Antioxidant properties from seaweeds *Kappaphycus alvarezii*, *Euchema spinosum* and *Sargassum* sp. using different solvent

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Abstract. Antioxidants are substances produced by the body in small amounts. Antioxidants could be obtained from seaweeds’ bioactive compounds. Seaweeds’ bioactive materials include sulfate polysaccharides, proteins, pigments carotenoid, polyunsaturated fatty acids (PUFA), essential minerals, vitamins and other secondary metabolites. The aim of this research is to determine the antioxidant properties using DPPH radical scavenging method from the extracts of *K. alvarezii*, *E. spinosum* and *Sargassum* sp.. The experiment was conducted using three species of seaweeds (*K. alvarezii*, *E. spinosum* and *Sargassum* sp.) which were extracted with three different polarity solvents (*n*-hexane, ethyl acetate and ethanol). The results showed the presence of the antioxidant activity in various extraction of seaweeds. Inhibitor to DPPH Free Radical activity were shown in 100 ppm by extracts *Sargassum* sp. (etil asetat) 23.8±0.7 \%, *Sargassum* sp. (ethanol) 11.7±0.4 \%, *K. alvarezii* (etil asetat) 14.7±0.4 \%, *K. alvarezii* (ethanol) 14.9±0.4 \%, *E. spinosum* (etil acetate) 12.1±0.4 \%, *E. spinosum* (ethanol) 12.0±0.4 \%, and BHT 58.5±3.1 \%. All of the *n*-hexane extracts of the seaweeds had no antioxidant activities when being treated by qualitative of DPPH methods.

1. Introduction
Free radicals were caused by oxidation process in the body. Free radicals in the human body caused by unstable compound that called Oxygen Species (ROS) [1]. ROS materials for example, are hydrogen peroxide (H\(_2\)O\(_2\)), Superoxide Anion (O\(_2^−\)) and Hydroxyl Radical (OH) [2]. Free radicals are naturally needed in small amount to protect the human cell and tissue from bacterial attack and parasites. The availability of excessive free radicals can damage the cell membrane, cell structure and genetic structure such as deoxyribose nucleic acid (DNA) [3].

Prevention and protection against the effect of free radicals could be prevented by the antioxidant. Antioxidant are substances that produced by the body in small amount or from the human diets [4]. Seaweed is one of common food contain many bioactive ingredient that have function as antioxidant compound The chemical compound could donor electron toward compound of free radicals to stable and lead to inhibit oxidation process [5,6].
The purpose of this research is to determine the potential of antioxidant properties from seaweed. Antioxidant properties of seaweed could be determine using DPPH scavenging activities test from three species of seaweed which were extracted with different solvents. The research were conducted by using three species of seaweed: *K. alvarezii*, *E. spinosum* dan *Sargassum* sp. This study could give information about comparation of antioxidant properties of three edible seaweed that culture (*K. alvarezii*, *E. spinosum*) and wild type (*Sargassum* sp.) extract using different solvent. Moreover, the highest antioxidant properties from selected seaweed could be applied as functional food by local communities.

2. Materials and methods
2.1 Materials
*K. alvarezii* (farmed) and *Sargassum* sp. (wild) seaweed were collected from coastal area of Tanjung village, Saronggi, Sumenep Residence, East Java Province, Indonesia and *E. spinosum* (farmed) were collected from Pekandangan Barat Village, Bluto, Sumenep Residence, East Java Province, Indonesia. Seaweed samples were identified by Biology Department, Faculty of Science and Technology, Universitas Airlangga, Indonesia.

The seaweed sample were washed and then dried in room temperature (±25 °C) and stored in dry place (undirect sunlight) until exceeded water content less than 11%. Sample were cut and ground then extracted used maceration method for 24 hours using three different solvents (*n*-hexane, ethyl acetate, and ethanol) then concentrated used rotary evaporator (temperature: <40 °C). Extract samples were stored in brown umber bottles and keep in refrigerator at 4°C until further analysis.

Antioxidant properties from various seaweed extract were determined using DPPH scavenging activity test followed methods described by [7]. The concentrated seaweed extract was dissolved in 10 mL methanol (pro-analyst) with several variety of concentrate (30, 50, 80, 100, and 1000 mg/L, w/v). Seaweed extract sample (0.3 mL) was mixed with 2.70 ml of DPPH (0.004% in methanol) then were incubated for 30 minutes in the dark room temperature (±25°C). The result of the reaction was read using UV-Vis spectrophotometer at 497, 517, and 537 nm. Butylated hydroxytoluene (BHT) was used as standard.

The percentage of radical scavenging activity of seaweed extract was calculated by following formula:

$$Absorbance\ 517 = \left[ A_{517} - \frac{(A_{479} + A_{537})}{2} \right]$$

$$Inhibition\ (%) = \left[ 1 - \frac{Absorbance\ Sample\ 517}{Absorbance\ DPPH} \right] \times 100\%$$

2.2 Data analysis
The antioxidant properties data were analysed using Analysis of Variance (ANOVA) (α=0.05) and continued with the Duncan’s Multiple Range Test to determined significant differences between treatments.

