heptamethyl-1,3,5,7,2,4,6,8-Tetraoxatetrasilocan-2-Yl)Oxy]-	C16H48O10Si9	0.28	80	652	-
37	17.162	1,1,1,3,5,7,9,11,11,11-Decamethyl-5-[(Trimethylsilyl)Oxy]	$C_{13}H_{42}O_6Si_7$	0.09	73	490	-
38	17.289	Myristic acid	$C_{14}H_{28}O_2$	0.47	93	228	R-COOH
39	17.473	2,4,4,6,6,8,8,10,10-Nonamethyl-1,3,5,7,2-[(2,4,4,6,6,8,8-Heptamethyl-1,3,5,7,2,4,6,8-Tetraoxatetrasilocan-2-Yl)Oxy]	$C_{16}H_{48}O_{10}Si_9$	0.07	81	652	-
40	17.748	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	0.13	89	270	R-COOR
41	18.261	methyl Cyclopropanebutanoic acid, 2-[[2-[[2-[(2-pentylcyclopropyl)methyl]cyclopropyl]methyl]cyclopropyl]methyl]	$C_{25}H_{42}O_2$	0.19	61	374	R-COOR
42	18.633	Tetradecanoic acid, trimethylsilyl ester	$C_{17}H_{36}O_2Si$	1.49	68	300	-
43	18.824	2,4,2-((1,1,3,3,5,5-Hexamethyl-5-[(2,4,4,6,6-Pentamethyl-1,3,5,2,4,6-Trioxatrisilinan-2-Yl)Oxy]Trisiloxanyl)Oxy)	$C_{16}H_{48}O_{10}Si_9$	0.06	74	652	-
44	18.897	Pentadecanoic acid	$C_{15}H_{30}O_2$	0.26	89	242	R-COOH
45	19.088	2,4,2-((1,1,3,3,5,5-Hexamethyl-5-[(2,4,4,6,6-Pentamethyl-1,3,5,2,4,6-Trioxatrisilinan-2-Yl)Oxy]Trisiloxanyl)Oxy)	$C_{16}H_{48}O_{10}Si_9$	0.12	74	652	-
46	19.171	2,4,2-((1,1,3,3,5,5-Hexamethyl-5-[(2,4,4,6,6-Pentamethyl-1,3,5,2,4,	$C_{16}H_{48}O_{10}Si_9$	0.14	62	652	-

		6-Trioxatrisilinan-2-Yl)Oxy]Trisiloxanyl)Oxy)					
47	19.320	Propyl Myristate	$C_{17}H_{34}O_2$	0.17	88	270	R-COOR
48	19.380	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	0.07	89	270	R-COOR
49	19.641	trimethylsilyl ester 1H-Indole-2-carboxylic acid, 1-(trimethylsilyl)-5-[(trimethylsilyl)oxy]	$C_{18}H_{31}NO_3Si_3$	0.21	83	393	C=C, C=O
50	19.953	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	5.59	91	270	R-COOR
51	20.252	trimethylsilyl ester 1H-Indole-2-carboxylic acid, 1-(trimethylsilyl)-5-[(trimethylsilyl)oxy]	$C_{18}H_{31}NO_3Si_3$	1.06	89	393	C=C, C=O
52	20.338	3,7,11,15-Tetramethyl-1-Hexadecen-3-Ol	$C_{20}H_{40}O$	0.14	95	296	C=C, C-OH
53	20.897	Pentadecanoic acid	$C_{15}H_{30}O_2$	16.65	83	242	R-COOH
54	20.958	Ethyl 9-Hexadecenoate	$C_{18}H_{34}O_2$	0.09	78	282	R-COOR
55	21.077	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	3.63	88	284	R-COOR
56	21.546	1-Methylethyl Ester Hexadecanoic Acid	$C_{19}H_{38}O_2$	0.15	90	298	R-COOR
57	21.607	15-methyl-, methyl ester Hexadecanoic acid	$C_{18}H_{36}O_2$	0.30	88	284	R-COOR
58	21.774	3-Octeneoic Acid 1tms	$C_{11}H_{22}O_2Si$	0.27	86	214	C=C, C=O
59	21.913	trimethylsilyl Hexadecanoic acid	$C_{19}H_{40}O_2Si$	4.52	91	328	C=O
60	22.123	14-Pentadecenoic Acid	$C_{15}H_{28}O$	0.12	83	240	R-COOH
61	22.308	Heptadecanoic acid	$C_{17}H_{34}O_2$	1.93	89	270	R-COOH
62	22.545	Oxacycloheptadecan-2-one	$C_{16}H_{30}O_2$	1.83	82	254	R-COOR
63	22.699	Propyl Palmitate	$C_{19}H_{38}O_2$	7.89	85	298	R-COOR
64	22.838	Ethyl (9z,12z)-9,12-Octadecadienoate	$C_{20}H_{36}O_2$	1.17	91	308	R-COOR
65	22.970	(Z,Z,Z 9,12,15-Octadecatrien	$C_{19}H_{32}O_2$	3.99	93	292	R-COOR,

