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COMPARISON OF PHYTOCHEMICAL CONTENT AND TOXICITY OF N-HEXANA EXTRACTS AND FRACTIONS OF *Padina australis*

Riong Seulina Panjaitan*, Herni Farida

Faculty of Pharmacy, Universitas 17 Agustus 1945 Jakarta, Jl. Sunter Permai Raya No.1,Jakarta 14350, North Jakarta, Indonesia

*Email: riongpanjaitan@yahoo.co.id

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Abstract: *Padina australis is a brown macroalgae that is abundant in Indonesian coastal waters.* It is known to contain bioactive compounds such as alkaloids, flavonoids, steroids, saponins and tannins. This research aims to produce and determine the best extraction technique to increase the % yield and test the toxicity of the methanol extract and the n-hexane fraction of *Padina australis*. This study began with macroalgae sampling, determination, extraction (maceration and *Ultrasound-Assisted Extraction* (UAE)), phytochemical screening, liquid-liquid fractionation, thin layer chromatography, and BSLT (Brine Shrimp Lethality Test) toxicity testing. According to the research results, it is known that the UAE method provides the highest % yield of methanol extract at 10.86% compared to the maceration method, namely 4.76%. Furthermore, there was no difference in the phytochemical content of the two extraction methods. Meanwhile, there are differences in the phytochemical content of the n-hexane fraction, namely that the maceration method contains steroids and the UAE contains steroids and saponins. Then the toxicity test for the methanol extract obtained the highest LC_{50} value for the UAE method at 352.508 ppm, while for the n-hexane fraction, the highest LC_{50} value was 54.99 ppm. The methanol extract and n-hexane fraction in the toxicity test were included in the very toxic and toxic categories. The benefit of this research is to provide information about the comparison of the two extraction methods and toxicity tests of the methanol extract and n-hexane fraction of *Padina australis*.

Keywords: brown macroalgae; BSLT*; Ultrasound Assisted Extraction*

Abstrak: *Padina australis* merupakan makroalga coklat yang melimpah di perairan pesisir Indonesia. *Padina australis* diketahui mengandung senyawa bioaktif seperti alkaloid, flavonoid, steroid, saponin dan tanin. Penelitian ini bertujuan untuk menghasilkan dan menentukan teknik ekstraksi terbaik untuk meningkatkan % rendemen dan menguji toksisitas ekstrak metanol dan fraksi n-heksana Padina australis. Penelitian ini diawali dengan pengambilan sampel makroalga, determinasi, ekstraksi (maserasi dan *Ultrasound-Assisted Extraction* **(**UAE)), skrining fitokimia, fraksinasi cair-cair, kromatografi lapis tipis, dan uji toksisitas BSLT (*Brine Shrimp Lethality Test*). Berdasarkan hasil penelitian diketahui bahwa

metode UAE memberikan % rendemen ekstrak metanol tertinggi yaitu sebesar 10,86% dibandingkan dengan metode maserasi yaitu 4,76%. Selain itu, tidak terdapat perbedaan kandungan fitokimia pada kedua metode ekstraksi. Sedangkan kandungan fitokimia fraksi n-heksana terdapat perbedaan yaitu metode maserasi mengandung steroid dan UAE mengandung steroid dan saponin. Kemudian uji toksisitas ekstrak metanol diperoleh nilai LC_{50} tertinggi untuk metode UAE sebesar 352,508 ppm, sedangkan untuk fraksi n-heksana nilai LC_{50} tertinggi sebesar 54,99 ppm. Ekstrak metanol dan fraksi n-heksana pada uji toksisitas termasuk dalam kategori sangat toksik dan toksik. Manfaat dari penelitian ini adalah untuk memberikan informasi mengenai perbandingan kedua metode ekstraksi dan uji toksisitas ekstrak metanol dan fraksi n-heksana *Padina australis*.

Kata kunci: BSLT*;* makroalga cokelat; *Ultrasound Assisted Extraction*

INTRODUCTION

Indonesia has the largest marine diversity in the world or commonly called marine mega-biodeversity. One example of marine biota that has economic value in Indonesia is *Padina australis* (macroalgae). *Padina australis* is generally distributed in Indonesian waters, especially in Tidung Island, District of Seribu Islands. It grows on rocky substrates because it has disc-like rhizoid holdfasts, which are usually used to attach to dead coral fragments. It is shaped like a fan and has a brown color, forming thin sheet segments. The substance is gelatinous, the color is yellowish brown, the upper part of the lobe is slightly widened, and the roots are fibrous (Rosdiana et al., 2017). *Padina australis* is huge and visible with the naked eye, with a fan-like shape (Wijayanti et al., 2020).

