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Cytotoxicity of leaves, stems, and flowers of Kecubung (*Datura metel*) extracts using the Brine Shrimp Lethality Test (BSLT) method

Rindhira Humairani[1,*], Nanda Rizki Purnama[2], Herpandi Herpandi[3], Mochamad Syaifudin[4], Ilham Zulfahmi[2], Yusrizal Akmal[1], Muliari Muliari[1], Agus Putra AS[5], Agung Setia Batubara[6]

[1]*Department of Aquaculture, Faculty of Agriculture, Universitas Almuslim, Bireuen 24261, Indonesia.*
[2]*Department of Fisheries Resources Utilization, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Banda Aceh, Indonesia.*
[3]*Department of Fisheries Products Technology, Faculty of Agriculture, Universitas Sriwijaya, Indralaya Ogan Ilir, South Sumatera 30662, Indonesia.*
[4]*Department of Aquaculture, Faculty of Agriculture, Universitas Sriwijaya, Indralaya Ogan Ilir, South Sumatera 30662, Indonesia.*
[5]*Department of Aquaculture, Faculty of Agriculture, Universitas Samudra, Langsa 24416, Indonesia.*
[6]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Medan, Indonesia.*

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ABSTRACT

Brine shrimp (*Artemia salina*) cytotoxicity assays (BSLT) are one of the commonly used cytotoxic test methods to assess a plant extract's pharmacological activity and toxicity. Thus, this study aimed to examine the toxic levels of Kecubung (*Datura metel*) leaves extracted using different solvents, namely ethanol and ethyl acetate. The results showed that the LC_{50} value of flower ethanol, flower ethyl acetate, leaf ethanol, and stem ethyl acetate extracts had an LC_{50} value <1000, which was included in the toxic category. The LC_{50} value of flower ethanol is 121.044 ppm, flower ethyl acetate 105.89 ppm, leaf ethanol 639.589 ppm, and stem ethyl acetate 635.276 ppm. Ethanol leaf extract at a concentration of 1000 ppm showed the highest mortality with a percentage of 60% of the total number of *A. salina*. The flower ethanol extract showed the highest mortality at a concentration of 250 ppm with a percentage of 67% and at a concentration of 1000 ppm with a percentage of 70% of the total number of *A. salina*. Meanwhile, flower ethyl acetate and stem ethyl acetate extract at a concentration of 1000 ppm obtained 100% mortality of *A. salina* in the first 6 hours. The result shows that the flower ethyl acetate and stem ethyl acetate extract at a concentration of 1000 ppm is very toxic compared to other concentrations.

Introduction

Kecubung (*Datura metel*) is one of the wild plants that can be used as herbal medicine which is widespread in lowland areas up to an altitude of 800 meters above sea level. All parts of the *D. metel* plant have active compounds consisting of roots, stems, leaves, flowers, and fruits. Behind these active compounds, *D. metel* also has benefits as traditional medicines and has been used as an anti-bacterial, antiseptic, narcotic, and sedative for centuries (Ganesh *et al.*, 2015).

Bioactive compounds and alkaloids in the *D. metel* included fatty substances, steroids, phenolic saponins, tannins, and tropane alkaloids: such as atropine, hyoscyamine, scopolamine, hyoscine, metosdina, norhiosiamina, norscopolamine, cuschohygrine, and nicotine (Huong, 1990; Thomas, 2003). The high content of alkaloids in the *D. metel* can be used as a natural pesticide for pest control in the fish ponds. In addition, alkaloids can also be used in fish as an anesthetic in the transportation processes. The goal is to reduce stress levels and fish deaths on the transportation processes. Natural anesthetic from *D. metel* with the compounds contained can give the fish unconscious or fainting effect.

Toxicity is the ability of a substance that has toxic properties, so that it can cause organ damage to organism. Rahmawati and Romi (2017) stated that toxicity is an effect that causes functional,

* Corresponding author.
Email address: humairanirindhira@gmail.com

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biochemical, or physiological (structural) disorders that can cause pain that interferes with the health of the organism's body. The tropane alkaloids contained in the *D. metel* plant are anticholinergic alkaloids that can be toxic to the nervous system, so that the safety limit for their use needs to be set (Sharma *et al*., 2021).

