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Antioxidant activity of dried and rehydrated *Kappaphycus alvarezii* from Langkawi, Kedah and Semporna, Sabah

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Abstract : The aim of this study was to determine the antioxidant activity of dried and rehydrated *Kappaphycus alvarezii* from Langkawi, Kedah and Semporna, Sabah. The antioxidant activity in *K. alvarezii* was determined by using total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, Trolox equivalent antioxidant capacity (TEAC), Ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC). Comparison between dried and rehydrated of *K. alvarezii* indicated that the rehydrated was significantly ($p<0.05$) high in antioxidant activity compared to dried. While, the Langkawi's rehydrated seaweed (119.3 mg GAE/100 g of dried weight (DW) sample) has significantly ($p<0.05$) higher TPC value compared to Semporna (89.2 mg GAE/100 g of DW sample). The DPPH value of rehydrated seaweed from Semporna was 34.6% and significantly ($p<0.05$) higher compared to seaweed from Langkawi which was 27.5% in value. Antioxidant activity for FRAP and ABTS assay of rehydrated seaweed from both locations showed no significant different ($p>0.05$) value. While, the Langkawi's rehydrated seaweed (73 µmol TE/100 g of DW sample) showed significantly ($p<0.05$) higher ORAC value compared to Semporna (53.8 µmol TE/100 g of DW sample). Thus, the finding in this study, demonstrated that rehydrated *K. alvarezii* possesses higher antioxidant activity compared to dried seaweed.
Keywords : Total phenolic content (TPC), antioxidant activity, seaweed, *Kappaphycus alvarezii.*

Introduction

In Malaysia, *Kappaphycus alvarezii* is the most common species that were cultivated for food as well as for carrageenan production, where almost all of them were cultivated in fishing community around Semporna, Sabah[1-2]. Due to high market value of *K. alvarezii*, the variety of seaweed from Semporna, Sabah was

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transferred and planted to Langkawi, Kedah. Langkawi was chosen as the seaweed farming place due to environmental conditions such as water temperature and salinity level that are conducive for farming. Therefore, Langkawi was expected to transform the seaweed industry into a larger and more profitable scale.

Seaweed has low calorie content, high fibre and mineral content, and significant amount of protein, vitamins and trace elements[3]. Furthermore, seaweeds are capable to generate essential defence mechanisms against oxidation. Therefore seaweeds are important source of antioxidant that may able to protect human body against reactive oxygen species[4]. While, a lot of studies have reported about significant amount of antioxidant activity in the *K. alvarezii*[5-6].

Normally, the dried seaweeds are rehydrated in water to restore their original structure and the excess water is removed before they can be used[7-8]. During the rehydration process, the dry porous material submerged in water undergoes several changes towards its moisture, porosity and volume[9]. However, the dehydration and rehydration process may affect the nutritional value of *K. Alvarezii,* by causing changes in chemical composition as well as bioactivities such as antioxidant activity. Therefore, in this study we determined the effects of rehydration on antioxidant activity using *K. alvarezii* from Langkawi, Kedah and Semporna, Sabah.

Materials and Methods

Sample preparation

Dried seaweed of *K. alvarezii* from Langkawi was supplied by Maya System Enterprise, Malaysia. While sample from Semporna was purchased from Sabah local supplier in November 2014. The samples were prepared as method described by[10] with slight modification. The seaweeds were rinsed using distilled water. Then, they were rehydrated three times using distilled water in the ratio 1: 5. A total of 200 g seaweed was soaked into 1 L of distilled water in biker for 1 min at 28°C. After rehydrated, the water was discarded and seaweeds were dried for 15 mins to remove-excess water. The dried and rehydrated seaweeds were dried in the oven at 50°C for 72 hr. Finally, they were ground into powder form using universal cutting mill (Fritsch Industries, Germany) and was stored at 4°C until further analysis.

Antioxidant extraction

The extraction of antioxidant was done as described by[11] with slight modification. About 0.5 g of seaweed powder was mixed with 10 mL of 50% (v/v) acetone (HmbG Chemicals, German). Then, samples were homogenized using high speed homogenizer (T250, IKA, Germany) at 24,000 rpm for 1 min. After that, the samples were mixed using magnetic stirrer (Heidolph, MR3001, K, Germany) for 24 hrs at 1,000 rpm. All extracted samples were centrifuged for 10 mins at 1580 rcf. The supernatant was collected and stored at -20°C.

Determination of total phenolic content (TPC)

The determination of total phenolic content analysis was conducted according to the method by[11] with slight modification. 200 µL of seaweed extract was mixed with 0.5 mL diluted FC (Merck, Germany) reagent (1 mL FC reagent: 10 mL distilled water) and the mixture was left for 5 mins. Then, 0.4 mL of 7.5% (w/v) sodium carbonate (Sigma, Germany) was added to the mixture and the solutions were incubated in room temperature for 2 hrs. After that, the absorbance was taken at 765 nm wavelength using spectrophotometer (BMG Labtech, Germany). Standard calibration curve of gallic acid (Sigma, Germany) was set up to estimate the activity capacity of samples. TPC was expressed in terms of mg gallic acid equivalents per 100 g dried weight (DW) sample (mg GAE/100 g of DW sample).

