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Short Communication

Phytochemical Screening and Toxicity Test of Various Extracts from Microalgae *Dunaliella salina*

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Abstract

Microalgae are single celled microorganisms as the primary producers in the water food cycle. Microalgae bioactive compounds was estimated to be 10 times more diverse than compounds produced by land plants. Microalgae use nutrients more efficiently to grow, metabolize, and produce chemical compounds. *Dunaliella salina* is a species of chlorophyte microalgae with a lot of potential to be used in various fields. This study aimed to determine the phytochemical compound content and the value of lethal toxicity (24-hour LC50) in microalgae *D. salina* extract with different solvents. The multistage maceration method uses n-hexane, ethyl acetate, and methanol to extract samples. Phytochemical screening uses reagents according to the content of secondary metabolites. The *Brine Shrimp Lethality Test* method is used to test toxicity. The extracts were tested by using 10 *Artemia salina* against five concentrations, namely 0, 1, 10, 100, and 1000 ppm. Toxicity data were processed through probit analysis to get the 24-hour LC50 value. The results showed that alkaloids, steroids, triterpenoids, and phenols were found in the methanol, ethyl acetate, and n-hexane extracts. Saponin were found in the methanolic extracts. Flavonoid were found in the methanol and ethyl acetate extracts. The 24-hour LC50 value of the n-hexane extract was 276 ppm, the methanol extract was 811 ppm, and ethyl acetate extract was 673 ppm. The three extracts were included in toxic category. Extracts of microalgae *D. salina* have plenty secondary metabolite, that can be used in various fields and holds the potential as an anticancer.

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1. Introduction

Microalgae are single-celled microorganisms that act as primary producers in the food cycle in waters. Microalgae increase their biomass up to 30 times and the diversity of components from microalgae bioactive compounds was estimated to be 10 times more diverse than compounds produced by land plants (Raposo *et al*., 2013). This includes secondary and basal metabolites specific to certain environmental conditions. Microalgae use nutrients more efficiently to grow, metabolize and produce chemical compounds, so that many bioactive compounds and secondary metabolites are contained in microalgae. The benefits of microalgae bioactive compound products can be a solution to various health issues such as antioxidants, antifungals, antitumor, antibacterial, and anti-inflammatory agents (Suryaningtyas, 2019).

Dunaliella salina is a species of chlorophyte microalgae or green algae that has a lot of potential to be used in various fields. Microalgae *D. salina* produces chlorophyll, carotenoids, β-carotene, amino acids, fatty acids, and glycerol (Zainuddin, 2017). Microalgae *D. salina* are known for producing carotenoids around 1,100-2,100 mg and β-carotene per 100 grams of dry weight (Campo *et al*., 2009). Carotenoids are used as additive coloring agents, antioxidants, and pro-vitamin A, used as health supplements. B-carotene has benefits as an anticancer, antiaging, and immune system. In the research of Rusmawanto *et al.* (2019), *D. salina* was macerated using n-hexane as a solvent; found to contain alkaloids, flavonoids, steroids, terpenoids and saponins, but terpenoids were not found in extracts with ethyl acetate solvent. Flavonoids, tannins, alkaloids, saponins, steroids, and quinnons contained in *D. salina* macerated with methanol solvent (Widowati *et al*., 2017). *D. salina* macerated with acetone as a solvent contained flavonoids, alkaloids, steroids and saponins (Rajendran *et al*., 2014). Flavonoids, phenols, alkaloids, and carotenes were also found in fresh samples of *D. salina* extract, used as antioxidants and antimicrobials (Cakmak *et al*., 2014). These microalgae bioactive compounds also have the potential as anticancer and antioxidants (Singh *et al*., 2016). The lower the LC50 value, the compound has more significant potential as an anticancer/tumor (Meyer *et al*., 1982).