According to the results of phytochemical screening, methanol extract of *Padina australis* contains alkaloids, flavonoids, triterpenoids, saponins, tannins, and phenols (Haryani et al., 2014). Compared to Maharany et al., (2017) research, the n-hexane extract of *Padina australis* contained flavonoids, phenol hydroquinone, triterpenoids, saponins, and tannins. Nuzul et al., (2018) described the ethanol, ethyl acetate, and diethyl ether extracts of *Padina australis* positively contained saponins, flavonoids, quinones, and tannins and no alkaloids. Another research reported that only alkaloid metabolite was presence in *Padina australis* (Pohuwato, 2018).

Based on literature study above, research on *Padina australis* has so far focused on the use of extracts from various solvents for further bioactivity testing purposes. Besides, the extraction method used is still relatively common, namely the maceration method (Haryani et al., 2014; Maharany et al., 2017; Nuzul

et al., 2018; Pohuwato, 2018). This maceration method has disadvantages in terms of time efficiency and the amount of solvent used (Khoddami et al., 2013). Therefore, it is necessary to carry out further research regarding the appropriate method for increasing the % yield by paying attention to time and solvent efficiency factors. This study was designed to compare the maceration and UAE methods for increasing the % yield and efficiency of solvent use and time. The *Ultrasonic Assisted Extraction* (UAE) method is an extraction technique that involves applying ultrasonic waves to the material to be extracted (Chemat et al., 2017). The advantages of this method compared to the maceration method are the increasing penetration of the liquid into the cell walls (Kanifah et al., (2015)), faster mass transfer rate, enlargement extraction yield, low temperatures, small solvent volumes, and a short time (Dey & Rathod, 2013).

In addition to phytochemical testing, toxicity tests were performed on the methanol extract and n-hexane fraction of *Padina australis.* Toxicity tests are carried out to determine the safety level of an extract and fraction. *Brine Shrimp Lethality Test* (BSLT) method utilize *Artemia salina* Leach larvae for toxicity test puposes and is expressed in the Lethal Concentration 50 (LC_{50}) value. The advantages of using this larvae is its high sensitivity to sample, easy cultivation, shorter life cycle.

Based on the explanation above, this research was designed to obtain a comparison of the highest % yield (between maceration and UAE) and a comparison of the secondary metabolite content and toxicity of between methanol extract and the n-hexane fraction of *Padina australis.*

METHODS

The tools used in this research were analytical balance (Boeco Germany), rotary evaporator, water bath (Memmert®), glassware (Pyrex), Whatman No. 41 filter paper, aluminum foil, evaporating cup, separating funnel, spatula, blender, equipment maceration, glass bottles, stopwatches, sonicators and micro pipettes, aquariums and lights.

The materials used in this research were samples of macroalgae (*Padina australis*), n-hexane, methanol p.a (Merck), silica gel GF_{254}, aquadest, yeast, *Artemia salina* Leach larvae (*Supreme Plus*) and iodized salt.

Collecting Macroalgae

The macroalgae used in this research was *Padina australis,* which was

sampled from Harapan Island, North Seribu Islands (Kepulauan Seribu), North Jakarta, Indonesia. All parts of the macroalgae were collected in fresh condition, at 08.00 p.m. Then, they were cleaned and stored in a cooler box containing ice cubes to maintain their freshness during the transportation process from the beach to the laboratory.

Determination of Macroalgae

The determination of macroalgae was carried out at the Oceanographic Research Center (LIPI) on Jl. Pasir Putih I, East Ancol, Jakarta. This work aims to determine the species of brown macroalgae so errors do not occur in conducting research.

Sample Preparation

The sample used in this research was 10 kg of fresh *Padina australis*. The samples were washed thoroughly to remove sand, then air dried without exposure to direct sunlight. Then, they were grinded using a blender (Miyako bl 101), weighed and stored them in the freezer for use at the next stage.

Extraction

Maceration Method

100 grams of *Padina australis* samples were put into a maceration bottle and then extracted using 500 mL methanol solvent (1:5 (w/v)). The maceration process lasts for 7x24 hours with the solvent changed every 24 hours, repeated in triplicate. The maceration process will be stopped after 7 days because the color of the extract is colorless. The maceration results were then filtered and evaporated at 40°C with a rotary evaporator until a concentrated extract was obtained. Then, the concentrated extract were stored in the refrigerator for the next stage (Maharany et al., 2017).