Based on this description, the researcher aims to calculate the effect of *D. metel* toxicity on *Artemia salina* by using the BSLT (Brine Shrimp Lethality Test) method. In addition, the purpose of this study was to calculate and analyze the toxicity levels and compare the toxicity levels of stem, leaf, and flower extracts. BSLT is a toxicity test method using one of the aquatic animals, in the form of *A. salina* larvae (Meyer *et al*., 1982). This method is most commonly used because of the easy, fast and low cost of treatment.

Several studies have shown that BSLT can be used to measure the toxicity of herbal materials for medical purposes. BSLT has also been used to measure the toxicity of herbal compounds that have the potential to be used as anesthetic ingredients (Purbosari *et al.* 2022). The BSLT test is generally used to see 50% mortality of test animals exposed to herbal extracts or the effectiveness of the concentration of compounds that cause toxicity in test animals. However, the use of the BSLT method can only be used to determine the initial concentration to be used for the test because the toxicity effect is not able to describe physiological damage due to compound toxicity (Setiani *et al.* 2023).

Materials and Methods

Location and time of research

This research is an experimental study with white flowered *D. metel* plant extract with an extraction solution using ethanol and ethyl acetate. The plant parts of *D. metel* used include; stems, leaves and flowers. The method used in the toxicity test is the Brine Shrimp Lethality Test (BSLT) method based on the method of Mayer *et al.* (1982). This method uses *A. salina* larvae as an organism toxicity test. This research was conducted from December 2021 to February 2022. All stages of extraction and anesthesia testing were conducted at the Aquaculture Laboratory, Faculty of Agriculture, Almuslim University, Bireun Regency, Indonesia.

Materials and tools preparation

The materials used in this study were *D. metel*, sea water, fresh water, *A. salina*, yeast, ethyl acetate and 90% ethanol. The tools used in this research are aquarium, blender, measuring cup, beaker, filter paper, test tube, soxhlet, aquarium, and aerator.

D. metel plants used as samples consisted of stems, leaves, and flowers obtained in the districts of Aceh Tengah and Bener Meriah. *D. metel* collected according to the part of the plant, then cleaned of dirt and aphids by washing under running water until clean. Then drained, then chopped into small pieces. Dry by airing in a room until completely dry. *D. metel* has been dried is mashed by means of a blender to obtain *D. metel* powder.

Preparation of *D. metel* extract

In this extraction step of *D. metel* using maceration method with two treatment solutions of the compound, namely ethyl acetate and 90% ethanol. This extraction has been modified from the study of Zulfahmi *et al.* (2018). *D. metel* powder was weighed according to the dose and put into a macerator then soaked with a solution of the compound used in the treatment (ethyl acetate or 90% ethanol), then covered with aluminum foil for 48 hours while stirring occasionally. The maceration results were filtered using filter paper (Whatman no.1). The results of the *D. metel* filter were weighed, then mixed with the solution (ethyl acetate or 90% ethanol) into one. The result of the filtration was evaporated with Soxhlet, so that a clear thick colored *D. metel* extract was obtained. The maceration process can be repeated twice using the same solvent. The results of the D. *metel* extract were weighed and ready for testing.

Identification of active compounds

GC-MS analysis was carried out to identify the active compounds contained in each extract. GC-MS analysis was performed using a Shimadzu GC-MS-QP2010 Ultra equipped with a 30-m × 0.25-mm × 0.25-µm Rxi-1MS column (Restek), and the initial temperature of the 100°C column was heated for 5 minutes, then the temperature was gradually increased up to 250°C at a rate of 10°C min^{-1}. The split injector and the GC-MS interface are each at a temperature of 250°C. The detectors used were mass-selective and electron-impact mass ionization spectrometry programmed at 70 eV and a temperature of 250°C. The carrier gas used was helium with a flow rate of 2.0 mL min^{-1}, and an injection volume of 2 L. Data was recorded using GC-MS Solution Software (Shimadzu). The components extract detected compared to the activity of the components with potentiality as anaesthetic agents in the literature existed.

Preparation of *A. salina* larvae

Artemia eggs are incubated in hatching funnels that have been filled with seawater and carried out under light. At the time of hatching, water quality

parameters that must be considered are temperature, pH, salinity 28-30 ppt, and DO.