Plants with secondary metabolites can be toxic at certain levels, so it is necessary to use phytochemical screening to indicate the secondary metabolites in the sample and toxicity test to determine the minimum concentration of a sample to be toxic (Fatimah and Santoso, 2020). Brine Shrimp Lethality Test method can be used as a preliminary stage in screening materials suspected of having potential as anticancer/antitumor before conducting in vitro tumor cell testing (Ramachandran *et al*., 2011). BSLT aims to determine the level of toxicity using *A. salina* (Muaja *et al*., 2013). The mortality of Nauplius *A. salina* are calculated against the tested compounds in BSLT. *A. salina* is an organism commonly used to fulfill the nutritional needs of fish larvae in hatcheries. They often used as an ideal test sample in research on toxicity because of its resistance (Djokosetiyanto *et al*., 2007).

Based on the many studies that have been carried out on *D. salina*, the diversity of phytochemical compounds in microalgae can be influenced by the solvent used in the maceration stage. So, this study will analyze the phytochemical compounds and the lethal toxicity value of the *D. salina* extract obtained from BPBAP Situbondo using 3 different solvents. The solvents used were n-hexane, ethyl acetate, and methanol, in order to obtain a comparison of the compound content and LC50 values of the three *D. salina* extracts. This research hopes that the information regarding the content of phytochemical compounds and the toxicity value of *D. salina* microalgae extract can be used for potential exploration and further research on the utilization of *D. salina* microalgae compounds in various fields such as pharmacology and health in the future.

2. Materials and Methods

This research was conducted from March to April 2022. Sample extraction, phytochemical screening, and toxicity tests was done at the Marine Natural Product Laboratory of UPT Diponegoro University. The material used in this research was 100 grams of dry *D. salina* microalgae obtained from BPBAP Situbondo, East Java.

2.1 Sample Extraction

Extraction of the microalga *D. salina* was carried out by using the multistage maceration method. One sample was immersed in three solvents with different polarity levels, starting with n-hexane (non-polar), and subsequently followed by ethyl acetate (semi-polar) and methanol (polar). This method aims to determine the content of phytochemical compounds and the effect of the toxicity of microalgae extracts with different solvents. The ratio of dry sample to solvent in this extraction method is 1:5 (Setha *et al*., 2013). One hundred grams of dry *D. salina* microalgae was immersed in 500 mL of n-hexane solvent for 24 hours and then filtered with Whatman paper to separate the filtrate and residue. The residue was macerated again using 500 mL of ethyl acetate for 24 hours. After 24 hours, the residue was fil-

tered again, separating the precipitate from the filtrate. The microalgae residue was macerated again with 500 ml of methanol for 24 hours and then filtered to separate the filtrate from the residue. The filtrate obtained from the threefold maceration process was collected and concentrated at a temperature of 40°C using a rotary evaporator. This produced concentrated extracts of n-hexane, ethyl acetate, and methanol. The yield of a concentrated extract was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{Extract weight}}{\text{Initial sample weight}} \text{ x } 100\%$$

2.2 Phytochemical Screening

2.2.1 Alkaloid test

The *D. salina* extract was dissolved in a few drops of 2 N H_2SO_4 and then tested with two alkaloid reagents, namely Meyer's reagent and Dragendorff's reagent. The test results are positive with Meyer's reagent if the extract has a yellowish-white precipitate and positive with Dragendorff's reagent if the extract has a red to orange precipitate (Riyanto *et al*., 2014).

2.2.2 Triterpenoid and steroid

Another extract of *D. salina* microalgae was dissolved in 2 ml of $CHCl_3$. Then, 10 drops of $CH_4H_6O_3$ and 3 drops of concentrated H_2SO_4 were added. The test results are positive for triterpenoid if the extract has formed a brownish or violet ring on the border between the two solvents. In contrast, a bluish-green color is indicative of steroids (Riyanto *et al*., 2014).

2.2.3 Saponin test

The *D. salina* sample extract was added a little into a test tube. Then added warmed distilled water, shaken vigorously, and let stand for 10 minutes. Positive test results contain saponin compounds in extract if the foam is formed and does not disappear after adding 2N HCl for 15 minutes (Septiadi *et al*., 2013).

2.2.4 Flavonoid test

The extract of *D. salina* was dissolved a little in 2 ml of methanol, then added magnesium powder and 5 drops of HCl. Positive test results showed by the appearance of a red or orange color (Widowati *et al*., 2017).