Ultrasound-Assisted Extraction (UAE) Method

A total of 30 grams of *Padina australis* was dissolved in 300 mL of methanol solution (1:10; (w/v)). Then, extraction was carried out using ultrasound every 1 hour. The extraction process will be stopped after 5 cycles, until the solution turns colorless. This extraction was carried out at a frequency of 42 KHz and 50% ultrasonic amplitude, the filtrate was evaporated using a rotary evaporator at a temperature of ± 40℃ then calculated to determine the % yield (Sasongko et al., 2018)

Liquid– Liquid Fractionation

The methanol extract that was obtained from the maceration and UAE process was carried out by a liquid-liquid

fractionation process using a separating funnel. 10 g of concentrated *Padina australis* methanol extract was first dissolved with methanol and followed by 100 mL of distilled water. The solution was then partitioned by adding 100 mL of n-hexane, shaking in a separating funnel and allowed to stand until there were two layers (distilled water at the bottom and n-hexane at the top). The two layers formed were then separated, taking the n-hexane layer. Refractionation was carried out until three (3) times with the same solvent until it became colorless. Further, all the n-hexane fractions were collected and evaporated using a rotary evaporator at a temperature of 40℃ to obtain the n-hexane fraction (Lisi et al., 2017).

Phytochemical Screening of Methanol Extract and n-Hexane Fraction of Padina australis

The further stage is phytochemical screening of the methanol extract and n-hexane fraction (maceration and UAE) (Harborne, 1987) Phytochemical screening includes alkaloid test, flavonoid test, saponin test, steroid and triterpenoid test and tannin/polyphenol test.

Identification of the Chemical Content of the n-Hexane Fraction Using Thin Layer Chromatography (TLC)

The n-hexane fraction of *Padina australis* was identified using thin-layer chromatography (TLC). Thin layer chromatography analysis was carried out on silica gel GF_{254}, 10 µL of each was spotted on a 1x10 cm TLC plate. The results obtained are observed, and the eluent that produces the best separation is then used as an eluent in column chromatography. (Bustannussalam et al., 2014) .

Toxicity Test of Padina australis Extracts and Fractions using the Brine Shrimp Lethality Test (BSLT) Method

Toxicity test of the extract and n-hexane fraction of *Padina australis* using the *Brine Shrimp Lethality Test* (BSLT) method was aimed to obtain data and processed using a probit analysis model to determine the LC_{50} value.

Preparation of Artificial Sea Water

The material used in making artificial sea water is sea salt without iodine as much as 38 grams in 1000 mL of distilled water to obtain a hygienic level of 38 ppt. Preparation media of hatching *Artemia salina* Leach eggs was carried out by dissolving sea salt without iodine in a glass beaker until completely

dissolved, then putting it in a 1000 mL measuring flask and adding distilled water to the mark (Panjaitan & Natalia, 2021).

Hatching of Artemia salina Leach Eggs

Hatching of *Artemia salina* Leach eggs was done by soaking 1 gram of *Artemia salina* Leach eggs in a container divided into two rooms, namely a light room and a dark room containing 1 liter of artificial sea water. The bright room was illuminated by incandescent lamps and equipped with an aerator, while the dark room was covered with black cloth and black duct tape. After 24 hours, *Artemia salina* Leach eggs hatched into larvae. 0.06% yeast solution was given to 24-hour-old larvae in the hatching container. After 48 hours, *Artemia salina* Leach larvae were collected to testing purposes. The larvae used in this research were larvae that were still actively moving and were 36 - 48 hours old (Wulandari, 2018).

Preparation of Test Solution for Methanol Extract and n-Hexane Fraction of Padina australis

In preparation of the test solution, it was prepared from methanol extract and n-hexane fraction of *Padina australis* with concentrations of 1000, 100, and 10 µg/mL. 4 vials were prepared for each concentration of the test solution, so in total there were 3 test vials and 1 vial for the control.

To obtain a concentration of 10,000 µg/mL stock solution, 100 mg of sample were dissolved in 10 mL distilled water. From stock solution (10,000 µg/mL), test solutions were created for 1000, 100 and 10 µg/mL (Panjaitan & Natalia, 2021).

Brine Shrimp Lethality Test (BSLT) Testing

48-hour-old *Artemia salina* Leach (10 larvae) were put in each vial and added 10 mL of *Padina australis* n-hexane fraction in each concentration namely 1000, 100 and 10 µg/mL respectively. Then all the vials were placed under the light. After 24 hours, the number of dead larvae was counted. Seawater controls have also been carried out where 10 larvae were put into the vial and add up to 10 mL of artificial seawater without adding the *Padina australis* n-hexane fraction. The test was carried out in triplicate to get accurate results. The data were analyzed using the probit analysis method to determine the LC_{50} value (R. Hamidi et al., 2014)

Determination of LC_{50} Value

Measurements were carried out by counting the number of dead *Artemia salina* Leach as much as 50% of the total

test larvae (10 larvae in the vial). Then the LC_{50} value is calculated by entering the probit number (50% death of test larvae). The toxicity effect was calculated from the percent death of *Artemia salina* Leach larvae and the linear regression equation (Rizqillah, 2013). LC_{50} is the antilog value of x, when y = 50%.

a. Formula of % Mortality

$$\% \text{ Mortality} = \frac{\text{number of dead larvae}}{\text{number of test larvae}} \times 100\%$$

b. Linear Regression Equation

$$y = a + bx$$

Where:
y = Probit Value
x = log concentration
a = Intercept
b = Slope

RESULTS AND DISCUSSIONS

Padina australis macroalgae was obtained from Harapan Island, District of Seribu Island, North Jakarta, Indonesia. Based on visual observations, the *Padina australis* macroalgae obtained from Harapan Island was found to be shaped like a fan, in the form of thin, segmented sheets with lines that tend to be circular. The edges of the talus tend to curve inward. The thallus is light brown-greenish.

