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Antioxidant Activity, Toxicity Effect and Phytochemical Screening of Some Brown Algae *Padina australis* Extracts from Dutungan Island of South Sulawesi Indonesia

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Abstract :
Brown algae is one of the bioactive compound sources producing secondary metabolites as antioxidant. Padina australis is a brown algae (Phaeophyceae) specific in Dutungan island Barru, South Sulawesi suspected of secondary metabolic compound. This study aims to determine the level of toxicity and antioxidant active compounds of Padina australis. Extraction of active compounds in P.australis was carried out by maceration method using a solven of methanol, ethyl acetate, and n-hexane. Each extract was tested for its toxicity against Artemia salina L and DPPH as radicals. The results showed that the toxicity test of methanol, ethyl acetate, and n-hexane extract against A. salina L obtained LC_{50} values, respectively 785.03 ppm, 73.3 ppm and 300 ppm. Antioxidant activity of methanol, ethyl acetate and n-hexane extract with IC_{50} values at 446.88 ppm, 259.48 ppm, and 495.67 ppm.

Keywords - *Antioxidant, Toxicity, P.australis, Brown algae, Phytochemical screening*

I. INTRODUCTION

Indonesia is a maritime country whose land surface is dominated by the ocean (maritime). Algae is included in a coastal economic program that is being developed in South Sulawesi. Marine algae are the country's foreign exchange earner and South Sulawesi is one of the largest contributors to the national marine algae. The ability of algae and marine organisms to produce secondary metabolites is related to their interactions with the environment. The presence of high salinity in the algae environment stimulates algae to produce secondary metabolites, which will be used to defend themselves from predatory threats[1].

Secondary metabolites found in brown algae include; alkaloids, glycosides, phenolic, and steroids which are also used as medicine and in the pharmaceutical industry[2],[3]. Several phenolic compounds and flavonoids are known to have activity in inhibiting the oxidation of LDL, angiotensin converting enzyme (ACE), α-

glucosidase[4], antioxidant[5], and these compounds also provide protection against various degenerative diseases, especially cancer[6], and as a therapeutic[7]. Flavonoids are among the largest phenolic or polyphenolic compounds, these compounds have biological activity that has been widely studied, one of which is their ability to inhibit or reduce free radicals[8],[9]. One of the brown algae species that is very abundant in Indonesian waters is *Padina australis*. This is the bioactivity potential of *P. australis* which is very interesting to research and develop[10]. The toxicity of a natural extract can be carried out by screening *Artemia salina* L shrimp larvae, the mortality of shrimp larvae is related to the active compounds contained in an extract.

The purpose of this study was to determine the antioxidant activity and toxicity found in several extracts of *P. australis* extract from Dutungan Island, South Sulawesi. So that it can be used as a source of scientific information regarding the antioxidant activity and toxicity of brown algae *P. australis* in the pharmaceutical field.

II. MATERIAL AND METHODS

2.1 Material

Some of the tools used in this study include a set of laboratory glassware, an analytical balance, a rotary evaporator, and a UV-VIS spectrophotometer. Brown algae *(Padina australis)* sample was obtained from the Dutungan Island, South Sulawesi. The materials used include methanol, ethyl acetate, n-hexane, DMSO, yeast, seawater, DPPH, 2% HCl, concentrated H_2SO_4, acetic acid anhydride, 1% $FeCl_3$, Meyer reagent, Dragendorff, Wagner, and Mg metal powder.

2.2 Methods

2.2.1 Sample Preparation

Brown algae *(Padina australis)* was cleaned of dirt with seawater, then washed under running water. The sample was then dried in a simplicia oven at 38^0C for 48 hours, and then pulverized using a blender until it was small/powdered.

2.2.2 Extraction

The extraction process was carried out by graded maceration by increasing the polarity of n-hexane, ethyl acetate and methanol. The algae powder of *P. australis* was macerated with n-hexane solvent for 3 x 24 hours, and was filtered. The residue was re-macerated for 24 hours, then the filtrate was evaporated with a rotary evaporator to obtain n-hexane extract. The residue was extracted using the same method of ethyl acetate and methanol as solvent.