Determination of the free radical scavenging activity

Scavenging activity determination of the seaweed extracts on the stable free radical DPPH were done as described by[11] with slight modification. The DPPH stock solution was prepared by dissolving 40 mg DPPH (Sigma, Germany) in 100 mL methanol (Merck, Germany). The DPPH working solution was freshly prepared by diluting the stock solution with methanol until the absorbance reached 0.7 ± 0.01. Then, 300 uL extract or blank were mixed with 1 mL DPPH working solution and the mixture were incubated for 24 hrs at room

temperature. Absorbance of the mixture was measured using spectrophotometer (BMG Labtech, Germany) at 517 nm wavelength. The scavenging activity was determined using the following equation:

$$\text{DPPH scavenging activity} = \left[\frac{\text{Absorbance}_{\text{blank}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{blank}}}\right] \times 100$$

Determination of ferric reducing antioxidant power (FRAP)

The antioxidant capacity of each extract was measured according to procedure by[11] with some modification. FRAP reagent consists of 300 mM acetate buffer pH 3.6, 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) (Sigma Aldrich, USA) dissolved in 40 mM HCl (Merck, Germany), and 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$) (Merck, Germany). All chemical were mixed in the ratio of 10: 1: 1 (300 mM acetate buffer: 10 mM TPTZ: 20 mM $FeCl_3.6H_2O$). After that, 1 mL FRAP reagent was mixed with 200 µL seaweed extract or standards solutions. The mixture was incubated for 30 mins at room temperature. Then, the absorbance of the mixture was measured using spectrophotometer (BMG Labtech, Germany) at 595 nm. Calibration curve of trolox (Sigma, Germany) was set up to estimate the antioxidant activity of samples. The result was interpreted in terms of micromol trolox equivalents per 100 g of dried weight (DW) sample (µmol TE/100 g of DW sample).

Determination of trolox equivalent antioxidant capacity (TEAC)

Antioxidant activities of seaweed extract was determined using TEAC method as described by[6] with slight modification. The stock solution was prepared by mixing the 7 mM 2,2’-Azino-bis(3-ethylbenzolthiazoline-6-sulfonic acid) diammonium salt (Sigma, Germany) with 2.45 mM potassium persulphate (J. Kollin, UK) in the ratio of 1:1. Next, the stock solution was incubated for 16 hrs at room temperature. The working solution was prepared by diluting stock solution with methanol (Merck, Germany) until the absorbance reached approximately 0.7 ± 0.01 at 734 nm. An aliquot (200 µL) of extracts or standard solutions were mixed with 1 mL of working solution and the mixture were incubated for 1 hr at room temperature. The absorbance of the solution was measured using spectrophotometer (BMG Labtech, Germany). The calibration curve of trolox (Sigma, Germany) was plotted. The result was interpreted in terms of micromol trolox equivalents per 100 g of dried weight (DW) sample (µmol TE/100 g of DW sample).

Determination of oxygen radical absorbance capacity

The determination of antioxidant capacity in seaweed was determined by using ORAC method as described by[12] with slight adjustment. 150 µL of fluorescein solution (Sigma Aldrich, USA).was pipetted in the black plate. Then, 25 µL trolox (Sigma, Germany), sample or blank was added, and the plate was incubated for 30 min at 37 °C in the microplate reader (POLARstar Omega, BMG Labtech, Germany). After incubation, fluorescence measurements were taken every 90 sec at the emission 520 nm and excitation 485 nm. At the fourth cycles, 25 µL of 240 mM 2,2’-azo-bis(2-amidinopropane) dihydrochloride (AAPH) (Sigma, Germany) was injected using the onboard injector. The test was resumed and fluorescence intensity was recorded up to 120 min. The calibration curve of trolox were plotted and the result was interpreted in terms of micromol trolox equivalents per 100 g of dried weight (DW) sample (µmol TE/100 g of DW sample).

Statistical analysis

All data were analysed statistically using Statistical Package for Social Science (SPSS) version 20 (IBM, California, USA) using independent T test and Pearson’s correlation coefficient test. A significant difference was assumed at the level of $p<0.05$.

Results and Discussion

The effect of rehydrated procedure to the total phenolic content (TPC) of *K. alvarezii* from Langkawi, Kedah and Semporna, Sabah was shown in Table 1. As indicated in Table 1, comparing between two locations of Semporna and Langkawi, the TPC in rehydrated *K. alvarezii* was significantly ($p<0.05$) higher compared to dried *K. alvarezii* for both location. Our result is similar with[13] who reported significant increase in total phenolic contents when dry common beans and pinto beans were soaked in cold water.