Figure 1. *Padina australis* from Harapan Island

This is in accordance with research by Marcel (2015), which stated that *Padina australis* species is shaped like a fan with a diameter of 3–4 cm, grows in concentric circles, has many or sword-like leaves, is shaped like a fan and has a brown color, forming thin sheet segments, the substance is gelatinous, the color is yellowish brown, the upper part of the lobes is slightly wider, and the roots are in the form of fibers called holdfasts to stick firmly to the substrate so that they can be used to adapt to wave movements in the intertidal area. The holdfast structure is disc-shaped.

Results of Macroalga Padina australis Extraction Using Maceration and Ultrasound-Assisted Extraction (UAE) Methods

From the extraction results (maceration and UAE), there was differences in the color of the extract from the two methods, where in the maceration method, the color of the extract was brighter green, while UAE resulted brownish green.

Table 1. % yield of methanol extract (maceration and UAE)

Method	Time	Weight	Vol (mL)	Yield (%)
Maceration	7 days	100 g	500	4.76
UAE	1 hour	30 g	300	10.86

Based on Table 1, it can be concluded that the use of the UAE method is better than the maceration method, where the % yield resulting from the UAE method is 2x more than the maceration method. Besides, in using simplicia, the UAE method requires less simplicia than the maceration approach. The advantage of using UAE is that less solvent is used and the extraction time is faster and the % yield value is higher compared to the maceration method. Furthermore, Widyasanti et al., (2018) discovered that extraction using the UAE method provided a larger percentage yield than the maceration approach.

Phytochemical Compounds of Methanol Extract of Padina australis

Phytochemical screening aims to identify the content of secondary metabolite in natural or plant materials. In this study, a comparison was conducted between the phytochemical content of *Padina australis* methanol extract extracted using the maceration method (7 days) and the *Ultrasound-Assisted Extraction* (UAE) method (1 hour). The comparison results for the two methods are tabulated in Table 2.

Table 2. Comparison of Phytochemical Compounds of Methanol Extract of Macerated and UAE Methods-*Padina australis*

No	Compounds	Maceration	UAE
1	Alkaloid	+	+
2	Saponin	+	+
3	Tanin	-	-
4	Phenolic	-	-
5	Flavonoid	+	+
6	Glycoside	+	+
7	Triterpenoid	+	+
8	Steroid	+	+

Note:
(+) = presence
(-) = absence

Based on Table 2, it can be concluded that extraction using the maceration method and UAE contained the same phytochemical content. The results of this screening are in accordance with research by Haryani et al., (2014) where the methanol extract of *Padina australis* contains alkaloids, flavonoids, triterpenoids, saponins, and tannins.

n-Hexane Fraction

Fractionation is carried out to separate one main group of content from another main group based on differences in polarity. The solvent used in this research was n-hexane. The organoleptic properties of the n-hexane fraction are liquid with a slightly unpleasant odor, volatile, and colorless. The n-hexane fraction of *Padina australis* obtained was 9 mL. Then, the fractions were identified for chemical content using phytochemical screening.

Phytochemical Contents of the n-Hexane Fraction

The following stage is phytochemical screening of the n-hexane fraction from the maceration and UAE methods. The phytochemical screening carried out in this study included the examination of alkaloids, flavonoids, tannins/polyphenols, and steroids/triterpenoids (Harborne, 1987) The comparison results for the two extraction methods are tabulated in Table 3.

Table 3. Comparison of the Phytochemical Content of the n-Hexane Fraction of *Padina australis*