Toxicity test

Ten larvae of *A. salina* were transferred to each treatment tank using a 9-inch disposable pipette, and seawater was added to reach 5 mL. A drop of dry yeast suspension was added as feed to each tank. The treatment tank is placed under the light. Surviving *A. salina* was counted with the help of a 3x magnifying glass lens after 6 hours and 24 hours. If a control death occurred, the data were corrected by the Ordaz-Silva *et al.* (2016) formula, as follows:

$$\text{Mortality (\%)} = \frac{\text{treatment/control}}{\text{control}} \times 100$$

Determination of LC_{50}

LC_{50} at doses of 100 ppm, 250 ppm, 500 ppm and 1000 ppm with 95% confidence intervals determined from a 24-hour count using the probit analysis method. If the data is not sufficient to perform this technique, then the dose-response data is transformed into a straight line using the logit transformation. The LC_{50} value is derived from the best line obtained from the regression analysis.

Results

Identification of active compounds

GC-MS analysis data on ethanol extracts of leaves and flowers can be seen in Table 1, while ethyl acetate extracts of stems and flowers of amethyst plants, can be seen in Table 2.

Secondary metabolic compounds were identified for ethanol extracts only on the leaves and flowers. In the stem part, the yield produced is very small so it does not reach the minimum number of samples needed for GC-MS testing. A total of 19 compounds were identified in the leaves and 13 compounds in the flowers. In the leaves, the main components identified in the extract were hexadecanoic acid, ethyl ester (CAS) ethyl ester (15.37%), phytol (34.38%) and elaidic acid, (E)-9-octadecenoic acid ethyl ester (14.49%). In flowers, the main components identified were hexadecanoic acid, ethyl ester (CAS) ethyl ester (34.39 %) and elaidic acid, (E)-9-octadecenoic acid ethyl ester (30.88 %).

Table 1. Bioactive compounds of ethanol extracts determined by GC-MS Analysis.

No	Compounds	Ethanol Extract			
		Leave		Flowers	
		Ret	Area (%)	Ret	Area (%)
1.	1-methyl-2-pyrrolidineethanol	7.455	1.34	7.466	2.18
2.	1,2-Dimethylpyrrolidine			8.839	1.99
3.	1-Methyl-pyrrolidine-2-carboxylic acid	9.020	4.16		
4.	2H-Inden-2-one, octahydro-	14.420	0.66		
5.	(-)-Loliolide	14.481	1.45		
6.	9-oxa-bicyclo[4.2.1]non-7-en-3-on	15.042	2.37		
7.	2-Pentadecanone, 6,10,14-trimethyl-	15.980	2.48		
8.	NEOPHYTADIENE	16.038	4.90	16.033	6.90
9.	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-,	16.497	1.32	16.494	2.74
10.	Hexadecanoic acid, methyl ester	16.810	1.30	16.810	2.23
11.	Palmitic acid, n-Hexadecanoic acid	17.268	1.45		
12.	Hexadecanoic acid, ethyl ester (CAS) Ethyl ester	17.554	15.37	17.547	34.39
13.	1-Octadecanol (CAS) Stenol	18.476	2.00		
14.	9,12-Octadecadienoic acid (Z,Z)-, methyl e			18.519	0.88
15.	Phytol	18.844	34.38	18.797	2.96
16.	Linoleic acid ethyl ester	19.192	2.83	19.180	2.47
17.	Elaidic acid, (E)-9-Octadecenoic acid ethyl ester	19.278	14.49	19.270	30.88
18.	Stearic acid, 9-Octadecenoic acid (Z)-, ethyl			19.315	5.43
19.	Stearic acid, Octadecanoic acid, ethyl ester	19.525	3.10	19.517	5.59
20.	Borane, diethylmethyl- (CAS) Methyldieth	20.141	1.14		
21.	1-Octadecanol (CAS) Stenol	20.426	4.04		
22.	Hexadecanoic acid, 2-hydroxy-1-(hydroxy			22.594	1.34
23.	Palmitic acid. beta.-monoglyceride, Hexade	22.600	1.20		

Table 2. Bioactive compounds of ethyl acetate extracts determined by GC-MS Analysis.

