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Research Article

Identification of Bioactive Compounds of Seaweed *Sargassum* sp. and *Eucheuma cottonii* Doty as a Raw Sunscreen Cream

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Abstract: This study aimed at determining proximate value, vitamin E, antioxidant activity and active components extracts of *Sargassum* sp. and *Eucheuma cottonii* Doty. Extraction was done by maceration stratified method. The yield of *Sargassum* sp. used solvent *n*-hexane, ethyl acetate and methanol and amounted to 0.027 3 %, 0.133 3 %, 7.332 8 %. The yield of *E. cottonii* used solvent *n*-hexane, ethyl acetate and methanol and amounted to 0.025 7 %, 0.078 8 %, 6.758 6 %. Proximate value of *Sargassum* sp. is a row of moisture, ash, fat, protein and crude fiber that amounted to 82.26 %, 5.09 %, 1.26 %, 0.41 %, 0.43 %, respectively, and *E. cottonii* amounted to 77.27 %, 5.84 %, 2.39 %, 0.12 % and 0.67 %, respectively. Vitamin E value of *Sargassum* sp. was 165.19 μg mL^{-1} while vitamin E value of *E. cottonii* amounts to 160.01 μg mL^{-1}, using HPLC method. The antioxidant activity of *Sargassum* sp. and *E. cottonii* from the methanol extract were 57.050 μg mL^{-1} and 105.040 μg mL^{-1}. The active components of *Sargassum* sp. and *E. cottonii* contained in the methanol extract were flavonoids, phenols hydroquinone and triterpenoids.

Keywords: Antioxidant, extraction, *E. cottonii* Doty*, Sargassum* sp.*,* sunscreen

1. INTRODUCTION

Indonesia is widely known as an archipelago, of which 2/3 of its territory is ocean and has the longest coastline in the world which is ± 80 791.42 km. One potential commodity that grows and thrives in the ocean is kelp [1]. Seaweed is one of the commodities of pro and its production increased by 20.9 % in 2013 to 7.5 × 10^6 t, and in 2014 the its production was 10 × 10^6 t. Seaweeds are taxonomically classified as algae, pertaining to four main classes, namely *Rhodophyceae* (red algae), *Chlorophyceae* (green algae), *Chyanophyceae* (blue green algae), and *Phaeophyceae* (brown algae) [2].

Brown algae is a photoprotective agent that is capable of absorbing UV rays. Genetically, brown algae is more frequently induced by sunrays so that it is able to synthesize the compounds that have a capability of absorbing UV rays. [3]. One type of brown algae that has potential as raw material for sunscreen cream is *Sargassum* sp.

Sargassum sp. has greater antioxidant activity than other types of *Caulerpa racemosa* (Forsskal) J. Agardh, *Ulva lactuca* Linn. and *Gracilaria tenuistipitata* (C.F. Chang & B.M. Xia) with IC_{50} values of each (1.08 ± 0.83) μg mL^{-1}, (15.05 ± 0.61) μg mL^{-1}, (103.73 ± 0.59) μg mL^{-1}, (24.22 ± 0.87) μg mL^{-1} [4]. *Sargassum* sp. contains ascorbic acid, as well as *phlorotannin* compounds that play a role in inhibiting the formation of melanin. *Phlorotannin* is a *polyphenolic* compound that is only found in brown algae, but is not found in terrestrial plants, and is formed from units of *phloroglucinol* (*1,3,5-trihydroxybenzene*) [5]. Based on several studies that have been

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carried out, it proved that phlorotannin has activity Biological broad as anti-proliferative and antioxidant [6], inhibitors of matrix metalloproteinases [7], and an ability to absorb UV radiation [8].

E. cottonii is red seaweed that can be cultivated and consumed. Seaweed is growing very fast and can be harvested every 45 d for consumption. Seaweed cultivation is abundant in Southeast Asia potentially in Africa and the islands located in the Pacific region [9].