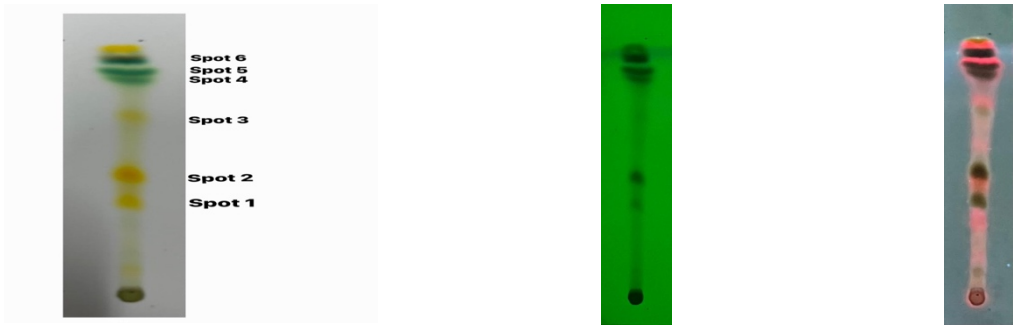
No	Compounds	n-hexane Fraction	
		Maceration	UAE
1	Alkaloid	-	-
2	Saponin	-	+
3	Tannin	-	-
4	Phenolic	-	-
5	Flavonoid	-	-
6	Glycoside	-	-
7	Triterpenoid	-	-
8	Steroid	+	+

Note:
(+) = presence
(-) = absence

From the results of phytochemical screening on the n-hexane fraction using the maceration and *Ultrasound Assisted Extraction* (UAE) methods, differences in results were obtained, where the maceration was positive for containing steroid compounds, while the UAE was positive for containing saponins and steroids. Compared to methanol extract above, methanol extract has more phytochemical compounds than n-hexane fraction.

Results of Thin Layer Chromatography (TLC) Analysis

Thin-layer chromatography (TLC) was used to separate the metabolites of n-hexane fraction of *Padina australis.* Commonly, TLC is used for bioactive compound identification and to determine the phytochemicals from plants (Appamaraka et al., 2022). This method utilized mobile phase (eluent n-hexane: ethyl acetate (6:4)) and stationary phase (silica gel GF_{254}).



(a) (b) (c)

Figure 2. Detected Spot on TLC of the n-hexane fraction of *Padina australis* (a); After Sprayed at UV 254 nm (b); After Sprayed at UV 366 nm.

Figure 2 shows a TLC profiling of n-hexane fraction of *Padina australis*. The fluorescent image was analyzed under UV 254 and 366 nm. The result of TLC fingerprinting of n-hexane fraction of *Padina australis* showed six (6) metabolites (Fig. 2).

Table 4. Results of preparative TLC separation of n-hexane fraction

Spot	Rf (cm)	Visual Colour	Predicted Compounds	References
1	0.769	Yellow	Steroid	Al-Quais (2015)
2	0.211	Yellow	Steroid	Al-Quais (2015)
3	0.578	Yellow	Saponin	Harwoko (2014)
4	0.252	Green	Steroid	Al-Quais (2015)
5	0.115	Green	Saponin	Harwoko (2014)
6	0.106	Green	Steroid	Al-Quais (2015)

From Table 4, the TLC profiling of n-hexane fraction revealed the presence of steroid and saponin metabolites. The metabolites spots formed will have different polarity properties. The metabolites spots that have a small Rf value will have more polar properties because they are more retained in the stationary phase (silica plate), while spots with a large Rf value will tend to have non-polar properties because they are carried away by the mobile phase, which has non-polar properties. Commonly, steroid compounds will produce large Rf values because they have non-polar properties (Rustanti et al., 2013). This is due to differences in the distribution of compounds and structures in the mobile and stationary phases used.

Toxicity Test of Methanol Extract and n-Hexane Fraction of Padina australis Against Artemia salina Leach Larvae

In the research on the toxicity test of methanol extract and n-Hexane fractionation of *Padina australis,* probit analysis was used to obtain a straight line curve with the LC_{50} value determined. The results of the toxicity test of *Padina australis* methanol extract are presented in Tables 5 and 6.

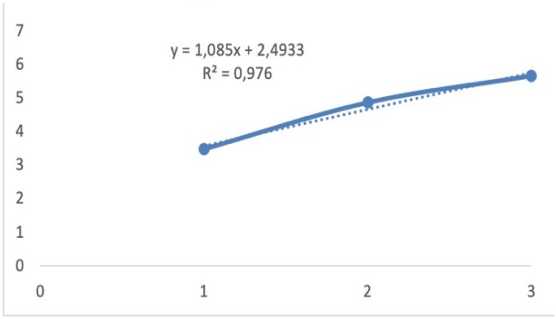
Table 5. Toxicity test of *Padina australis* methanol extract using the maceration method

Concentration	Repeat					average	% died	Probit value of % mortality
	1	2	3	4	5			
1000 ppm	7	7	8	7	8	7,4	74	5,65
100 ppm	4	4	5	4	4	4,4	44	4,86
10 ppm	1	1	0	0	1	0,6	6	3,48
Blanco	0	0	0	0	0	0	0	0