No	Compounds	Ethyl Acetate Extract			
		Stem		Flowers	
		Ret	Area (%)	Ret	Area (%)
1	2-Hexen-1-ol, acetate,(E)- (CAS) trans-2-	4.218	1.67		

No	Compounds	Ethyl Acetate Extract			
		Stem		Flowers	
		Ret	Area (%)	Ret	Area (%)
2	(+)-3-Carene, 2-(acetylmethyl)-	5.212	3.56		
3	1,2-Epoxy-3-propyl acetate			7.181	3.47
4	1,3-Cyclopentanedione, 4-butyl-(CAS) 4-			7.535	0.63
5	1,2,3-Propanetriol, monoacetate			8.139	18.44
6	2-Decenal, (E)-	8.379	1.40		
7	Indole			8.821	1.26
8	Methyl 2-(2-butoxyethoxy)acetate	8.854	1.98		
9	3-Quinuclidinol			8.990	1.17
10	2-Methoxy-4-vinylphenol			9.150	1.31
11	2(3H)-Furanone, dihydro-5-pentyl-	9.644	2.02		
12	Cyclopropanecarboxamide, 2,2-dimethyl-N			9.912	0.94
13	Benzaldehyde, 4-hydroxy-3-methoxy- (CA	10.244	2.35		
14	1,2,3-Propanetriol, 1-acetate			10.300	3.26
15	4,7-Methanoazulene, 1,2,3,4,5,6,7,8-octahy	10.523	1.75		
16	Guaia-1(10),11-diene			10.525	1.35
17	1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octane-			10.774	2.04
18	3-(trans-2'-acetoxy-cyclohexy			10.999	3.32
19	Seychellene	11.37	7.51	11.372	7.46
20	Ethanone, 1-(4-hydroxy-3-methoxyphenyl)	11.456	2.63		
21	1H-3a,7-Methanoazulene, 2,3,6,7,8,8a-hexa	11.566	5.78	11.573	2.75
22	1,2-Cyclohexanediol, 1-methyl-,Trans-			11.731	3.1
23	Hexadecane, 1-chloro- (CAS) 1-Chlorohex	11.957	0.85		
24	CIS-Limonene oxide			12.089	3.83
25	bicyclo[4.1.0]hept-3-en, 2-isoprop	12.384	0.93		
26	Teloidinone $$ 8-Azabicyclo[3.2.1]octan-3			12.592	4.33
27	Tricyclo[8.6.0.0(2,9)]hexadeca-8,16,head,ta	12.850	0.69		
28	Ledol	12.906	1.28	12.925	2.33
29	(-)-Caryophyllene oxide	13.067	2.86	13.073	2.12
30	1-Pentadecene	13.125	4.11		
31	Humulene epoxide 2, 12-Oxabicyclo[9.1.0]			13.376	1.99
32	Ethanone, 1-(3,4,5trimethoxyphenyl)-	13.784	1.70		
33	Nitrobenzene, 3,4,5-trimethoxy-	14.263	1.24		
34	7-Oxabicyclo[4.1.0]heptane, 1-(1,3-dimeth	14.455	1.90		
35	Limonene dioxide 1	14.539	3.75	14.525	2.83
36	Ethyl L-menthyl carbonate			15.079	1.21
37	Pyrimidine, 2,4-dihydrazino-5-nitro-6-meth	15.179	8.10		
38	(-)-Caryophyllene oxide	15.327	0.88		
39	2-(Hexyloxy)benzaldehyde			15.425	1.63
40	1-Octadecene	15.530	5.58		
41	Cyclotetradecanol, 1,7,11-trimethyl-4-(1-m	15.774	0.77		
42	Neophytadiene	16.044	0.84		
43	Phytol, acetate			16.050	1.2
44	Pentadecanoic acid, 14-methyl-, Methyl est			16.847	3.79
45	Hexadecanoic acid, methyl ester	16.852	10.74		
46	Phthalic acid, dibutyl ester $$ Dibutyl phth	17.019	1.16		
47	Palmitic acid, n-Hexadecanoic acid	17.388	5.53	17.547	5.14
48	1-Octadecanol	17.692	3.15		
49	Linoleic acid, 9,12-Octadecadienoic acid (Z	18.540	1.32	18.550	3.95
50	Elaidic acid, 9-Octadecenoic acid, methyl e	18.623	2.23		
51	Phytol	18.832	2.99	18.826	2.73
52	Methyl stearate	18.894	3.06		