Manufacture of sunscreen formulations is also needed in gelling agent and stabilizer. Carrageenan has been widely used in food industry, medicine, textiles, and cosmetics, because it is as an emulsifier, thickener, stabilizer, and gelling [10]. *E. cottonii* is a commercial source of carrageenan which is a gelling agent and stabilizer in food industry. *E. cottonii* contains nutrients comprising proteins, lipids, carbohydrates, vitamin C, α-tocopherol, and minerals that can be used as a medium for the growth of lactic acid bacteria [11]. Bioactive components found in seaweed are very prospective in cosmetics because they contain terpenoids, carotenoids, and polysaccharides (fucoidan, carrageenan, alginate, and jelly). This study aimed at determining chemical characterization proximate value, vitamin E and the antioxidant activity as well as active components extracts of *Sargassum* sp. and *Eucheuma cottonii* Doty. This study aimed to determine proximate value, vitamin E, antioxidant activity and active components in extracts of *Sargassum* sp. and *Eucheuma cottonii* Doty.

2. MATERIALS AND METHODS

2.1 Sampling

The sample used comprised of two types of natural seaweeds, i.e., *Sargassum* sp. and *E. cottonii. Sargassum* spp., obtained from the "*Kepulauan Seribu*" beach. Samples were obtained in wet conditions. The *Sargassum* sp. samples were transported manually to the laboratory by using a cool box and then were dried under the sun. Samples were dried and then were cut into pieces using scissors to simplify the process of destruction. *E. cottonii* seaweed sample was obtained from the beach Serang, Banten by collecting the cultivation of local communities. *E. cottonii* samples were obtained in the dry state to avoid the risk of damage. The samples of *E. cottonii* were washed first to remove dirt and salt content. All the samples were identified prior to enumeration or cutting.

2.2 Extraction [12]

The method of extraction was done in some stages using the solvent n-hexane, ethyl acetate and methanol. *E. cottonii* and *Sargassum* sp. were chopped each as much as 100 g and then inserted into Erlenmeyer and the solvent was added with a ratio (1:5 (w/v). Samples were soaked with solvent n-hexane, then covered with cotton, and wrapped in aluminum foil. Samples macerated and mixed using a shaker at a speed of 150 rpm (1 rpm = 1/60 Hz) for 3 d.

The obtained extract solution was filtered with Whatman filter paper number 42 to separate the filtrate and the residue. Residues were soaked again with 500 mL of solvent ethyl acetate and stirred for three days using a shaker with a speed of 150 rpm. The filtrate and the residue were filtered using filter paper, and then the same process using methanol. The filtrate was evaporated by rotary evaporator at 40 °C.

2.3 Yield Analysis [13]

Yield was calculated as a percentage of the weight of seaweed extract that had been obtained from the weight of the initial sample.

2.4 Proximate Analysis [14]

Proximate analysis is a method of chemical analysis to identify the nutritional content of the sample. Proximate analysis was conducted on the levels of ash, fat, protein and crude fiber.

2.5 The Content Analysis of Vitamin E [15]

Qualitative method used for analyzing vitamin E compounds in *E. cottonii* and *Sargassum* sp. seaweed is a comparison between the retention time (RT) and vitamin E with standard samples.

Quantification method used for quantitative analysis in this study is an external standard.

2.6 Antioxidant Activity Analysis [16]

Testing the antioxidant activity uses DPPH method (method of reduction of free radicals). Test activities include the manufacture stock DPPH antioxidant, vitamin C stock, stock samples, blank, and testing the activity using DPPH method. The antioxidant activity was calculated based on the linear regression equation and expressed in IC_{50} (mg L^{-1}). Ascorbic acid is used as a benchmark commercial antioxidant compound at a concentration of 0.1 mg L^{-1} to 0.5 mg L^{-1}.

2.7 Phytochemical Analysis [17]

Phytochemical analysis was performed to determine the bioactive components contained in the extracts of seaweed *Sargassum* sp. and *E. cottonii*. Phytochemical composition comprised of steroids / triterpenoids, flavonoids, phenols hydroquinone.

2.8 Steroids / Triterpenoids

Samples were dissolved in 2 mL of chloroform in a test tube. As many as 10 drops of acetic anhydride and sulfuric acid as much as three drops added to the mixture. Positive test results of samples containing steroids and triterpenoids was the formation of a solution of the red for the first time and then changed to blue and green.