2.2.5 Phenolic test

The extract of *D. salina* dissolved a little in 1 ml of ethanol. Then add 3 drops of 1% $FeCl_3$ solution. If there is a color change, it shows a substituted hydroxyl group for the benzene group or a phenolic compound (Hanani, 2015).

2.3 Artemia Setup and Hatching

The total of 0.25 grams of *A. salina* cyst was used with the brand Supreme Plus. *A. salina* cysts hatched into nauplius according to Panggabean (1984), namely by inserting Artemia eggs into seawater at a salinity of 30 ppt and oxygen levels of about five ppm for 24 hours. The development of *A. salina* cysts during hatching was observed and aerated. The oxygen content for hatching was adequate, and the cysts did not settle at the bottom of the incubator, which could inhibit cyst development. During the hatching process, radiation is also given to stimulate light, which is effective for cyst hatching. The density of the cyst also needs to be considered during the hatching process so that it is not too dense so that the cyst can develop properly. Around 48 hours after hatching, nauplius *A. salina* will be actively mobile and phototropic so they can use as toxicity test animals in BSLT (Mangirang *et al*., 2019).

2.4 Brine Shrimp Lethality Test (BSLT)

*Brine Shrimp Lethality Tes*t (BSLT) are use in toxicity test. BSLT aims to determine the toxic potential of 24-hour LC50 extract against Nauplius *A. salina*. A toxicity test was done based on Meyer *et al.* (1982) method, in which Nauplius *A. salina* tested to various concentrations of extract solutions. The concentration was 0, 1, 10, 100, and 1000 ppm. Preparation of a stock solution with a concentration of 1000 ppm was done by diluting 50 mg with 50 ml of seawater. The test was carried out by adding ten larvae of *A. salina* into a vial bottle that already contained a stock extract solution according to the concentration. A toxicity test was done for 24 hours for 3 repetitions and calculated the mortality of *A. salina* larvae. The 24-hour LC50 value in this test was obtained through probit analysis.

2.5 Data Analysis and Calculation of LC50

The toxicity effect of *D. salina* microalgae was analyzed from observations with percent mortality (Kurniawan dan Ropiqa, 2021).

$$\text{\% Mortality} = \frac{\text{Total of dead larvae}}{\text{Total of larvae}} \text{ x } 100\%$$

Once the mortality of *A. salina* larvae was determined, look for the probit value in the respective table and make a line equation:

$$Y = a + bX$$

Where:

Y = Probit Value of Test Animal Mortality, X = Logarithm of Test Animal, a = Constant, b = Slope m – X value at Y = 5 (probit value of 50% mortality of Test Animals.

The value of LC50-24 hours was obtained, and the value of antilog m. The value of m is the value of X at the time of death by 50%, so the linear function is:

$$5 = a + bX.$$

3. Result and Discussion

Species of Chlorophyceae microalgae that has a lot of potential to be used in various fields is *D. salina*. Microalgae *D. salina* produces chlorophyll, carotenoids, β-carotene, amino acids, fatty acids, and glycerol (Zainuddin, 2017). Carotenoids are used as antioxidants and pro-vitamin A health supplement. Carotene has benefits as an anticancer, antiaging, and immune system. *D. salina* also contain secondary metabolites such as flavonoids, alkaloids, steroids, triterpenoids, saponins and phenols. These microalgae bioactive compounds also have the potential as anticancer and antioxidants (Singh *et al*., 2016). The lower the LC50 value, the compound has a greater potential as an anticancer/tumor (Meyer *et al*., 1982).

3.1 Microalgae Extract

Three solvents were used to extract the *D. salina* microalgae, namely n-hexane (non-polar), ethyl acetate (semi-polar), and methanol (polar). By using three solvents with different polarities, three targets could be obtained—unknown bioactive compounds, compounds that are known to exist within an organism, and groups of compounds in organisms that are structurally related according to the polarity of the solvent (Sarker *et al*., 2006). The results of the *D. salina* extraction show weight, form and color from the yield extract (Table 1).

