

## **Red seaweed (*Kappaphycus alvarezii* DOTY) from Mollucas island water as potential flavonoid resource of natural antioxidant**

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### **Abstract**

The purpose of this research was to find out the best extraction solvent from the point of total flavonoid content and antioxidant activity of *Kappaphycus alvarezii*. The total flavonoid content of various solvent extracts were determined by spectrophotometer, and calculated as mg QE/g while its antioxidant activity was analysed using DPPH method and calculated as % radical scavenging activity. Total flavonoid content and antioxidant activity data were presented as means  $\pm$  standard deviation from three replicated experiment.

The hexane extract of *Kappaphycus alvarezii* showed higher total flavonoid content (15.93 %) and water extract showed lowest content (0.187 %). The Radical scavenging activity of various solvent extract was increasing with increased solvent extract concentrations and the highest radical scavenging activity could be obtained in 5 ug/ml hexane extract. It can be concluded that hexane extract of *Kappaphycus alvarezii* could be potential as a rich resource of natural antioxidant.

**Keywords:** Scavenging DPPH radicals, solvent extraction

### **Introduction**

*Kappaphycus alvarezii* as an economically important red tropical seaweeds, which is highly demanded for its cell wall polysaccharides, is the most important source of kappa carrageenan. This seaweed accounts for the largest consumption worldwide (Kumar et al 2008) has also been widely cultivated in Indonesia and becoming production of seaweed priority target within the domestication fisheries revitalitation program of 2009. Some location in Indonesia where this program was carried out are in Lombok, Sumba, South-East Sulawesi and Mollucas islands. However the species originated from Eastern Indonesia has not been investigated scientifically

Flavonoids as the largest group of phenolic compound are known to contain a broad spectrum of chemical and biological activities including antioxidant and free radical scavenging properties (Kahkonen et al 1999). Santoso et al (2002) and Yoshie et al (2000) reported that series of flavonoid compounds such as catechin (e.g gallic acid, epigallocatechin gallate), flavonols and flavonol glycosides have been identified from methanol extracts of red and brown algae. Many researches also reported that a positive correlation has

been documented between total flavonoid content and antioxidant activity of different seaweeds extracts. (Athukorala et al 2006, Jimenez et al 2001).

Antioxidant are classified by the products they formed on oxidation (these can be antioxidants themselves, inert, or prooxidants), or by what happens to the oxidation products and its oxidized form may be broken down by the organism as well as how effective the antioxidants is against specific free radicals. The most common procedure for antioxidant activity measurement was 2,2 diphenyl-1-picrylhydrazyl (DPPH) assay (Kumar et al 2008).

The purpose of this research was to find out the best extraction solvent from the point of total flavonoid content and antioxidant activity of *Kappaphycus alvarezii* from Mollucas island.

## **Materials and Methods**

### **Collection of Samples**

*Kappaphycus alvarezii* were taken from the cultivation area of seaweed in Wael Village, western Seram regency, Mollucas province. Samples at the age of 30-35 days were harvested from the area at 33 °C, salinity 33 ‰ and pH 7.

### **Preparation Extract**

Sample were washed using tap water to clean up from sand, salt and other dirt, followed by sun drying for three days until its moisture content was 27 %. The dried sample was ground to powder using grinder and strained with diameter of 100 mm. The powder samples (400 g) was then put into percolator (ratio samples and solvent used 1 : 2) and extracted using various solvents namely hexane, chloroform, ethyl acetate, methanol and water.

### **Laboratory Analysis**

Total flavonoids were determined using colorimetric method as described by Quettier (2000). The ability of the extracts to scavenge DPPH radicals was determined by the method of Duan et al (2006).

### **Statistical Analysis**

Data were expressed as means of three determination ± standard deviation. Data were analyzed using one-way analysis of variance (ANOVA) followed by student t test using SPSS system version 16. A significant difference was considered at the level of  $p < 0.05$ .

## **Results and Discussion**

### **Total Flavonoid Content**

The highest total flavonoid of *K. alvarezii* could be obtained in hexane extract (15.93 %), followed by chloroform extract (12.93%), ethyl acetate extract (8.92 %), methanol extract (1.43 %) and aquades extract (0.187%) (Table 1)

**Table 1:** Mean values for total flavonoid content using hexane solvent, chloroform, ethyl acetate, methanol and aquades of *Kappaphycus alvarezii*

	Hexane	Chloroform	Ethyl acetate	Methanol	Aquades
Total flavonoid content #	0.159±0.002 <sup>a</sup>	0.128±0.0051 <sup>b</sup>	0.0892±0.0040 <sup>b</sup>	0.0143±0.0030 <sup>b</sup>	0.00187±0.0011 <sup>c</sup>

# mg quercetin equivalent/g of dry extract

<sup>abc</sup> Means in the same row without common letter are different at  $P < 0.05$

Total flavonoid was determined by colorimetric method using aluminium chloride where the principle involved is aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminium chloride forms acid labile complexes with the ortho-dihydroxyl group in the ring A or B ring of flavonoids. (Rajanandh and Kavitha 2010).

### Scavenging Effect on 1,1-diphenyl-2-picrylhydrazyl radical (DPPH)

DPPH is a useful reagent for investigating the free radical scavenging activities of compounds. The method is based on the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H by the reactions (Shon et al 2003).

The results (figure 1) indicated that scavenging activity of seaweed extract were depended on its concentration. It was founded that the scavenging activity of sample increased with increasing extract concentration and solvent polarity. DPPH radical scavenging activity of various extracts were in the range of 4.54% -70.31%. The highest DPPH radical scavenging activity could be obtained in 5 mg/ml hexane extract (70.31%) and the lowest one could be obtained in 0.5 mg/ml water extract (4.54%).

**Figure 1.** The DPPH radical scavenging activity of concentration extract of *Kappaphycus alvarezii*

The research result indicated that non polar compound found in extract possesses strongest ability to scavenge DPPH radicals. In this study, the extract concentration showed positive and significant correlation with DPPH scavenging activity ( $R^2$  0.93). Futher studies will elucidate the identity of the antioxidant molecules in the hexane extract.

### Conclusion

- The crude extract of *Kappaphycus alvarezii*, hexane extract possessed the highest total flavonoid and DPPH scavenging activity.

- The hexane extract of *Kappaphycus alvarezii* from Mollucas islands water could be a potential rich resource of natural antioxidant.

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