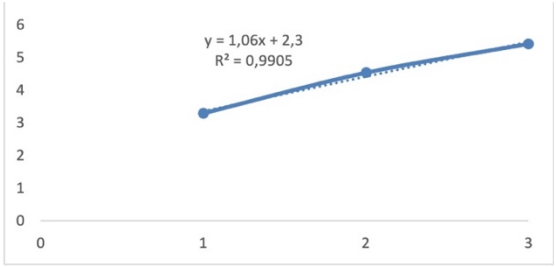
Table 6. Toxicity test of *Padina australis* methanol extract using the UAE method

Concentration	Repeat					average	% died	Probit value of % mortality
	1	2	3	4	5			
1000 ppm	6	6	7	7	7	6,6	66	5,42
100 ppm	4	2	3	4	3	3,2	32	4,54
10 ppm	0	1	0	0	1	0,4	4	3,30
Blanco	0	0	0	0	0	0	0	0

The percentage of death obtained can be displayed in the form of a curve based on the ratio of the percentage of death of *Artemia salina* L. larvae to the extract concentration of all research samples. The linear regression curve can be seen in Figure 3.



(A)



(B)

Figure 3. Probit curve of LC_{50} analysis of *Padina australis* methanol extract using maceration (A) and UAE (B) methods

The LC_{50} results obtained are put into categories, LC_{50} less than 1000 ppm is in the non-toxic category; 500 - 1000 ppm is in the medium category; 250 - 500 ppm is in the toxic category; and 0 - 250 ppm is in the very toxic category. Based on the calculation of the first probit curve analysis using the maceration method as shown in Figure 3 (A), after analysis the results show an LC_{50} value <1000 ppm, namely 204.325 ppm in the very toxic category, while in Figure 3 (B) using the UAE method the LC_{50} value is 352.508 ppm in the toxic category.

Afterwards, a toxicity test was carried out on the n-hexane fraction of *Padina australis* using probit analysis to determine the LC_{50} value. The results of the toxicity test of the n-hexane fraction of *Padina australis* are presented in Tables 7 and 8.

Based on the calculation of the probit graph analysis of the n-hexane fraction using the maceration method, it can be seen in Figure 4 (A). After analysis, the results show an LC_{50} value < 1000, namely 13.61 ppm in the very toxic category, and in Figure 4 (B) with the UAE method, the LC_{50} value is 54.99

ppm, which is included in the very toxic category.

This shows that differences in extraction techniques can influence the toxicity of an extract and fraction. The presence of flavonoid compounds in the methanol extract of *Padina australis* which have the potential to be toxic can be determined based on the results of phytochemical tests. Flavonoid secondary metabolite compounds can reduce the activity of digestive enzymes and food absorption and act as stomach poisoning when these compounds enter the larvae's bodies. Compounds or extracts also inhibit taste receptors in the larvae's mouth area, which results in the larvae failing to receive a taste stimulus, so that the larvae are unable to recognize their food and the larvae eventually die of starvation (Panjaitan & Natalia, 2021). Meanwhile, steroid compounds are known to have toxic properties for shrimp larvae (Azizah, 2016). Therefore, in this study, the methanol extract and n-hexane fraction of *Padina australis* can be very toxic to *Artemia salina* Leach larvae.

Table 7. Toxicity test of the n-hexane fraction of *Padina australis* using the maceration method

Concentration	Repeat					Average	% died	Probit value of % mortality
	1	2	3	4	5			
1000 ppm	8	9	8	8	8	8,2	82	5,93
100 ppm	7	6	6	6	7	6,4	64	5,37
10 ppm	5	5	4	5	5	4,8	48	4,96
Blanco	0	0	0	0	0	0	0	0

Table 8. Toxicity test of the n-hexane fraction of Padina australis using the UAE method

Concentration	Repeat					Average	% died	Probit value of % mortality
	1	2	3	4	5			
1000 ppm	8	8	7	8	8	7,8	78	5,78
100 ppm	5	6	5	5	4	5	50	5,01
10 ppm	4	4	4	3	3	3,6	36	4,65
Blangko	0	0	0	0	0	0	0	0

The percentage of death can be displayed in the form of a curve based on the ratio of the percentage of death of *Artemia salina* L. larvae to the concentration of the n-hexane fraction of all research samples. The linear regression graph can be seen in Figure 4.