In this research, the highest yield of *D. salina* microalgae extract was produced by methanol extract, which was 6.74%. The yield of ethyl acetate extract was 0.96%, and n-hexane extract was 0.27%. The yield results showed that the extract produced by methanol solvent was the most abundant compared to ethyl acetate and n-hexane solvents. That happens because the methanol is universal solvent, so it can attract semi-polar and polar compounds (Novianti, 2019). The n-hexane solvent extracted the least number of microalgae because n-hexane is a non-polar solvent, which can only attract non-polar compounds. The yield results that differ greatly between methanol with n-hexane and ethyl acetate are also suspected because the samples tested are crude extracts. The immature extract still contains other compounds from the sea such as salt, nutrients, and other minerals (Leksono *et al*., 2018). At the extraction stage, polar methanol not only takes secondary metabolites from microalgae, but also attracts other compounds.

D. salina microalgae extract produced various extract colors. Methanol extract has a green color, ethyl acetate extract has a dark green color, and n-hexane extract produces a yellow color. The yellow color of the extract indicates that the *D. salina* microalgae contain carotenoid pigments. The green extract indicates that the microalgae *D. salina* contains chlorophyll. Rizkina *et al*. (2013) support these findings in their research, which notes that chlorophyll a is bluish green, chlorophyll b is yellowish green, and carotenoids are yellow-orange.

The aim of using different solvents during the extraction process was to obtain compounds with various levels of polarity. Using different solvents in the extraction process aims to obtain compounds based on various levels of polarity. The extraction is expected to extract the compound with suitable polarity to the solvent in each step. Using three solvents with different polarities is used to obtain three targets, including unknown bioactive compounds, compounds that are known to exist in an organism, and groups of compounds in organisms that are structurally related (Sarker *et al*., 2006). The selection of the correct solvent according to the substance to be extracted is crucial during the extraction process. In addition, solvents with different polarities can attract compounds under the level of polarity from non-polar, semi-polar, and polar. Polar compounds will dissolve easily in polar solvents and non-polar compounds will dissolve easily into non-polar solvents (Fithriani *et al*., 2015).

3.2 Phytochemical Screening

Phytochemical screening was carried out to determine the compounds or active ingredients of secondary metabolites from *D. salina* microalgae extract. Phytochemical tests carried out were alkaloids, flavonoids, steroids, triterpenoids, saponins, and phenols against three *D. salina* extracts (Table 2).

A phytochemical test of *D. salina* microalgae showed that n-hexane, ethyl acetate and methanol extracts contained alkaloids, steroids, triterpenoid, and phenol compounds. Flavonoid compounds were present in methanol and ethyl acetate extracts but not in n-hexane extracts. Saponin compounds were present in the methanol extract but not in the n-hexane and ethyl acetate extracts. Alkaloid compounds are used as anes-

thetic agents, stimulate the nervous system, and fight microbial infections (Fithriani *et al*., 2015; Widowati *et al*., 2017). Steroid compounds have the effect of lowering cholesterol, anticancer, controlling metabolism, and improving the function of sexual organs (Nasrudin, 2017). Triterpenoid compounds can be antimicrobial, anticancer, antiviral, and anti-inflammatory (Balafif *et al*. 2013). Phenol compounds respond to different stimuli, protect cells against UV radiation and cell death to prevent damage (Cotas *et al*., 2020).

Flavonoid compounds exist in ethyl acetate and methanol extracts. Flavonoids are secondary metabolites of the polyphenol group, which have various potentials. They can be antioxidant, anti-inflammatory, anticancer, antiaging, and antimicrobial. It has multiple types and is present in the bound form as glycosides and free forms (aglycones). Polymethoxy aglycones are nonpolar while polyhydroxy aglycones are semipolar. Flavonoid glycosides are polar because they contain several hydroxyl groups and sugars (Santi *et al*., 2014). The flavonoid compound content in *D. salina* was thought to be polyhydroxy aglycone and glycoside because flavonoid was only found in ethyl acetate and methanol extracts. Flavonoids compounds found in ethyl acetate and methanol extracts.

