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Determination of Element Compositions and Antioxidant Activities of *Kappaphycus alvarezii* Found in the Waters of Langkawi and Sabah, Malaysia

XirenGuli Keyimu[1*], Aminah Abdullah[1, 2]

[1]School of Chemical Sciences and Food Technology, Faculty Science & Technology, UniversitiKebangsaan Malaysia, 43600 UKM Bangi, Selangor DarulEhsan , Malaysia
[2]Natural Medicine Research Group, Universiti Islam Malaysia, 63000 Cyberjaya, Selangor, Malaysia

Abstract : The element composition and antioxidant properties of the two red seaweeds *Kappaphycus alvarezii*, from Lankawi and Sabah in Malaysia were determined. The results indicate that the two red seaweeds contained high levels mineral such as K. There were no significant differences in TPC (26.81-28.04 umol /100g GAE), DPPH (18.75-22.76%), ORAC (235.12-530.24umol/100g TE) between the two seaweeds. This study suggested that *Kappaphycus alvarezii* from both habitats could be used as ingredients to improving nutritive value and functional properties in human diets.
Keywords : *Kappaphycus alvarezii*, element composition, DPPH, TPC, ORAC, antioxidant activities.

Introduction

Seaweed is one of the potential export commodities to be developed[1.]Seaweeds are rich in soluble dietary fibers, proteins, minerals, vitamins, antioxidants, phytochemicals, and polyunsaturated fatty acids, with low caloric value. They are an excellent source of vitamins A, Bl , B2, B3, B12, C, D and E[2].According to Fayaz*et al*[3]*Kappahycusalvarezii* contains ascorbic acid and polyphenols, which are hydrophilic which might also contribute to the antioxidant activity of the sample[4]. Seaweeds have antioxidative defense system due the absence of oxidative damage in basic components[5] and have the ability to prevent the oxidation during storage when exposed to combination of light and oxygen[6].Previous literature reported the potential antioxidant compounds such as some pigments (i.e. fucoxanthin, astaxanthin, carotenoid) and polyphenols (i.e. phenolic acid, flavonoid, tannins), that are widely distributed in seaweeds and are known to exhibit higher antioxidative activities[4]. Antioxidants are potent scavengers of free radicals and have beneficial effects on human health and disease prevention[7].In addition, seaweedis identified as valuable sources of elements[5]which are useful for metabolic reactions in human and animal such as enzymatic regulation of lipid, carbohydrate and protein metabolism[9, 10]. The red *Kappaphycusalvarezii* seaweed falls under the class of Rhodophyceae. Drying seaweed with heating has a variety of purposes, one of which was to extend the shelf life [11]. It is economically important specie which has been extensively cultivated in more than 20 countries for a source of carrageenan[12].

Kappaphycus alvarezii is found abundantly in Sabah and Langkawi. Utilisation of seaweeds from Langkawi is limited to people living in the coastal areas only.However, the nutritional composition of seaweeds vary depends on their species, maturity, environmental growth conditions and seasonal period[13,14].Changes in

their ecological conditions have an influence on the synthesis of nutrients[10, 15], and the biochemical composition of *Kappaphycus alvarezii* from Langkawi is poorly known.

The purpose of this study was to determine the element composition and antioxidant activities of seaweeds from the two different places. In order to provide more intensive nutrient information, the samples were collected from both Langkawi and Sabah for analysis.

Materials and Methods

Samples

Samples were collected from the Langkawi Island in the state of Kedah Peninsular Malaysia, and from the coastal area of Sabah, East Malaysia. Samples were thoroughly rinsed and soaked in water for 117 minutes, and then soaked in 5% lemon juice overnight to eliminate a fishy odour[16].

Antioxidant Activity

Extraction of antioxidant

1.0g of each sample was weighed in universal bottles and mixed with 10 mL of 50% acetone. Samples were then homogenized using homogenizer (T 250, IKA, Germany) at 24,000rpm for 1 minute. Followed by centrifugation using table top centrifuge (MLX 210, Thermo-line, China) at 1000rpm for 10 minutes. The supernatants were collected for further analysis.