2.2.2 Toxicity Test

Sea water as a hatching medium is placed in the container and the lights are illuminated. 3 mg of *Artemia salina* L eggs are put into a container filled with seawater. The hatched shrimp larvae were used as test animals, after the eggs were aerated for 48 hours[11]. Each extract was made into a concentration series; 1000 ppm, 800 ppm, 600 ppm, 400 ppm and 200 ppm for methanol extract, 20 ppm, 40 ppm, 80 ppm, 100 ppm for ethyl acetate extract. Whereas for the n-hexane extract 100 ppm, 200 ppm, 300 ppm, 400 ppm, and 500 ppm, use sea water if the sample is not dissolved, drop it using DMSO, then add 10 shrimp larvae and yeast. The control was made by entering into a vial glass of 2 mL of sea water, 100 µL of dimethyl sulfoxide, and 1 drop of solution, then sufficient with sea water to a volume of 10 mL. 10 larvae of *Artemia salina* L. shrimp were put into the vial. To find out the LC_{50} value, observations were made by counting *Artemia salina* L. shrimp larvae that died after 24 hours under light lighting[12],[13]. The percentage of shrimp larvae mortality is calculated by the formula:

$$\% \text{ Mortality} = (\text{number of dead larvae}) / (\text{number of live larvae}) \times 100\%$$

2.2.3 Antioxidant Activity Test

The DPPH solution was prepared by weighing 0.01577 g DPPH, and dissolving it with p.a ethanol up to 100 mL in a volumetric flask. Methanol extract was made a solution with a concentration of 200 ppm, 400 ppm, 600 ppm, 800 ppm and 1000 ppm, ethyl acetate extract was made with a concentration of 100 ppm, 200 ppm, 300 ppm, 400 ppm, 500 ppm, and 100 ppm, 200 ppm n-hexane extract. ppm, 300 ppm, 400 ppm, 500 ppm. Then each extract concentration is pipette as much as 1 mL, 1 mL of DPPH reagent solution is added, and it is sufficient to add up to 5 mL with methanol p.a in the volumetric flask. Homogenize the mixture and leave it for 30 minutes, the absorption at a wavelength of 515 nm was measured by a UV-VIS spectrophotometer[14]. The antioxidant activity is calculated using the formula:

Antioxidant activity = (Abs Blank sample) / (Blank Abs) x 100%

A total of 1 mL of DPPH solution is pipette and 5 mL of methanol p.a is sufficient in a volumetric flask. Absorbance was measured on a UV-Vis Spectrophotometer with a wavelength of 515 nm.

2.2.4 Phytochemical Test[15],[16]

2.2.4.1 Phenolic Test

A total of 2 mL of sample was added 10 drops of 1% $FeCl_3$. Positive results are indicated in blue to blackish blue

2.2.4.2 Flavonoid Test

A total of 2 mL of hot water is added to 2 mL of the sample, then heated for 5 minutes, and filtered. Add 0.05 mg of Mg powder and 1 mL of concentrated HCl to the filtrate, homogenize the solution. Positive results are indicated by the color red, yellow or orange.

2.2.4.3 Terpenoid / Steroid Test

A total of 2 mL of each extract was added with 1 mL of chloroform, 0.5 mL of anhydrous acetic acid, and 2 mL of H_2SO_4. A positive result for triterpenoids is indicated by the formation of a purple ring on the border of two solvents, whereas if steroids are formed a bluish green color, if it is red it indicates a terpenoid.

2.2.4.4 Alkaloid Test

The extract was inserted into the test tube and then added with 0.5 mL of 2% HCl, then the solution was divided into two tubes. Tube I is added 2-3 drops Dragendorff reagent, tube II is added 2-3 drops Mayer reagent. The orange sediment found in tube I and yellowish precipitate in tube II indicate positive for alkaloids.

2.2.4.5 Saponin Test

In a test tube filled with water (1: 1), each extract is added and shaken for 1 minute, add 1 N HCl if foam is formed, saponin positive if the foam formed can last for 10 minutes with a height of 1-3 cm.