Saponin compounds of *D. salina* were only found in methanol extracts because saponins are polar. So, only polar solvents can attract them. Astarina *et al.* (2013) found that saponin was polar because it is a glycoside form of sapogenins. The appearance of foam in the saponin test indicates that glycosides in the extract can form foam in water, which is hydrolyzed into glucose and other compounds. A polar solvent such as methanol can extract saponin compounds. Saponins have antimicrobial, anticancer, and bone health effects and stimulate the body's immune system (Fithriani *et al*., 2015).

Several factors influence the content of phytochemical compounds in a sample, including species, varieties, seasonal variations, growing conditions, and processing and storage methods (Novianti, 2019). Using solvents with different polarities also led to the various compound contents in the three D. salina extracts. Polar solvents can bind polar, nonpolar, and semipolar compounds. Semipolar solvents can bind semipolar compounds, whereas nonpolar solvents can bind nonpolar compounds. A compound will easily dissolve in a solvent that has a similar level of polarity.

3.3 Toxicity Test of Various Extracts of D. salina with Brine Shrimp Lethality Test (BSLT)

Percent mortality of *A. salina* to extracts of n-hexane, ethyl acetate, and methanol of microalgae *D. salina* was obtained after 24 hours of observation with three repetitions. Tests were done using five different extract concentrations to compare the toxicity values of 10 *A. salina*. The mortality percentage was obtained by dividing the total of dead larvae by the total of live larvae multiplied by 100% (Table 3).

Brine Shrimp Lethality Test (BSLT) is a test for toxicity analysis by describing the level of toxicity of an extract to *A. salina* larvae (Zuraida, 2018). The sample concentrations of the three extracts used were based on Rizkina *et al.* (2013), 0, 1, 10, 100, and 1000 ppm, using 10 nauplius *A. salina* in each test. The results of observations for 24 hours after 3 experiments showed that the n-hexane extract of *D. salina* caused the percent mortality at a concentration of 1 ppm to be 13.3%, 10 ppm to be 20%, 100 ppm to be 40%, 1000 ppm to be 63.3%, while concentration 0 ppm (control) did not cause death in test larvae. The highest mortality of *A. salina* to n-hexane extract of *D. salina* was found at a concentration of 1000 ppm with a mortality percentage of 63.3%. The lowest was at a concentration of 1 ppm with a mortality percentage of 13.3%. The ethyl acetate extract caused mortality at a concentration of 1 ppm at 20%, 10 ppm at 20%, 100 ppm at 43.3%, at 1000 ppm at 53.3%, and at a concentration of 0 ppm (control) did not cause death in test larvae. The highest mortality of *A. salina* to the *D. salina* ethyl acetate extract was found at a concentration of 1000 ppm with percent mortality of 53.3%. The lowest was at a concentration of 1 ppm with percent mortality of 20%.

The methanol extract of *D. salina* caused mortality at a concentration of 1 ppm at 23.3%, 10 ppm at 26.7%, 100 ppm at 40%, 1000 ppm at 53.3%, and a concentration of 0 ppm (control) did not cause death in test larvae. The highest mortality of *A. salina* to the methanolic extract were at a concentration of 1000 ppm with a percent mortality of 53.3%. The lowest was at a concentration of 1 ppm with a percent mortality of 20%. The regression graph of concentration with % mortality in methanol extract, ethyl acetate and n-hexane (Figure 1) also shows that the extract concentration was directly proportional to the percent mortality, where the higher the concentration of extract, then higher the probit percent mortality. Based on the results of the three extracts, these results are in accordance with Harborne (1987), which states that the higher the concentration of the extract, then higher the toxicity.

3.4 Probit Analysis and LC50 BSLT

Value processing of the mortality probit of the three extracts of *D. salina* resulted in a correlation between log concentration regression analysis and the percent mortality probit of the extract. It shows that they are directly related the concentration of extract to the percent mortality. The toxic nature of the extract dissolved in the live medium of the test animal during the toxicity test caused the death of the test animal. The type of extract and the compounds in the extract also affect the toxicity of a sample.