Determination of Total Polyphenol Content

The total polyphenol content (TPC) was determined by spectrophotometry technique using gallic acid as a standard in accordance with the method described by Musa*et al*[17] Briefly, 100 µL of each seaweeds extract, was mixed with 0.5 mL diluted Folin-Ciocalteu reagent. The mixture was left for 5 minute before 1 mL7.5% sodium carbonate (w/v) was added. Absorbances were taken at 765 nm wavelength with spectrophotometer after 2 hours. Calibration curve of gallic acid was set up to estimate the activity capacity of samples. The result was expressed as mg of gallic acid equivalents per 100 g of fresh sample (mg GA/100 g of FW).

Determination of the Free Radical Scavenging Activity

Scavenging activities of the extracts on the stable free radical DPPH were assayed based on the procedure described by Musa*et al*[17]. Stock solution of DPPH was prepared by dissolving 40 mg DPPH in 100 mL methanol and kept at -20°C until used. About 350 mL stock solution was mixed with 350 mL methanol to obtain the absorbance of 0.7±0.01 unit at 516 nm wavelength by using spectrophotometer (Epoch, Biotek, USA). 100 µL seaweed extracts with 1 mL methanolic DPPH solution prepared were kept overnight for scavenging reaction in the dark[18]. Percentage of DPPH scavenging activity was calculated by measuring the absorbance of the sample and applying the following equation[19]:

$$\text{DPPH scavenging activity (\%)} = [(A_{blank} - A_{sample})/ A_{blank}] \times 100$$

ORAC Antioxidant Activity Assay

The oxygen radical absorbance capacity (ORAC) assay was carried out based on the procedure described by Musa*et al*[17] with slight adjustments. 175 µL of the sample/blank were dissolved with PBS at a concentration of 160 µg/Ml and pH 7.4. Subsequently, 150 µL of fluorescent sodium salt solution was added, and the plate was incubated for 45 minutes at 37°C. AAPH solution (25 µL) was added to make up a total volume of 200 µL /well. Fluorescence was recorded at 37°C until it reached 0. Data were collected every 2minutes for 2 hours and were analysed by computing the differences of areas in the fluorescent falloff curve (AUC) between the blank and the sample. Calibration curve of Trolox were prepared and values were expressed as Trolox equivalents [17].

Heavy Metals and Mineral Element Analytical

The dried seaweed samples were digested using the hot-block digestion procedure for total metal concentration[20]. Sample weighting 1g were ground using digestion tube, 10ml concentrated HNO_3 (Merack Germany) was added to the digestion tube and covered with watch glass in the mouth of it. The mixture was left overnight and after that the digestion tube was placed into a block digester (ATM600 BLOCK, Australia) and heated at 95 °C for 1.5 hours then allowed to cool before it was added 7 ml H_2O_2 and continue to digest the sample for another 2 hours until the digest sample was clear . Additional HNO_3 not exceeding 5 ml was added to maintain a wet digest. The diluted equaregia (mixture of nitric acid and hydrochloric acid with a volume ratio of 1:3) was added to the dissolved digest residue and the solution was transferred into a 50 ml volumetric flask. Deionized water was added to bring the volume of the solution up to 50 ml and the solution was filtered using 0.45 μm filter paper (Whatman, U.S.) to remove any particulates. Finally, the solution was analyzed using the inductively coupled plasma mass spectrometry ICP-MS.

Results and Discussion

Antioxidant Activity

Two seaweed have been tested for their antioxidant activities by the DPPH scavenging system, ORAC and TPC. The value of all antioxidant assays showed in Table 1.

Table 1. Antioxidant activity of *Kappaphycus alvarezii* from Langkawi and Sabah

Sample	TPC umol/100g GAE	DPPH %	ORAC umol/100g TE
Kappaphycus alvarezii (Lankawi)	19.46^{a}	18.75^{b}	530.24^{a}
Kappaphycus alvarezii (Sabah)	20.76^{a}	22.76^{a}	235.12^{b}

Different letters indicate significant differences ($P<0.05$). Values are expressed as mean (n=2).

