Total phenolic, antioxidant activity and toxicity effect of *Turbinaria decurrens* extracts from South Sulawesi

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Abstract. *T.decurrens* is a species of brown algae (*Phaeophyta*) that has bioactivity potential. This study aims to determine the total phenolic, antioxidant activity, and toxicity effects of n-hexane, ethyl acetate, and methanol extract. The effect of toxicity was carried out using the *Artemia salina* L death test method. Meanwhile, the antioxidant test uses the DPPH (2,2-diphenyl-1-picrylhydrazyl) method, and Folin Ciocalteu method is used for total phenolic content. The output of antioxidant activity of *T. decurrens* in ethyl acetate extract has an IC_{50} value of 180.54 µg/mL. Similar results have been found in the effects of toxicity and total phenolic content, which showed that ethyl acetate extracts gave LC_{50} values of 25.41 µg/mL, and total values of phenolic content of 4.8091 mg EAG /g, respectively. This indicated that ethyl acetate extract from *T. decurrens* has the potential as anticancer.

1. Introduction

Marine algae are abundant marine resources in Indonesia, utilization of marine algae has been widely developed. Thus, algae are one of the income sources for those who live on the coast of Indonesian waters with high algae potential. Demand for algae in the world is increasing along with the increased use of algae for various purposes, including in the fields of industry, food, textiles, paper paint, cosmetics, and medicine [1]. One of the waters in South Sulawesi that has a high diversity of algae is Dutungan Island, Barru Regency. This location is a habitat of brown algae with a location in the form of a coral reef path. One of brown algae species found there is *Turbinaria decurrens*.

Turbinaria is a genus of brown algae (*Phaeophyta*) which contains several micronutrients as though polyphenols, vitamins A, C, E, and folic acid. According information of some references, those micronutrients have the ability to capture free radicals in order to be used as natural antioxidants [2]. Studies on *T.decurrens* had been conducted by several researchers regarding bioprospection of brown algae from Binuangeun as reported. Both studies examined the potential of *T. decurrens* as an anti-cancer ingredient [3,4].

Those previous studies on *T.decurrens* showed that algae were also potential antioxidants and anticancer. However, there have not been many references about total phenolic content, toxicity effect, and antioxidant activity of *T.decurrens* especially those originating from South

Sulawesi waters. Therefore, it is necessary to know the total phenolic, antioxidant activity, and toxicity effect from the extract of *T.decurrens.*

2. Experiment

2.1 Extraction

The extraction process was carried out by multilevel maceration by increasing its polarity from n-hexane, ethyl acetate, and methanol. After, algae powder of *T. decurrens* was macerated with n-hexane solvent for 3 days (3 x 24 hours) then, the filtering was carried out. The residue was re-macerated for 24 hours, next the filtrate was evaporated by a rotary evaporator to obtain n-hexane extract. Nonetheless, the residue was extracted with ethyl acetate and methanol by the same method.

2.2 Toxicity Test Using the BSLT Method

Seawater as a hatching medium was placed in the container that got enough light from the lamp. As much as 3 mg of *Artemia salina L.* eggs were put in a vessel filled with sea water. After the eggs were aerated for 48 hours, the hatched shrimp larvae were then used as test animals [5].

Each extract was made a concentration series of 100 - 500 µg/mL for methanol and n-hexane extract, and a concentration series of 10 - 50 µg/mL for ethyl acetate extract. Whereas each extract using sea water if the insoluble sample was dripped using DMSO, nevertheless 10 shrimp larvae and yeast were put [6]. The control was made by putting 100 µL dimethyl sulfide and 1 drop of solution into a 2 mL vial glass of seawater. Afterward, the volume of seawater was increased into 10 mL. 10 shrimp larvae of *Artemia Salina* L. were included in the vial. In order to find out the LC_{50} value, shrimp larvae of *Artemia Salina L.* which died after 24 hours with light beam lighting were observed. The percentage of shrimp larvae deaths was calculated by the following formula:

$$\%\ \text{Mortality} = \frac{\text{total of death larvae}}{\text{total of live larvae}}$$

2.3 Antioxidant Activity Assay

DPPH was weighed as much as 0.01577 g, dissolved with methanol, its volume was satisfied with methanol p.a up to 100 mL in a measuring flask [7,8]. DPPH solution was piped as much as 1 mL and was sufficient volume up to 5 mL with methanol p.a in a measuring flask. This solution was then measured for its absorbance with a Vis Spectrophotometer at a wavelength of 515 nm.

Methanol and ethyl acetate extracts were made a concentration of 100 - 500 µg/mL, and n-hexane extract was in the concentrations of 200 - 1000 µg/mL. Each extract concentration was piped as much as 1 mL. Then, 1 mL of DPPH reagent solution was added up to 5 mL with methanol p.a in a measuring flask. The mixture was homogenized and left for 30 minutes before it was measured by a Vis spectrophotometer at a wavelength of 515 nm. The amount of antioxidant activity was calculated using the following formula:

$$\%\ \text{Inhibition} = \frac{Abs\ Blank - Abs\ sample}{Abs\ blank} \text{x}100\%$$