No	Compounds	Ethyl Acetate Extract			
		Stem		Flowers	
		Ret	Area (%)	Ret	Area (%)
53	9,12-Octadecadienoic acid (Z,Z)-(CAS) Li			19.197	3.33
54	Benzeneacetic acid, .alpha.-[[(trimethylsilyl			19.471	1.73
55	1,4-Methanoazulen-7-ol, decahydro-1,5,5,8	19.485	2.42		
56	1-Tricosanol	19.659	1.28		
57	1-Octadecanol (CAS) Stenol			20.431	0.54
58	Scopolamine			21.098	3.83

Table 3. LC_{50} values in *A. salina* treated with ethanol and ethyl acetate extracts of leaves, stems and flowers of *D. metel* after 24 hours of observation.

Extract	*A. salina* mortality (%)				LC_{50} ppm
	100	250	500	1000	
Leaf ethanol	14	34	42	60	639.589
Leaf ethyl acetate	26	34	38	44	2040.887
Flower ethanol	44	68	54	70	121.044
Flower ethyl acetate	70	64	74	100	105.89
Stem ethanol	28	28	24	44	3905.27
Stem ethyl acetate	32	32	34	100	635.276

Identification of secondary metabolite compounds in ethyl acetate extract was carried out on the stem and flower of amethyst. In the leaves, the yield produced is also very small, so it does not reach the minimum number of samples needed for GCMS testing. The total compounds identified were 35 in the stem and 31 in the flower. The main compound components identified in the stem are Seychellene (7.51%), Pyrimidine, 2,4-dihydrazino-5-nitro-6-meth (8.10%), Hexadecanoic acid, methyl ester (10.74%). While the main compound components identified in the flower are 1,2,3-Propanetriol, monoacetate (18.44%) and Seychellene (7.46%).

Determination of LC_{50}

Figure 1 shows that in the control, *A. salina* can live up to 100% without the addition of any extract. In contrast to the test tank which has been added to the ethanolic leaf extract at concentrations of 100 ppm, 250 ppm, 500 ppm and 1000 ppm, the survival rates of *A. salina* are 78%, 66%, 58% and 38%, respectively. Furthermore, leaf ethyl acetate extract at concentrations of 100 ppm, 250 ppm, 500 ppm and 1000 ppm showed the survival rates of *A. salina* reached 74%, 66%, 62% and 55%, respectively.

In the control, *A. salina* can live up to 100% without the addition of any extract (Figure 2). In contrast to the test tank which has been added to the ethanolic stem extract at concentrations of 100 ppm, 250 ppm, 500 ppm and 1000 ppm, the survival rates of *A. salina* are 72%, 72%, 76% and 56%, respectively. Furthermore, stem ethyl acetate extract at concentrations of 100 ppm, 250 ppm, 500 ppm and 1000 ppm showed the survival rates of *A. salina* reached 68%, 68%, 66% and 0%, respectively.

A. salina can live up to 100% without the addition of any extract in the control (Figure 3). In contrast to the test tank which has been added to the ethanolic flower extract at concentrations of 100 ppm, 250 ppm, 500 ppm and 1000 ppm, the survival rates of *A. salina* are 56%, 32%, 46% and 30%, respectively. Furthermore, flower ethyl acetate extract at concentrations of 100 ppm, 250 ppm, 500 ppm and 1000 ppm showed the survival rates of *A. salina* reached 30%, 36%, 30% and 0%, respectively.

Discussion

Identification of active compounds

Both hexadecanoic acid, ethyl ester (CAS) ethyl ester and elaidic acid, (E)-9-octadecenoic acid ethyl found as main components in the ethanol extracts are fatty acid compounds. It is known to have biological activities such as antitumoral, antimicrobial, antioxidant, decrease blood cholesterol, anti-inflammatory, hypocholesterolemic nematicide, pesticide, antiandrogenic flavour, hemolytic, 5-Alpha reductase inhibitor (Isbilen and Volkan, 2021). While phytol is a diterpene compound known to have biological activities as antimicrobial, cytotoxic, antioxidant, anticancer, apoptosis induction and autophagic protection, anxiolytic and anticonvulsant, immune-modulating, antinociceptive and anti-inflammatory properties (Islam *et al.* 2018).