2.9 Flavonoids

A mixture between 0.1 mg of magnesium powder and 0.4 ml of amyl alcohol (a mixture of hydrochloric acid 37 % and 95 % ethanol by volume each) were added to the sample and 4 ml of alcohol, and then the mixture was shaken. Positive test results of samples containing flavonoids was the formation of red, yellow or orange in the lining of amyl alcohol.

2.10 Phenol Hydroquinone

The sample, which is 1 g, was extracted with 20 mL of 70 % ethanol. The resulting solution was taken as 1 mL then two drops of 5 % $FeCl_3$ solution were added. Positive test results of samples containing phenol compounds were shown the formation of green or blue green solution.

The experimental data were analyzed by using statistic descriptive with Ms Excel.

3. RESULTS AND DISCUSSION

3.1 Yield of *Sargassum* sp. and *E. cottonii*

Extraction is the separation of one or more ingredients of a solid or a liquid with the aid of solvents. Separation occurs on the basis of different solubility of the components in the mixture [18]. Extraction with organic solvents can be done by percolation, maceration and soxhletation [19].

Maceration is a crude drug extraction process by using a solvent with some time shaking or stirring at room temperature (room temperature). The procedure is done by soaking bulbs in a suitable solvent in a sealed container. Occasional or constant stirring can increase the speed of reaction [20]. Maceration has several advantages, such as the amount of organic solvent used is not too much and the extraction temperature used below the boiling point of the solvent so that the degradation of oil components due to heat can be avoided [19].

Selection of solvents and extraction methods will affect the results of the content of secondary metabolites that can be extracted. Selection of solvent extraction generally uses principles where the non-polar compounds will dissolve in non-polar solvents, while the polar compounds will dissolve in polar solvents [21]. The extraction is done in some stages. The organic solvents used in this study are n-hexane, ethyl acetate and methanol. Evaporation process is used to separate the solvent from the extract. The temperature used is 40 °C to 50 °C to prevent damage of the active components contained in the extract.

Yield is an important parameter to determine the economic value and effectiveness of a material or product. Yield is the percentage share of the raw materials that can be utilized. The yield of

Sargassum sp. using solvent n-hexane, ethyl acetate and methanol amounted to 0.027 3 %, 0.133 3 %, 7.332 8 % respectively. The yield of *E. cottonii* using solvent n-hexane, ethyl acetate and methanol amounted to 0.025 7 %, 0.078 8 %, 6.758 6 % respectively. The extracts of *Sargassum* sp. and *E. cottonii* using the methanol produces the greatest yield compared with the solvent n-hexane and ethyl acetate. Putri et al. [22], states that methanol can dissolve almost all organic compounds that exist in the sample, both polar and non-polar compounds. Harborne [23] reported the differences of the yield of extract depends on the natural conditions of the sample, the extraction method, the particle size of the sample, the conditions and the time of extraction, as well as a comparison sample with solvent. Setha et al. [24] states that methanol produce extracts with antioxidant potential is better than other organic solvents, which means that methanol is able to attract the active components in the *Sargassum* sp. and *E. cottonii* optimally. The extracts of *Sargassum* sp. generated in this study is brownish green. Limantara & Heriyanto [25], reported that the color of *Sargassum* sp. is caused by three main pigments such as chlorophyll (the green pigment bluish), carotenoids (red pigment), and fucoxanthin (brown pigment).

3.2 Chemical Composition of *Sargassum* sp. and *E. cottonii*

The test results of proximate seaweed *Sargassum* sp. and *E. cottonii* from Kepulauan Seribu are presented in Table 1.

Table 1. Chemical composition of *Sargassum* sp. and *E. cottonii* from Kepulauan Seribu.

Chemical component	*Sargassum* sp. (%)	*E. cottonii* (%)
Water	82.26	77.27
Ash	5.09	5.84
Protein	1.26	2.39
Fat	0.41	0.12
Crude Fiber	0.43	0.67

Being marine in nature seaweeds contain a large amount of water. In a fresh condition, they have 75 % to 85 % water and 15 % to 25 % organic components and minerals [26]. States that the drying process is uneven and fluctuating temperature changes affect the water content. The longer the drying time is done, the water content of a substance contained in the lower [27].