In this study, the Sabah's seaweed displayed the highest scavenging effects (22.76%), while the Langkawi's seaweed displaying the lowest scavenging effects (18.75%),they have significant difference ($P<0.05$) between the two seaweeds. The result of this study also shows that the total phenolic content was higher (20.76 umol /100g GAE) in Sabah's seaweed, lower (19.46umol /100g GAE) in Langkawi's seaweed, but they don't have significant difference ($P>0.05$) between the two seaweeds. In terms of ORAC value, the significant higher ($P<0.05$) oxygen radical absorption capacity was determined on seaweed which were collected from Langkawi (530.24 umol/100g TE), Besides, Sabah's seaweed (235.12 umol/100g TE) showed lower value of oxygen radical absorption capacity. The content of the phenols may differ depending on the occurrence and climatic conditions of seaweeds [21].

The correlation between the total phenolic contents and total antioxidant capacity of the seaweeds was studied by many researchers. Devi *et al*[22]have reported that there is a strong correlation between antioxidant activity and phenolic content. Also, the quantitative analysis of the total phenolic content of the seaweeds has indicated that Gelidella acerosa and Haligra spp. have high phenolic contents, which correlate to their respective antioxidant and antimicrobial activity. In the present study also, seaweed antioxidant properties were studied for total phenolic content, oxygen radical absorption capacity and free radical scavenging activity and a significant correlation was observed between them[20].

Element contents

The elements in the two seaweeds are listed in the Table 2. The means of macro elements (K, Mg, Na, Ca) and trace elements (Fe, Cu, Zn, Mn, Cr, Se) contents ranged from 23.39-555.23mg/100g DW and 0.09-8.33mg/100g DW, respectively. Thus the two seaweeds were rich in K and Na. From the above, it can be observed that Na/K ratio is below 1.0 which is interesting from the point of view of nutrition, since the intake of sodium chloride and diet with a high Na/k ratio have been related to the incidence of hypertension[23]. As for the trace elements, copper plus zinc contents were found within the range of 2.27-3.11 mg/100g and also below the maximum level allowed in seaweeds for human consumption in Japan and France (10 mg/100g)[10].

Table 2. The elements contents of *kappaphycus alvarezii* from Lankawi and Sabah

Minerals	***Kappaphycus alvarezii* (Lankawi)**	***Kappaphycus alvarezii* (Sabah)**
Macro elements (mg/100mg)		
K	351.72[b]	555.23[a]
Mg	95.49[a]	60.33[b]
Na	250.13[a]	156.01[b]
Ca	23.39[b]	37.34[a]
Trace elements (mg/100mg)		
Fe	8.30[a]	8.22[a]
Cu	0.21[a]	0.22[a]
Zn	2.06[a]	2.89[a]
Mn	0.18[b]	0.34[a]
Cr	0.33[a]	0.35[a]
Se	0.14[a]	0.09[b]
Toxic elements (mg/100mg)		
Co	0.03[a]	0.01[b]
Ni	0.67[a]	0.49[b]
As	0.34[b]	0.46[a]
Cd	0.04[a]	0.01[b]
Pb	0.08[a]	0.07[a]

Different letters indicate significant differences ($P<0.05$). Values are expressed as mean (n=2).

The toxic elements in the two seaweeds were Pb (0.07-0.08 mg/100g), Cd (0.01-0.04 mg/100g), As (0.34-0.46 mg/100g), Co (0.01-0.03 mg/100g) and Ni (0.49-0.67 mg/100g). The levels of detected elements fit within the allowed ranges in previous reports[10]. Mineral content will differ with each other because of several factors such as genetic species, sea condition, and seasons and also the physiology and morphology of the seaweed[24]. The present study shows the possibility of both seaweeds being used as food supplements to improve the nutritive value for the human diet.

Conclusion

This study showed that minerals and antioxidant composition of *Kappaphycus alvarezii* from Sabah were significantly higher ($p\leq0.05$) than those grown in Langkawi. Both seaweed contained various amounts of macro-minerals such as Sodium (976.56 mg/100 gm), Potasium (987.52 mg/100 gm), Magnesium (247.17-760.92 mg/100 gm), and rich in calcium (77.09- 120.76gm/100 gm). These both seaweeds contain an acceptable amount of antioxidant content. Therefore, both seaweeds appeared to be potential sources of ingredients in the production of functional food products and animal feeds. More studies should be conducted to determine the seasonal variation of seaweed.

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