As mentioned above, the ethyl acetate extracts main components were Seychellene, Pyrimidine, 2,4-dihydrazino-5-nitro-6-meth, Hexadecanoic acid, methyl ester and 1,2,3-Propanetriol, monoacetate. It is known that Seychellene is one of the components of patchouli oil and is the dominant component of *Valeria celtica* (Bicchi *et al.*, 1983), *Pogestemon cablin* (Swamy and Sinniah, 2015) and also identified in *Waldheimia glabra* (Giorgi *et al.*, 2013). Seychellene is known to have the ability as a non-selective inhibitor of cyclooxygenase, an enzyme that plays a role in the production of prostaglandins which are important mediators of pain and inflammatory responses

(Raharjo *et al.* 2017). While Propanetriol monoacetate was confirmed to be present in other plants exhibiting antimicrobial, anti-inflammatory, diuretic and anticancer effects (Foo *et al.*, (2015).

Determination of LC_{50}

Based on the results of the study, the LC_{50} value of flower ethanol extract, flower ethyl acetate, leaf ethanol and stem ethyl acetate of *D. metel* had an LC_{50} value of <1000 which was included in the toxic category. The LC_{50} value of flower ethanol reached 121.044 ppm, flower ethyl acetate 105.89 ppm, leaf ethanol 639.589 ppm and stem ethyl acetate 635.276 ppm. Leaf ethanol extract at a concentration of 1000 ppm showed the highest mortality with a percentage of 60% of the total number of *A. salina*. The flower ethanol extract showed the highest mortality at a concentration of 1000 ppm with a percentage of 70% of the total number of *A. salina*. Meanwhile, extracts of ethyl acetate from flowers and stems at a concentration of 1000 ppm can cause 100% mortality of *A. salina* in the first 6 hours. This indicates that the ethyl acetate extract of flowers and stems at a concentration of 1000 ppm has the highest toxic level compared to other concentrations.

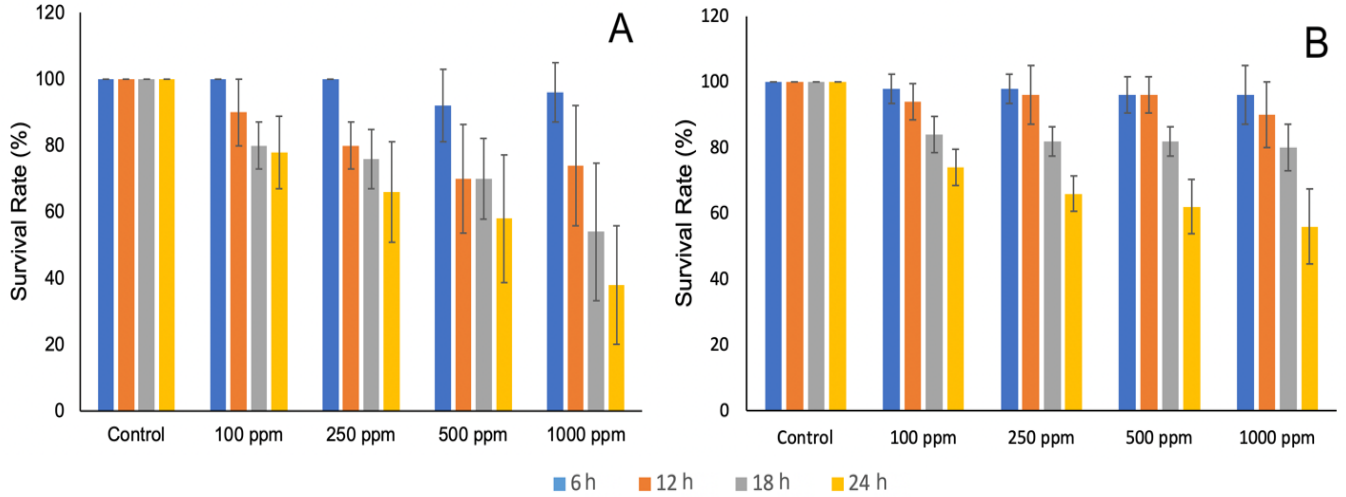

Figure 1. The survival rate of *A. salina* exposed to (A) ethanol and (B) ethyl acetate extracts of *D. metel* leaves.