The ash content value of *Sargassum* sp. and *E. cottonii* is 5.09 % and 5.84 %. The resulting ash content values still meet the standards of ash content in brown seaweed which is about 36 %. Winarno [28] states that the ash is inorganic substances waste products of combustion of an organic material. Ash has to do with mineral materials. High and low ash content contained in a material can be attributed to the amount of mineral elements [29], while the mineral content of seaweed can be affected by processing [30]. In addition, the level of each mineral component is determined by species, physiological factors, geographical conditions and frequencies, as well as the types of methods used in the mineralization process.

The protein content *Sargassum* sp. and *E. cottonii* in this study was obtained by 1.26 % and 2.39 %. The protein content *Sargassum* sp. and *E. cottonii* is lower when compared to the Burtin research [31], Brown seaweed that contains proteins of 3 % to 9 % of the weight of the wet, while the red and green seaweed containing protein at 6 % to 20 % of wet weight.

Environmental conditions, such as temperature, salinity, water transparency for synthesis of NFE and the nutrient uptake are the factors that can affect the crude fibre levels [32]. The variations in crude fibre of seaweeds can occur due to differences in growth stages and photosynthetic activity among seaweed species, and season brought about by changing environmental parameters that influence photosynthesis and uptake of nutrients [33].

The crude fat of seaweed was less than 5% reported on crude fat of seaweeds in other works [34]. Dharmananda [35], reported that seaweed generally contains fat by 1 % to 5 % of the dry weight. Seaweed contains very little fat.

3.3 Vitamin E of *Sargassum* sp. and *E. cottonii*

Vitamins based on their solubility properties are divided into fat soluble and water soluble. Vitamin E is a fat soluble vitamin in seaweed which contains many antioxidant activity [36]. Antioxidants are chemical compounds that can donate one or more electrons to free radicals, so that the free radicals can be suppressed [37]. Vitamin E (α-tocopherol) is widely used as an antioxidant in cosmetic preparations for preventing the aging process, maintenance and protection of normal biological processes such as anti-inflammatory. Vitamin E is an essential nutrient that functions as an antioxidant in the human body [38]. The results of the assay of vitamin E *Sargassum* sp. and *E. cottonii* use HPLC system. Levels of vitamin E obtained from samples of *Sargassum* sp. amounted to 165.19 mg L^{-1} and *E. cottonii* at 160.01 mg L^{-1}.

3.4 Antioxidant Activity of *Sargassum* sp. and *E. cottonii*

Antioxidants are defined as compounds that could delay, slow down and prevent the oxidation of lipids. In a special sense, antioxidants are substances that can delay or prevent the occurrence of free radical reactions in the oxidation of lipids [39]. Free radicals can be defined as a molecule or compound in a free state that has one or more unpaired electrons free. Electrons of unpaired free radicals are very easy to attract electrons from other molecules so that they become more reactive radicals. Therefore, highly reactive, free radicals are very easy to attack healthy cells in the body [40].

The different types of seaweed give differences in the content and type of the polyphenol compound. The differences of its compounds will affect its ability to reduce free radicals. The value of antioxidant activity *Sargassum* sp. and *E. cottonii* from the methanol extract is 57.050 mg mL^{-1} and 105.040 mg mL^{-1} respectively. The results showed that the antioxidant value of the *Sargassum* sp. and *E. cottonii* sample is very strong. Molyneux [41] states that a compound called active as an antioxidant when the IC_{50} value is less than 200 mg mL^{-1}. When the IC_{50} values obtained ranged from 200 mg mL^{-1} to 1000 mg mL^{-1}, the substance is less active but still has potential as antioxidants. Prior & Cao [42] suggest the lower the IC_{50} value, the higher the antioxidant activity of antioxidants. This was due to the use of lower concentrations can already inhibit DPPH by 50 %.