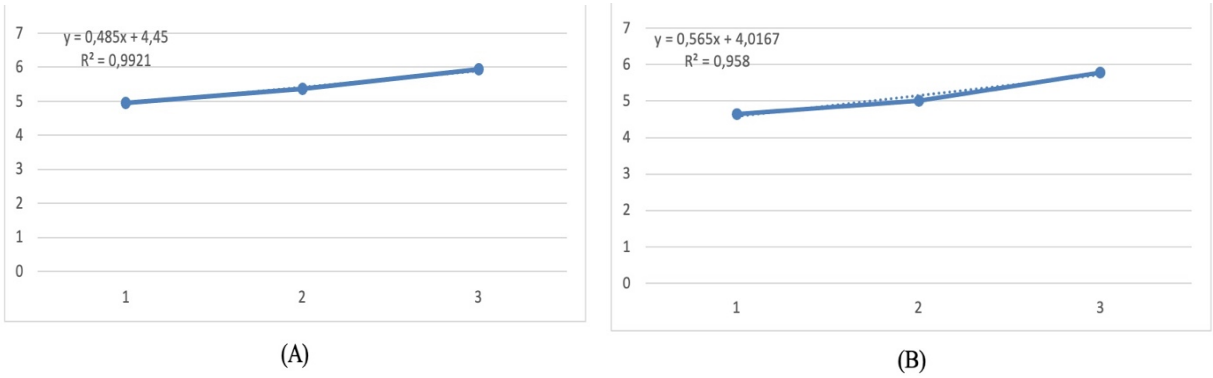


Figure 4. Probit graph of LC_{50} analysis of the n-hexane fraction using the maceration (A) and UAE (B) methods

CONCLUSION

The % yield of *Padina australis* methanol extract using the UAE method resulted in a relatively high % yield of 10.86% compared to the maceration method, which had a % yield of 4.76%. As well as having the same phytochemical content in the methanol extract, the n-hexane fraction has different phytochemical content; the maceration method is positive for containing steroids, while the UAE method is positive for containing saponins and steroids. Then in the toxicity test the value (LC_{50}) obtained for the methanol extract in maceration was 204.325 ppm (very toxic) and UAE 352.508 ppm (toxic), while for the n-hexane fraction in maceration it was 13.61 ppm (very toxic) and 54.99 ppm (very toxic). The methanol extract and n-hexane fraction in the toxicity test were found to be in the very toxic and toxic categories, respectively. Therefore, methanol extract and n-hexane fraction of *Padina australis* can be used to develop medical products.

DAFTAR RUJUKAN

Agustina, S., Ruslan, & Wiraningtyas, A. (2016). Phytochemical Screening Of Medicinal Plants In Bima Regency. Cakra Kimia (Indonesian E-Journal Of Applied Chemistry), 4(1), 71–76.

Al-Quais, K. (2015). Test of Antioxidant Activity of N-Hexane Extract And Identification Of Steroid Compounds In Bamboo Grass Roots (*Lophaterum*

gracile Brongn). [Thesis]. Malang: Uin Maulana Malik Ibrahim.

Appamaraka S, Senakun C, Saensouk S. TLC Profiling and Phytochemical Screening Of Various Extracts Of *Ochna integerrima* (Lour.) Merr. From Kog Dong Keng Forest, Thailand. Pharmacognosy Journal. 2022;14(4):273-277.

Azizah, L. N. (2016). Uji Toksisitas Isolat Steroid Hasil KLT Fraksi Petroleum Eter Hasil Hidrolisis Ekstrak Metanol Alga Merah *(Eucheuma spinosum)*. Universitas Islam Negeri Maulana Malik Ibrahim Malang.

Bustannussalam, Haryanto, S., & Nurhidayati, E. (2014). Identifikasi Senyawa Dan Uji Aktivitas Ekstrak Etil Asetat Kulit Kayu Massoi (*Cryptocarpa massoy*). *Phytopharmacy Journal*, *14*(2), 152–168.

Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A. S., & Abert-Vian, M. (2017). Ultrasound Assisted Extraction Of Food And Natural Products. Mechanisms, Techniques, Combinations, Protocols And Applications. A Review. *Ultrasonics Sonochemistry*, *34*, 540–560. Https://Doi.Org/10.1016/J.Ultsonch.2016.06.035

Dey, S., & Rathod, V. K. (2013). Ultrasound Assisted Extraction Of B-Carotene From *Spirulina platensis*. *Ultrasonics Sonochemistry*, *20*(1), 271–276. Https://Doi.Org/10.1016/J.Ultsonch.2012.05.010

Harborne. (1987). *M*etode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan (Edisi 1. T). Press Itb.

Haryani, T. S., Sari, B. L., & Triastinurmiatiningsih. (2014). Efektivitas Ekstrak Padina Australis Sebagai Antibakteri Escherichia Coli Penyebab Diare. *Fitofarmaka:Jurnal Ilmiah Farmasi*, *4*(2).

Kanifah, U., Lutfi, M., & Susilo, B. (2015). Metode Ekstraksi Non-Thermal Berbantukan Ultrasonik (Kajian Characterization Of Red Betel Leaf (Piper Crocatum) Using Ultrasonic Assisted Extraction (Uae) (Study Of Solvent And Extraction Time). *Jurnal Bioproses Komoditas Tropis*, *3*(1), 73–79. Https://Jbkt.Ub.Ac.Id/Index.Php/Jbkt/Article/View/164

Khoddami, A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques For Analysis Of Plant Phenolic Compounds. *Molecules*, *18*(2), 2328–2375.