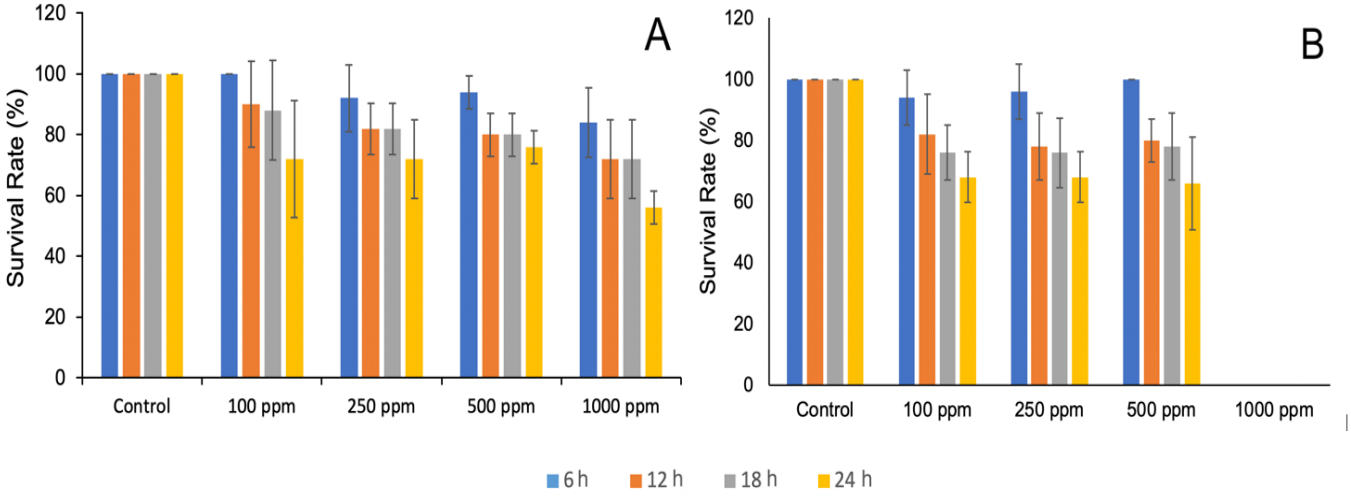

Figure 2. The survival rate of *A. salina* exposed to (A) ethanol and (B) ethyl acetate extracts of *D. metel* stems.

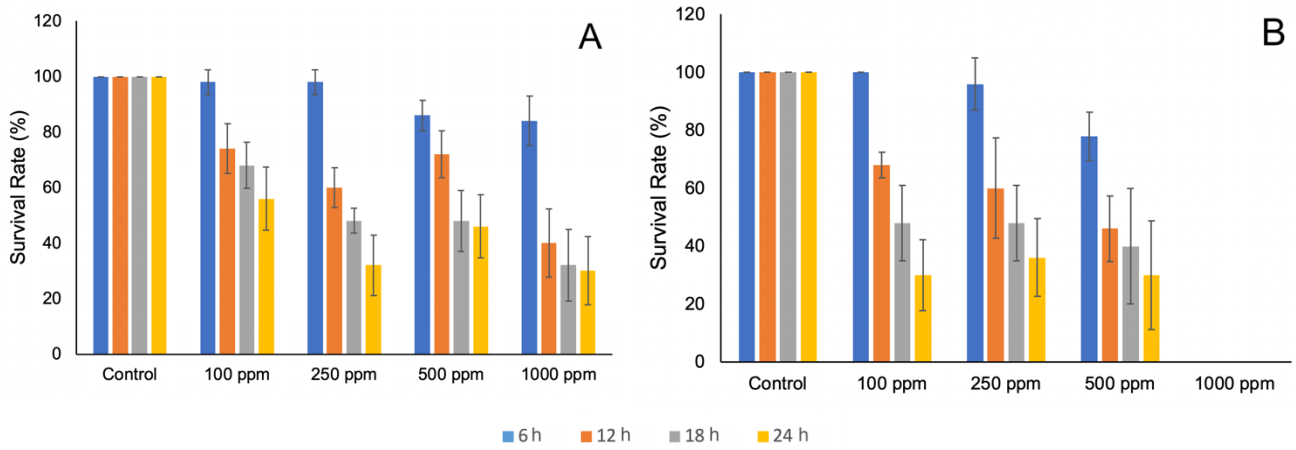

Figure 3. Survival rate of *A. salina* exposed to (A) ethanol and (B) ethyl acetate extracts of *D. Metel* flowers.