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		oic acid, methyl ester					C=O
66	23.092	Diallyl acetal Palmitaldehyde	$C_{22}H_{42}O_2$	0.17	82	338	R-OR, C=C
67	23.289	Heptadecanoic acid, 16-methyl-, methyl ester	$C_{19}H_{38}O_2$	4.25	89	298	R-COOR
68	23.722	(Z,Z)-9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	16.12	88	280	R-COOH
69	24.128	Octadecanoic acid	$C_{18}H_{36}O_2$	4.28	83	284	R-COOH
70	24.129	16-Methyl-heptadecanecarboxylic	$C_{18}H_{36}O_2$	0.06	75	284	R-COOH
71	24.334	1-methylethyl Hexadecanoic acid	$C_{19}H_{38}O_2$	0.18	73	298	R-COOR
72	24.384	15-methyl-, ethyl ester Heptadecanoic acid	$C_{20}H_{40}O_2$	0.37	87	312	R-COOR
73	24.525	Ethyl (9z,12z)-9,12-Octadecadienoate	$C_{20}H_{36}O_2$	0.11	79	308	R-COOR
74	24.827	Neophytadiene	$C_{20}H_{38}$	1.57	91	278	C=C
75	25.512	Ethyl (9z,12z)-9,12-Octadecadienoate	$C_{20}H_{36}O_2$	0.88	87	308	R-COOR, C=C
76	25.675	Ethyl (9z,12z)-9,12-Octadecadienoate	$C_{20}H_{36}O_2$	2.54	91	308	R-COOR, C=C
77	25.959	Propyl ester Octadecanoic acid	$C_{21}H_{42}O_2$	1.42	89	326	R-COOR
78	26.540	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	0.21	89	326	R-COOR
79	26.689	3-(4-methoxy-phenyl) -2-propenoic acid, 2-ethyl-hexyl ester	$C_{18}H_{26}O_3$	0.12	95	290	R-COOR, C-O, C=O
80	27.195	Eicosanoic acid	$C_{20}H_{40}O_2$	0.39	86	312	R-COOH
81	27.570	Heptadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$	0.17	82	284	R-COOR
82	29.138	Propyl Palmitate	$C_{19}H_{38}O_2$	0.27	68	298	R-COOR
83	30.395	2-([(2-Ethylhexyl)Oxy]Carbonyl)Benzoic Acid	$C_{16}H_{22}O_4$	1.13	96	278	R-COOH, R-COOR, C=C

Based on the data in Table 2, it can be seen that the chemical components contained in the isolated oil have different levels and similarity index. Of the 83 chemical components in the isolated oil, 63 compounds have levels as small as 1%, 16 compounds with levels between 1-5%, and 4 compounds with levels greater than 5%.

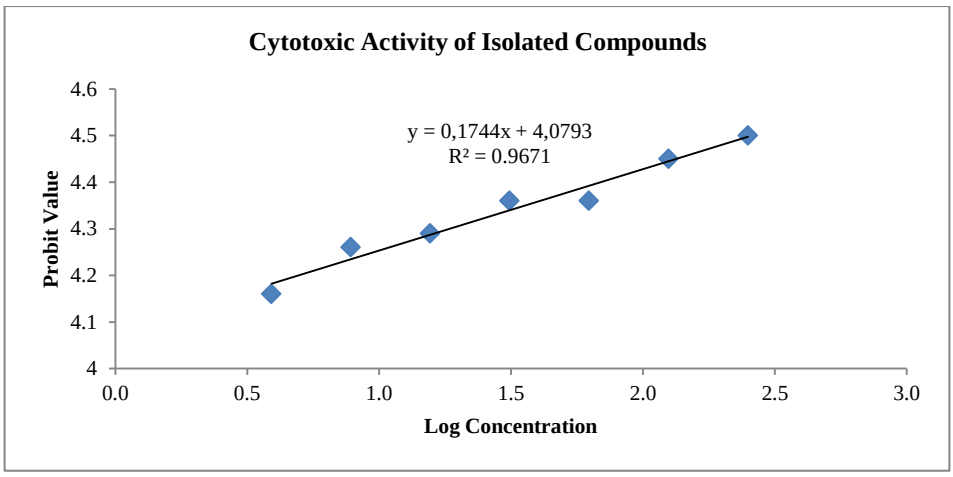
The main compounds that have the highest levels in this oil fraction are compounds that have area percent levels > 5%, namely pentadecanoic acid (16.65%), 9,12-octadecanoic acid (16.12%), propyl palmitate (7.89%), and hexadecanoic acid, methyl ester (5.59%). From the data in Table 2, it can also be seen that the compounds contained in this oil fraction consist of compounds from the monoterpenoid, sesquiterpenoid, diterpenoid, and lipid groups.