The best 24-hour LC50 value of *D. salina* extract in this study was n-hexane extract with an LC50 of 276 ppm. The lower the LC50 value of sample can be more toxic and compound has suggested to greater potential as an anticancer/tumor (Meyer *et al*., 1982). This is presumably because the microalgae extract *D. salina* with n-hexane as a solvent contains more carotenoid pigments than the methanol and ethyl acetate extracts. In the extraction process, the concentrated extract of n-hexane microalgae *D. salina* produces yellow. In contrast,

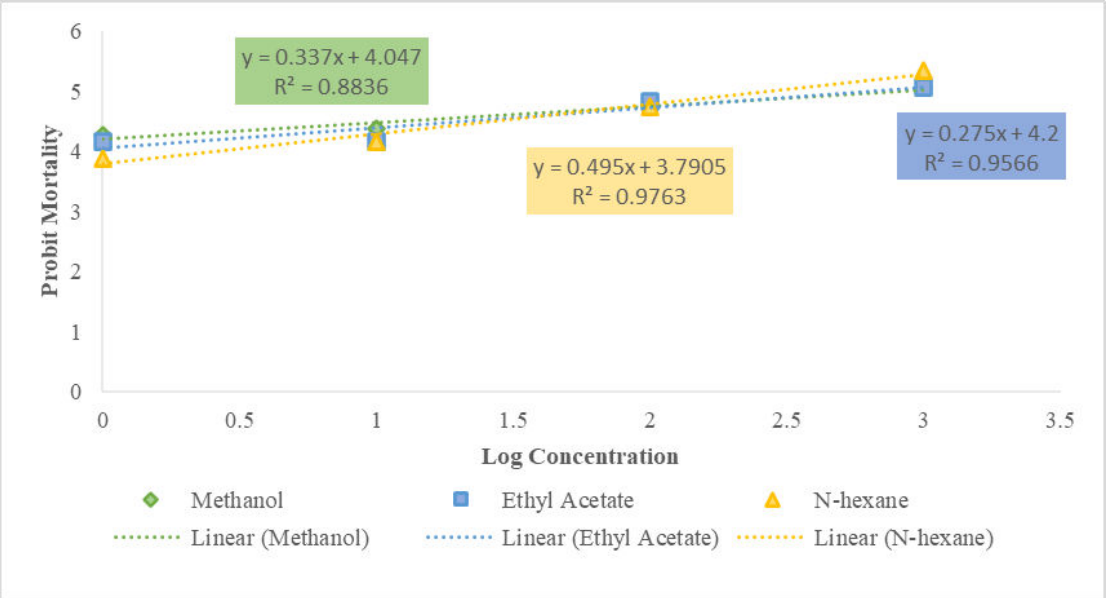

Figure 1. Concentration log regression graph with probit % mortality of *D. salina* extract

Based on the relationship between concentration and % mortality (Figure 1), the values of the linear regression equation for the *D. salina* microalgae were obtained, namely y = 0.4955x + 3.7905 in the n-hexane extract, y = 0.337x + 4.047 in the ethyl acetate extract, and y = 0.275x + 4.2 in the methanol extract. Probit analysis used to process the toxicity test data of methanol, ethyl acetate, and n-hexane extract *D. salina*. The calculation of probit analysis produces an intercept and x variable, which is used to calculate the 24-hours LC50 value, the toxicity value of the tested extract (Table 4).

The toxicity of a sample extract was determined by studying at the 24-hour LC50 value (Rizkina *et al*., 2013). According to Meyer *et al*. (1982), the BSLT method can determine that an extract having a 24-hour LC50 below 30 ppm is very toxic, 30-1000 ppm is toxic, and above 1000 ppm is non-toxic. By comparing the 24-hour LC50 value of the three microalgae *D. salina* extracts, the n-hexane extract was 276 ppm, ethyl acetate extract was 673 ppm, and methanol extract was 811 ppm, so that all the three were included in toxic category.

the ethyl acetate extract is light green. The methanol extract is dark green. According to Rizkina *et al*. (2013), extracts containing the pigment chlorophyll-a gave a dark green color, chlorophyll-b was yellowish-green; carotenoids were yellow-orange. Phytochemical compounds in a sample also can be toxic at certain levels (Puspa *et al*., 2017). In this study, the compounds contained in the n-hexane extract of *D. salina* that can be toxic are alkaloids, steroids, triterpenoids and phenols. Based on the phytochemical tests that have been done, the color change when tested with reagents was very clearly visible in alkaloid compounds compared to other compounds. So, it can be presumed that alkaloids at certain levels are the dominant toxic compounds that can cause death in *A. salina*.