Https://Doi.Org/10.3390/Molecules18022328

Lisi, A. K. F., Runtuwene, M. R. J., & Wewengkang, D. S. (2017). Uji Fitokimia Dan Aktivitas Antioksidan Dari Ekstrak *Saurauia bracteosa* (*Saurauia bracteosa* Dc.). *Pharmacon*, *6*(1), 53–61.

Maharany, F., Nurjanah, Suwandi, R., Anwar, E., & Hidayat, T. (2017). Kandungan Senyawa Bioaktif Rumput Laut *Padina australis* Dan *Eucheuma cottonii* Sebagai Bahan Baku Krim Tabir Surya. *Jphpi*, *20*(1), 10–17.

Nuzul, P., Lantang, D., Dirgantara, S., & Faculty, J. . (2018). Antibacterial Activity Test Of Brown Algae Type *Padina* sp. From Sorido Beach Biak Against *Staphylococcus aureus* and *Shigella dysenteriae* Bacteria. *Pharmacy Medical Journal*, *1*(1), 16–25.

Panjaitan, R. S., & Natalia, L. (2021). Ekstraksi Polisakarida Sulfat Dari *Sargassum polycystum* Dengan Metode *Microwave Assisted Extraction* Dan Uji Toksisitasnya. *Jurnal Pascapanen Dan Bioteknologi Kelautan Dan Perikanan*, *16*(1), 23–32. Https://Doi.Org/10.15578/Jpbkp.V16i1.692

Pohuwato, P. (2018). Characteristics Of Brown Seaweeds Shindy Hamidah Manteu, Nurjanah, Tati Nurhayati Characteristics Of Brown Seaweeds *Sargassum policystum* And *Padina minor* From Pohuwato Water. *Gorontalo Materials And Tools*, 21–397.

R. Hamidi, M., Jovanova, B., & Kadifkova Panovska, T. (2014). Toxicological Evaluation Of The Plant Products Using Brine Shrimp (*Artemia salina* L.) Model. *Macedonian Pharmaceutical Bulletin*, *60*(01), 9–18. Https://Doi.Org/10.33320/Maced.Pharm.Bull.2014.60.01.002

Rizqillah, N. (2013). *Uji Toksisitas Akut Ekstrak Metanol Daun Garcinia benthami* Pierre *Terhadap Larva Artemia salina* Leach *Dengan Metode Brine Shrimp Lethality Test (Bslt)* (Vol. 1, Issue 2). Universitas Islam Negeri Syarif Hidayatullah

Rosdiana, Nurgayah, & W, I. (2017). Macroalgae Community Structure In Waworaha Waters, Soropia Distruct. *Jurnal Sapa Laut (Journal Of Marine Science)*, *2*(3), 69–77.

Rustanti, E., Jannah, A., & Fasya, A. G. (2013). Uji Aktivitas Antibakteri Senyawa Katekin Dari Daun Teh (*Camelia sinensis* L.Var Assamica)

Terhadap Bakteri Micrococcusluteus. *Alchemy*, *2*(2). Https://Doi.Org/10.18860/Al.V0i0.2886

Sasongko, A., Nugroho, R. W., Setiawan, C. E., Utami, I. W., & Pusfitasari, M. D. (2018). Aplikasi Metode Nonkonvensional Pada Ekstraksi Bawang Dayak. *Jtt (Jurnal Teknologi Terpadu)*, *6*(1), 8. Https://Doi.Org/10.32487/Jtt.V6i1.433

Widyasanti, A., Nurlaily, N., & Wulandari, E. (2018). Karakteristik Fisikokimia Antosianin Ekstrak Kulit Buah Naga Merah Menggunakan Metode Uae (Physicochemical Characteristics Of Red Dragon Fruit Skin Anthocyanin Extracts Using Uae Method). *Jurnal Ilmiah Rekayasa Pertanian Dan Biosistem*, *6*(1), 27–38. Https://Doi.Org/10.29303/Jrpb.V6i1.63

Wijayanti, N., Sudjarwo, G. W., & Putra, O. . (2020). Phytochematic Screening Of Secoundary Metabolites Of Beown Algae (*Padina australis*) From The Potern Island, Madura. *J-Pham Journal Of Pharmaceutical Care Anwar Medika*, *2*(2), 2654–8364.

Wulandari, F. (2018). *Uji Toksisitas Akut Ekstrak Metanol Buah* Phaleria macrocarpa *(Scheff) Boerl Terhadap Larva Artemia salina Leach Dengan Metode Brine Shrimp Lethality Test (BSLT)*. Universitas Islam Negeri Syarif Hidayatullah Jakarta.