3. Results and Discussion
The result of DPPH scavenging activity test were showed the differences (p<0.05) between samples. The highest DPPH scavenging activity were demonstrated by *Sargassum* sp. (ethyl acetate extract) then were followed by *K. alvarezii* (ethyl acetate extract), *K. alvarezii* (ethanol extract), and the lowest DPPH free radicals scavenging activities were showed by *Sargassum* sp. (ethanol extract), *E. spinosum* (ethyl acetate extract) and *E. spinosum* (ethanol extract). Inhibition of free radical activities were summarized in Table 1 and Figure 1.

Seaweed is one of naturally material that can be recommended as source of natural antioxidant, which were demonstrated by the result DPPH scavenging activity from some of extract samples. DPPH free radical is compound that is used as reactor for process giving an electron donor to inhibited free radical’s
compound. This process can be used as a method to expressed the potential of antioxidant substance in active material [8].

Result of the research shows some seaweed extract that has potential as antioxidant. Addition of extract material causes bleaching process against DPPH free radicals as mechanism free radical inhibitor. It agreed with the statement Prakash et al. [8] that mechanism of inhibition to DPPH free radical compound were inhibited by the active ingredient. Seaweed extract could provide hydrogen atoms to inhibit DPPH compound. Addition of hydrogen atoms caused stability of DPPH that demonstrated by the bleaching color from purple become yellow during incubation. Donation mechanism of hydrogen atoms are antioxidant mechanism [2]. In this study, seaweed extract samples were demonstrated have antioxidant materials because it showed bleaching activities on DPPH color due to donor of hydrogen atoms to inhibit of DPPH as free radicals. Figure 1 showed bleaching process from purple to yellow as scavenging activities to inhibit DPPH as free radicals.

**Table 1.** Inhibitor Concentration (100 ppm) extract using DPPH scavenging activity test

| Sample                  | Inhibition ± SD (%) |
|-------------------------|---------------------|
| BHT                     | 58.5 ± 3.1<sup>a</sup> |
| *K. alvarezii* ethyl acetate | 14.7 ± 0.4<sup>c</sup> |
| *Sargassum* sp. ethyl acetate | 23.8 ± 0.7<sup>b</sup> |
| *E. spinosum* etil acetate | 12.1 ± 0.4<sup>d</sup> |
| *Sargassums* sp. ethanol  | 11.7 ± 0.4<sup>d</sup> |
| *K. alvarezii* ethanol    | 14.9 ± 0.4<sup>c</sup> |
| *E. spinosum* ethanol     | 12.0 ± 0.4<sup>d</sup> |

<sup>a</sup>The different superscript were showed significantly different of scavenging activity (p<0.05).

**Figure 1.** Changing color process of DPPH free radicals by seaweed extract.

BHT is one of standard that used as antioxidant [8]. BHT is synthetic antioxidant materials that is more used in supplement ingredient on food, but the use of BHT has banned in many various countries, which were reported could as carcinogenic compound [9].

Extract material that is used as antioxidant is crude extract materials. Crude extract is obtainable from extraction process with different levels of solvent polarity. Green color on *K. alvarezii* and *Sargassum* sp. more concentrated than *E. spinosum*. Green color shows presence of solute that suspected of chlorophyll and another photosynthetic pigment, which are antioxidant, and material of secondary metabolites that soluble in polar solvents.

The presence of chlorophyll gives suspected that seaweed extract has antioxidant activity. It is suitable with [10] that chlorophyll is one of potential antioxidant. Besides chlorophyll, another pigment which has *phycoerythrin* pigment (red color), *phycocyanin* pigment (green color), and *allophycocyanin*
pigment [11]. These pigments are addition pigment or accessories pigment which owned the red algae such as *K. alvarezi* and *E. spinosum*.

Flavonoid is one of material that has function as antioxidant. Nutrition substance from *K. alvarezi* and *Sargassum* sp. reported by [12], these two of seaweed contain of vitamin C and vitamin E, both of them are materials which functional as antioxidant.

The level of water in the beginning also affects active material, which extracted. The low level of water can simplify extraction process. It caused that active component is more concentrated and solvent easier to pull active compounds that be contained in a material. Level of water from *K. alvarezi* iaitu 6.5 % lower than *E. spinosum* by 11.412 % (11.4) and *Sargassum* sp. by 11.9 %, suspected as one of cause inhibition free radical bigger because the compound that extracted is better.

4. Conclusion

The research conclude that seaweed extract has potential as the highest antioxidant that is *Sargassum* sp. (ethyl acetate extract), followed by *K. alvarezi* (ethyl acetate extract), *K. alvarezi* (ethanol extract), *E. spinosum* (ethyl acetate extract), *E. Spinosum* (ethanol extract) and *Sargassum* sp. (ethanol extract). Seaweed extract *Sargassum* sp. (ethyl acetate extract), *K. alvarezi* (ethyl acetate extract) and *E. spinosum* (ethyl acetate extract).

5. References

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