Microalgae *D. salina* can produce carotenoids around 1,100 – 2100 mg a per 100 grams and contain 5 – 10 mg per 10 grams of dry weight (Campo *et al*., 2009). Carotenoids are used as additive coloring agents, antioxidants, anticancer agents, and pro-vitamin A, so they can be implemented as health supplements. β-carotene has benefits as an anticancer, antiaging, and immune system.

Table 1. The percentage yield of *D. Salina* extracts with different solvents

Solvents	Initial Weight (g)	Extract Weight (g)	Yield (%)	Form	Color
N-hexane	100	0.27	0.27	Pasta	Yellow
Ethyl Acetate	100	0.96	0.96	Pasta	Dark Green
Methanol	100	6.74	6.74	Solid	Light Green

Table 2. Phytochemical screening results of various extracts from *D. salina*

Phytochemical test		Results		
		N-hexane	Ethyl Acetate	Methanol
Alkaloid	Mayer	+	+	+
	Dragendroff	+	+	+
Flavonoid		-	+	+
Steroid		+	+	+
Triterpenoid		+	+	+
Saponin		-	-	+
Phenol		+	+	+

Table 3. The percentage mortality of *D. salina* microalgae extracts

Extract Concentration (ppm)	Percentage of Mortality (%)		
	N-hexane Extract	Ethyl Acetate Extract	Methanol Extract
0	0	0	0
1	13.3	20	23.3
10	20	20	26.7
100	40	43.3	40
1000	63.3	53.3	53.3

Table 4. The value of 24-hours LC50 *D. salina* extracts

Type Extract	LC_{50} Value (ppm)	Toxicity Category
Extract N-hexane	276	Toxic
Extract Ethyl Acetate	673	Toxic
Extract Methanol	811	Toxic

Description : LC50 < 30 ppm very toxic, LC50 30 – 1000 ppm toxic, LC50 >1000 ppm non-toxic (Meyer *et al*., 1982).

The phytochemical screening results show that *D. salina* contains secondary metabolites, such as flavonoids, alkaloids, steroids, triterpenoid, phenols, and saponins. All of that has many potentials in the health sector, such as anticancer, anti-inflammatory, and antioxidant. Toxicity test using test animals *A. salina* can be used as a preliminary test in research that leads to a cytotoxic test if the LC50 value of the initial toxicity test is < 1000 g/ml (Nerdy, 2021). Based on this research, methanol, n-hexane, and ethyl acetate extract of *D. salina* may be cytotoxic and can be further developed for anticancer treatment.

4. Conclusion

Secondary metabolites of alkaloids, steroids, triterpenoid, and phenols were found in the methanol, ethyl acetate, and n-hexane extracts of *D. salina*. Flavonoid compounds were found in the ethyl acetate and methanol extracts of *D. salina*. Saponin compounds were only found in the methanolic extracts of *D. salina*. The lethal toxicity value (24-hour LC50) of the n-hexane extract was 276 ppm, the methanol extract was 811 ppm, and the ethyl acetate extract was 673 ppm, so that all the three extracts were included in toxic category. The best 24-hour LC50 value was produced by the extract of n-hexane with the lower value among the others extracts. All of the three extracts were included in the toxic category so that further research can be carried out to isolate toxic compounds contained in *D. salina* microalgae extract and develop potential anticancer candidates and other potential.

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Authors' Contributions

All authors discussed and contributed from the start to the final manuscript. Each author's contribution is as follows, YY; collected the data and drafted the manuscript. WS and RH; critically revised the manuscript.

Conflict of Interest

All authors declares that they and this research have no conflict of interest.

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