Of the four main compounds, it is known that one compound has been reported to have cytotoxic activity, namely the compound pentadecanoic acid. This compound has cytotoxic activity on MCF-7/SC breast cancer cells. The pentadecanoic acid compound is the compound that has the largest composition compared to the 82 other compounds contained in the oil fraction of the leaves of the sungkai plant [23].

Cytotoxic test using the BSLT (Brine Shrimp Lethality Test) method

The cytotoxic test is used to determine the toxicity of isolated compounds (solids and oils) by determining the LC_{50} value. The LC_{50} value is determined based on the test animal's death percentage, namely *Artemia salina* L. larvae. The death rate of shrimp larvae will vary according to variations in the concentration of the test solution, where the more greater the concentration of the test solution, the greater the content of active compounds in the test solution. The results of the toxicity test of isolated oil using the BSLT (Brine Shrimp Lethality Test) method are shown in Figure 5.

From the linear regression equation (Figure 5), the LC_{50} value of the test solution can be determined. The calculation results show that the isolated compound has an LC_{50} value of 190214.2807 mg/L, while the isolated oil has an LC_{50} of 34.2452 mg/L. According to Clarkson (2004) LC_{50} values can be grouped into 4, namely LC_{50} values of 0-100 mg/L are categorized as strongly toxic, values of 100-500 mg/L can be categorized as moderately toxic, LC_{50} values of 500-1000 mg/L are categorized as weakly toxic and LC_{50} values > 1000 is categorized as non-toxic. So it can be concluded that the isolated compound is not toxic, while the isolated oil is very toxic[24][25]. The content of chemical compounds influences this cytotoxic ability in the test sample. The isolated oil has a composition of far more nonpolar compounds than the isolated compound. The lipophilic nature of this nonpolar compound makes it easier for the compound to enter the cell membrane in test animals, thereby changing the composition and fluidity of the membrane.



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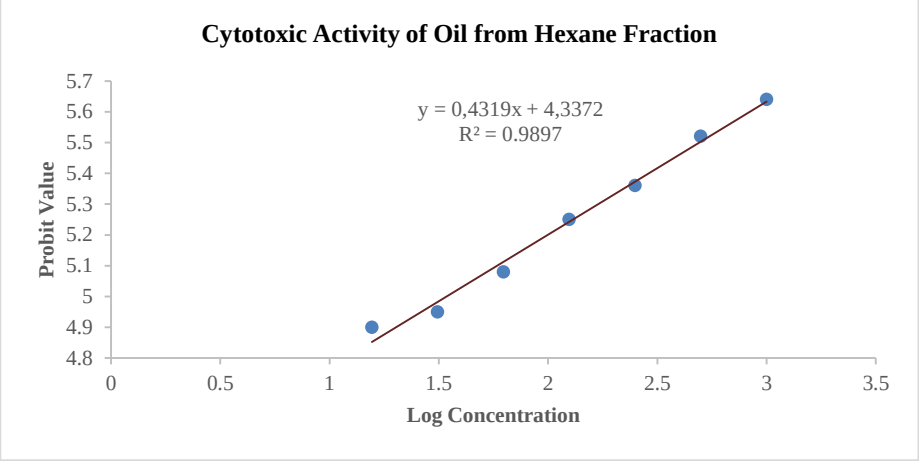

Figure 5. Relationship between log concentration of test solution and probit value

These changes cause leakage of cytoplasmic ions and molecules, as well as reduced ATP production and loss of mitochondrial function, causing death in *Artemia salina* L larvae [26].

Conclusions

Isolation of compound from the hexane fraction of ethyl acetate extract of sungkai leaves obtained compounds in solids and oils. The pure compound (solid) obtained is a triterpenoid with a melting point of 140˚C-142˚C. Meanwhile, the chemical components isolated from oil showed that there were 84 chemical components with 4 main compound components, namely pentadecanoic acid (16.65%), 9.12-octadecanoic acid (16.12%), propyl palmitate (7.89%), and hexadecanoic acid, methyl ester (5.59%). The results of the cytotoxic test using the BSLT method on *Artemia salina* L larvae showed that the isolated triterpenoid compounds did not show toxic properties, but the oil components showed very strong toxic properties with an LC_{50} value of 34.2452 mg/L.

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