The LC_{50} value of the stem ethanol extract and leaf ethyl acetate was >1000 ppm with the stem ethanol value 3905.27 ppm and the leaf ethyl acetate 2040.887 ppm. The highest mortality in stem ethanol extract and leaf ethyl acetate occurred at a concentration of 1000 ppm with a percentage of 44% of the total amount of *A. salina*. At the highest concentration it could not kill up to 50% of the tested *A. salina* samples, this indicates that the stem ethanol extract and leaf ethyl acetate extract are included in the non-toxic category. An extract is categorized as non-toxic if it has an LC_{50} value >1000 ppm, is categorized as toxic if it has an LC_{50} value <1000 ppm and is categorized as very toxic if the LC_{50} value <30 ppm (Meyer *et al.*, 1982).

The high toxicity found in plant extracts can be caused by the content of secondary metabolites of alkaloids, tannins, steroids, and triterpenoids. The most abundant alkaloid content found in *D. metel* was scopolamine and atropine, with variations in the content of stems, leaves, and flowers ranging from 0.001 to 0.66 µg/mL for scopolamine and 0.001 to 0.27 µg/mL to atropine (Sharma *et al.*, 2021). The compounds contained in these plants can kill *A. salina* by acting as stomach poisoning. Therefore, if these compounds enter the body of *A. salina*, the digestive system will be disturbed. In addition, the metabolites present in these plants will also inhibit receptors in the mouth of *A. salina*. This can result in *A. salina* not being able to stimulate the taste, so it is unable to recognize its food and causing *A. salina* to starve to death (Noviati *et al.*, 2012). The content of secondary metabolites in *D. metel* can have a variety of beneficial biological activities but can be toxic when administered in large quantities (Cinelli and Jones, 2021). Several studies reported damage to the kidney epithelial tissue of rats given ethanol extract of leaves, seeds, and fruit of *D. meter* (Imo *et al.*, 2018), and significant changes in blood biochemical parameters in rats given methanol, water, and diethyl ether extract of seeds *D. stramonium* for 14 days (Ogunmoyole *et al.*, 2019). The toxic effect of the aqueous extract of *D. metel* leaves has also been investigated to cause changes in the pathophysiological conditions of the gills and digestive tract of *Cyprinus carpio* (Tasneem *et al.*, 2016).

The effects caused by toxic secondary metabolites occur very quickly in just 24 hours and can cause 50% of deaths from *A. salina* (Rohmah *et al.*, 2019), even reaching 100% at a concentration of 1000 ppm (Al-Hadhrami *et al.*, 2016). Research conducted by Jihad *et al.* (2019) reported a 75% mortality rate of *A. salina* after exposure to *D. stremonium* extract for 24 hours. The toxicity of secondary metabolites found in plants is used for self-defense against predators. This self-defense mechanism occurs by protecting target organs or inhibiting cell division affected by pathogens (Cutler and Cutler, 2000), or by interfering with the nervous system, membrane transport system, protein synthesis and enzymatic activity from predators (Adibah and Azzreena, 2019).

Conclusion

The main components identified in the ethanol extract of the leaves were hexadecanoic acid, ethyl ester (CAS) ethyl ester (15.37%), phytol (34.38%) and elaidic acid, (E)-9-octadecenoic acid ethyl ester (14.49%). While in the flowers, the main components identified were hexadecanoic acid, ethyl ester (CAS) ethyl ester (34.39 %) and elaidic acid, (E)-9-octadecenoic acid ethyl ester (30.88 %). In the ethyl acetate extracts the main components identified in the stem were seychellene (7.51%), pyrimidine, 2,4-dihydrazino-5-nitro-6-meth (8.10%), hexadecanoic acid, methyl ester (10.74%). While the main compound components identified in the flower were 1,2,3-propanetriol, monoacetate (18.44%) and seychellene (7.46%). All of the main components

showed beneficial biological activities and may be toxic in certain doses.

Toxicity test for 24 hours showed that the ethanolic and ethyl acetate extracts of leaves, flowers, and stems of *D. metel* had LC_{50} values <1000 ppm, whereas ethyl acetate extracts of flowers and stems caused the highest mortality at concentrations of 1000 ppm compared to others.

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