III. Results and Discussion

3.1 Antioxidant Activity

Phenolic and flavonoids compounds have the ability to ward off free radicals, therefore the presence of these compounds in a plant can be used as a parameter of antioxidant activity. The wavelength of the DPPH is 515 nm, there is a change in the color of DPPH from deep purple to pale yellow or light purple, indicating that the extract can inhibit DPPH radicals. This inhibition depends on the antioxidant activity of the extracts contained in the plant[17]. The amount of antioxidant activity is determined by the value of IC_{50} < 50 ppm very strong antioxidant power, 50-100 ppm strong antioxidant power, 101-150 ppm moderate antioxidant power and > 150 ppm weak[18]. The antioxidant activity was obtained from the IC_{50} value of the *Padina australis* extract. IC_{50} is the concentration of an extract's ability to inhibit 50% of the oxidation process, as a comparison vitamin C is also used. The antioxidant activity of the extract of *Padina australis* can be seen in Table 1.

Table 1. IC_{50} values of methanol, ethyl acetate, n-hexane extracts and Vitamin C

Extracts *Padina australis*	IC_{50} (ppm)	Antioxidant category
Methanol	446,88	Weak
Ethyl Acetate	259,48	Weak
n-Hexane	495,67	Weak
Vitamin C	1,72	Very strong

DPPH radical reduction can also be influenced by the number of hydroxyl groups attached to the benzene ring such as the phenolic and flavonoid groups[19]. The results of this study showed that the antioxidant activity was weak, the dominant secondary metabolites of steroids and terpenoids in brown algae *P. australis* also influenced the results of measurements in inhibiting DPPH radicals.

3.2 Toxicity effect

The toxicity test of an extract can be carried out using test animals of *Artemia salina* Leach shrimp larvae. The nature of shrimp larvae which is very sensitive to the test material and relatively fast breeding time, it is proven that this method has a relationship with the cytotoxic power of anticancer cells. The mortality of larvae due to the test material indicates a toxic effect. The LC_{50} value < 30 ppm is very toxic, 30-1000 ppm is toxic, and > 1000 ppm is not toxic for the category of a single compound. Meanwhile, the extract is considered toxic if the LC_{50} value is < 1000 ppm[20]. The toxicity of an extract depends on the content of secondary metabolites, an extract can have a synergistic effect, that is the effect is very large if it is in many compounds or otherwise has an antagonistic effect. The LC_{50} values of *P. australis* extract are shown in Table 2.

Table 2. LC_{50} values of methanol, ethyl acetate and n-hexane extracts from *Padina australis*

Extracts	LC_{50} ppm	Toxicity
Methanol	785,03	Toxic
Ethyl Acetate	73,3	Toxic
n-Hexane	300	Toxic

3.3 Phytochemical Screening

The metabolite content of an extract can be determined by doing a phytochemical screening. The results of the phytochemical screening test showed that all *P. australis* extracts contained terpenoids, steroids, and alkaloids, these three classes of compounds were thought to provide the extract pharmacological effects. whereas flavonoids are minor compounds and phenols not found in n-hexane extract. Table 3 shows the results of the content of secondary metabolites in the methanol, ethyl acetate and n-hexane extracts of *P. australis*:

Table 3. Results of the *P. australis* phytochemical screening

Secondary metabolites	Methanol extract	Ethyl Acetate extract	n-Hexane extract
Alkaloid	++	++	++
Flavonoid	+	-	+
Phenol	+	++	-
Saponin	-	-	-
Terpenoid	++	+	+++
Steroid	+	+++	+

Information:
+++: more compound content (very dark color)
++: contains compounds (the color is quite thick)
+: contains compounds (slightly colored)
-: does not contain compounds.

IV. CONCLUSION

Based on the research results it can be concluded:

1. The toxicity test using the BSLT method of methanol, ethyl acetate and n-hexane *P.australis* extracts obtained LC_{50} values of 785.03 ppm, 73.3 ppm and 300 ppm respectively with toxic categories
2. Antioxidant test results of methanol, ethyl acetate, and n-hexane *P.australis* extracts obtained IC_{50} values of 446.88 ppm, 259.48 ppm, and 495.67 ppm with weak antioxidant category.
3. Phytochemical screening showed that the methanol, ethyl acetate, and n-hexane extracts of *P. australis* contained various secondary metabolites.

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