3.5 Bioactive Components of *Sargassum* sp. and *E. cottonii Extracts*

Sargassum sp. contains fucoidan and phenolic components. Type of phenolic components that are commonly found in brown seaweed is phlorotanin ranging from 0.74 % to 5.06 % [43]. *Sargassum* sp. contains ascorbic acid, as well as phlorotannin compounds that play a role in inhibiting the formation of melanin [5].

The extracts of *Sargassum* sp. and *E. cottonii* phytochemical were analyzed to determine the active component compound that acts as a good sunscreen to protect the skin. Phytochemical analysis undertaken includes flavonoids, phenols hydroquinone and triterpenoids. Phytochemical analysis results of extract *Sargassum* sp. and *E. cottonii* are presented in Table 2.

Table 2. Phytochemical analysis of extracts of *Sargassum* sp. and *E. cottonii.*

Compounds	*Sargassum* sp.	*E. cottonii*
Flavonoid	+++	+
phenol hydroquinone	++	+
triterpenoids	++	+

Description:
(+) = weak
(++) = strong
(++++) = very strong

Based on qualitative phytochemical analysis, it can be shown that the extracts of *Sargassum* sp. and *E. cottonii* contain the active components of flavonoids, phenols hydroquinone and triterpenoids which allegedly acted as a potential agent for raw materials sunscreen cream.

Flavonoids are generally found in all parts of the plant, including the fruit, pollen and roots in

the form of glycosides. Flavonoids are classified into flavones, flavonols, flavanones, flavanols, isoflavones, kalkon, dihidrokalkone, aurone, anthocyanidins, catechins and flavan-3,4-diol [44]. Flavonoids are good at reducing compounds, inhibiting many oxidation reactions, enzymatic and non-enzymatic [45] Flavonoids, one of polyphenols, have a big role in the activity of tyrosinase because they contain phenol group and ring pyren. The structure of flavonoids in principle is suitable as substrates and able to compete so that it can be inhibiting tyrosinase [8].

Phenolic component is an aromatic structure that binds to one or more hydroxyl groups which are some may be substituted with a methyl group or a glycosyl. Free phenolic compounds are usually are found in wood tissue, while the phenolic compounds are in another place usually in the form of glycosides [23]. Phenolic compounds are involved in electron transport in photosynthesis and in the regulation of certain enzymes. These compounds also have anti-inflammatory activity, as it can inhibit prostaglandin synthesis [46]. Kim et al. [7] reported the chemical structure of phenolic components has similarities with tyrosinase substrate so that the phenolic components are potential as competitive inhibitors of the tyrosine-tyrosinase reaction.

Triterpenoids are natural compounds formed by the process of biosynthesis and distributed widely in the world of plants and animals. Terpenoids structure is built by isoprene molecules with terpenoid skeleton which is formed of two or more units of isoprene (C5) [44]. Terpenoids consisting of several kinds of compounds are components of essential oils, diterpenoid, giberaline, triterpenoidem, sterid and carotenoids [47]. Three steroids isolated from Trifolium balansae were reported to have activity of tyrosinase inhibitor which isolates both have IC_{50} value of 2.39 μM and [48].

4. CONCLUSIONS

The yields of *Sargassum* sp. and *E. cottonii*, generated by methanol, amounted to 7.332 8 % and 6.758 6 %. The proximate value of *Sargassum* sp. comprsing of moisture, ash, fat, protein and crude fiber amounted to 82.26 %, 5.09 %, 1.26 %, 0.41 %, 0.43 %, respectively, and proximate *E. cottonii* amounted to 77.27 %, 5.84 %, 2.39 %, 0.12 % and 0.67 %, respectively. The concentration of vitamin E determined in *Sargassum* sp., using HPLC, was 165.19 mg L^{-1} and *E. cottonii* at 160.01 mg L^{-1}. The antioxidant activity of *Sargassum* sp. and *E. cottonii* from the methanol extract was 57.05 mg L^{-1} and 105 mg L^{1}, 04 mg L^{-1}. The active components of *Sargassum* sp. and *E. cottoni* contained in methanol extracts were flavonoids, phenols hydroquinone and triterpenoids compounds which can potentially be used as raw material for sunscreen cream.

5. ACKNOWLEDGEMENTS

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