2.4 Test of Total Phenolic Content

Each extract was dissolved with ethanol solvent in order to form a concentration of 1000 µg/mL. Then, the mixture was piped as much as 1 mL put in a 5 mL volumetric flask, added 0.5 mL Folin-ciocalteu, 3 mL Na_2CO_3 2%, and added aquadest until the volume of the mixture became 5 mL. The mixture was shaken until homogeneous with vortex. Absorbance samples was measured at a wavelength of 720 nm using a VIS spectrophotometer. The sample of total phenolic was set using standard gallic acid curves with various concentrations which were 2, 4, 6, 8, 10, 12 µg/mL. The results were equivalent to milligrams of gallic acid (mg/g EAG). The total phenolic content of each extract was expressed as equivalent to gallic acid (*Gallic Acid Equivalent*), a general reference for measuring phenolic compounds contained in an ingredient. Total phenolic was calculated by the following formula:

$$\text{Total Phenolic} = \frac{(a.\ V)/1000}{G} \times Fp$$

Description:

a = Gallic acid concentration in the test sample (mg/L)
V =Total volume of test solution (mL)
G = Weight of extract used (g)
Fp = Dilution factor
1000 = conversion factor for total solution volume

3. Results and Discussion

3.1 Toxicity Test with Brine Shrimp Lethality Test (BSLT)

BSLT testing was carried out to determine the toxicity of a sample of natural materials. This method was an initial test used to determine anticancer activity based on the ability of a sample to kill shrimp larvae.

Table 1. LC_{50} values of *T. decurrens* extract

T. decurrens extracts	LC_{50} Value (µg/mL)	Toxicity Level
Methanol	421.31	Toxic
Ethyl acetate	25.41	Very Toxic
n-hexane	364.08	Toxic

Shrimp larvae were used because they have a high sensitivity to the existing conditions of environmental changes and chemical contamination. Therefore, shrimp larvae were used as an initial parameter of changes in environmental conditions. Meanwhile, the data in Table 1 presented that the results of the BSLT test on semi-polar solvent extract (ethyl acetate) were more active than the other extracts. The toxic nature of brown algae was estimated to be related to the secondary metabolites contained. Larvae death due to the test ingredients indicates a toxic effect. The LC_{50}< 30 ppm was said to be very toxic, LC_{50} 30-1000 µg/mL was toxic, and LC_{50} > 1000 µg/mL was not toxic for the category of a single compound [9].

The toxic compounds in the extract were able to enter through the mouth of *A.salina* L, which were absorbed into the digestive tract by the absorption process through the cell membrane. The absorption process was followed by the process of distributing toxic compounds into the body of *A. salina* L which led into a process of damage to the metabolic reaction. The anatomical structure of the body of *A. salina* L at the stage of nauplii is still very

simple. It consisted of layers of skin, mouth, antenna, digestive tract or simple digestion, and prospective thoracopods [10]. The drastic change in concentration gradient between inside and outside the cell caused toxic compounds to spread well to the body of *A. salina* L. The effects of metabolic damage occurred quickly and could be detected within 24 hours. It caused 50% of *A. salina* L death. The mortality rate of larvae was closely related to its concentration [11]. Because a synergistic effect is the combined effect of several compound components which are mutually enhancing activities such as those that occur in ethyl acetate fractions.

3.2 Antioxidant Activity Test

The test of antioxidant activity with the DPPH method was carried out to determine how much activity a sample had to inhibit DPPH radical stability by donating hydrogen atoms. Samples that had antioxidant activity reduced DPPH to DPPH-H [7]. The reduction occurred marked with changes of color from purple to yellow. According to Molyneux (2004), the smaller of the IC_{50} means higher antioxidant activity. The results showed that the ethyl acetate extract sample had the smallest IC_{50} value compared to other extracts. However, it still had a greater IC_{50} value than the IC_{50} of vitamin C.

Table 2. IC_{50} values of *T. decurrens* extract and Vitamin C

T. decurrens Extracts	IC_{50} value (µg/mL)	Antioxidant Level
Methanol	340.06	Weak
Ethyl acetate	180.54	Weak
n-hexane	502.25	Weak
Vitamin C Control	1.72	Very Strong

The content of secondary metabolites in each extract affects its activity as an antioxidant. One of the compounds that acted as an antioxidant was phenolic, the largest group that was widely found in plants. The extract could also provide weak activity because it was pure and consisting of various components of the compound [12]. Another factor was the more dominant secondary steroid and terpenoid metabolite in brown algae *T. decurrens* also influenced the measurement results in DPPH radical inhibition.

3.3 Total Phenolic Content

Test of the total phenolic concept by using the Folin-Ciocalteu method was carried out based on the ability of Folin-Ciocalteu reagent to oxidize the hydroxyl (OH-) group from the phenol group compound. Phenolic compounds reduced phosphotungstate phosphomolybdate in Folin-Ciocalteu to form blue molybdenum.

Table 3. Total phenolic content of *T.decurrens* extract

T. decurrens extract	mg EAG/g
Methanol	3.16
Ethyl acetate	4.8
n-hexane	2.64

Based on the findings obtained, it was shown that more phenolic compounds *T.decurrens* were extracted in semi-polar (ethyl acetate) solvents than polar and non-polar. The highest total phenolic content was found in ethyl acetate extract of 4.8091 mg EAG/g. This occurred because phenol group compounds were polar or semi-polar [13]. It can be also inferred that antioxidant activity had a relationship with total phenolic content. Ethyl acetate extract of *T.decurrens* had the highest total phenolic content of 4.8091 mg EAG/g and the lowest IC_{50} value is 180.54 µg/mL. The lower of IC_{50} value, higher the total phenolic content. Therefore, antioxidant activity was directly proportional to total phenolic because of higher the phenolic content in a material, can high its activity as an antioxidant [14].

4. Conclusion

This study concluded that antioxidant activity, total phenolic content, and toxicity effects of *T. decurrens* with the highest values were found in ethyl acetate extracts, then n-hexane, and methanol. The greater total phenolic content means the greater antioxidant activity and toxicity.

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