Table 1 Antioxidant activities of dried and rehydrated *Kappaphycus alvarezii* that were obtained from Langkawi, Kedah and Semporna, Sabah

Antioxidative activities	Isolation Location of *Kappaphycus alvarezii*	
	Semporna, Sabah	**Langkawi, Kedah**
TPC (mg GAE/100 g of DW sample)		
Dried seaweed	41.4 ± 0.7^{bA}	33.8 ± 2.3^{bB}
Rehydrated seaweed	89.2 ± 4.8^{aB}	119.3 ± 10.4^{aA}
DPPH (%)		
Dried seaweed	31.7 ± 0.3^{bA}	17.2 ± 0.2^{bB}
Rehydrated seaweed	34.6 ± 1.0^{aA}	27.5 ± 3.1^{aB}
FRAP (µmol TE/100 g of DW sample)		
Dried seaweed	13.4 ± 0.7^{bA}	9.4 ± 1.9^{bB}
Rehydrated seaweed	22.5 ± 0.1^{aA}	17.7 ± 2.0^{aA}
TEAC (µmol TE/100 g of DW of sample)		
Dried seaweed	90.4 ± 12.0^{bA}	112.2 ± 15.0^{bA}
Rehydrated seaweed	138.4 ± 4.1^{aA}	130.9 ± 7.0^{aA}
ORAC (µmol TE/100 g of DW sample)		
Dried seaweed	31.4 ± 1.1^{bB}	36.4 ± 6.9^{bA}
Rehydrated seaweed	53.8 ± 7.3^{aB}	73.0 ± 18.2^{aA}

DW: Dried weight; ± Standard deviation

$^{A-B}$ Different capital letters at the same row shows significant differences ($p<0.05$) between location of the seaweeds were taken.

$^{a-b}$ Different small letters at the same column shows significant differences ($p<0.05$) between dried seaweed and rehydrated seaweed.

In the present study, the TPC of rehydrated seaweed from Langkawi (119.3 mg GAE/100 g of DW sample) was significantly ($p<0.05$) higher compared to rehydrated seaweed from Semporna (89.2 mg GAE/100 g of DW sample). The result obtained maybe was related to the environmental factor where *K. alvarezii* was harvested. The total phenolic compound can be affected by many factors such as season and agroclimatic conditions[14]. This finding was also supported by [15] who reported *Sargassum muticum* collected from different countries had a significant effect on the TPC value. No report has been obtained for *K. alvarezii* however the similar reason reported by [15] may explain in our result.

In Table 1 the DPPH scavenging percentages of rehydrated seaweeds (Semporna's seaweed; 34.6% and Langkawi's seaweed; 27.5%) are significantly ($P<0.05$) higher compared to dried seaweed for both locations (Semporna's seaweed; 31.7% and Langkawi's seaweed; 17.2%). Our finding was consistent with [16]. Though this study was not for *K. alvarezii* but [16] found that the radical scavenging activity of rehydrated *Himanthalia elongata* was higher than dried sample. Comparing between rehydrated seaweed from location, DPPH scavenging percentages of seaweed from Semporna (34.6%) was significantly ($p<0.05$) higher compared to seaweed from Langkawi (27.5%). [15] reported that the different of value DPPH radical scavenging activity of *Sargassum muticum* may due to this algae was collected from distantly geographical countries. This may explain why the different value DPPH radical scavenging activity between rehydrated *K. alvarezii* from Semporna, Sabah and Langkawi, Kedah.

For FRAP assay, rehydrated seaweed (Semporna; 22.5 µmol TE/100 g of DW sample and Langkawi; 17.7 µmol TE/100 g of DW sample) were significantly ($p<0.05$) higher than dried seaweed (Semporna; 13.4 µmol TE/100 g of DW sample and Langkawi; 9.4 µmol TE/100 g of DW sample) for both location. This result was similar with [17] who reported, the FRAP value of soaked beans were remained at 80% or higher compared to the raw beans. Similar assumption may explain the FRAP value in this study. There is no significant ($p>0.05$) difference between rehydrated sample from Semporna and Langkawi for FRAP.

In TEAC analysis, as presented in Table 1, the rehydrated seaweed (Semporna's seaweed: 138.4 µmol TE/100 g of DW sample and Langkawi's seaweed: 130.9 µmol TE/100 of g DW sample) significantly ($p<0.05$) has higher TEAC value compared to dried seaweed (Semporna's seaweed: 90.4 µmol TE/100 g of DW sample and Langkawi's seaweed: 112.3 TE/100 g of DW sample). There is no significant ($p>0.05$) difference between rehydrated sample from Semporna and Langkawi for TEAC assay.

ORAC analysis value for rehydrated seaweeds (Semporna; 53.8 µmol TE/100 g of DW sample and Langkawi; 73 µmol TE/100 g of DW sample) were significantly ($p<0.05$) higher compared to dried seaweed (Semporna; 31.4 µmol TE/100 g of DW sample and Langkawi; 36.4 µmol TE/100 g of DW sample) for both location. Similar observation was repoted by [18]. In their study they reported the ORAC value was significantly higher in rehydrated sea cucumber than in the fresh one with the internal organs included. Though the sample was not similar, this reason may also explain the value of ORAC in *K. alvarezii.* The ORAC analysis involves a hydrogen atom transfer (HAT) to peroxyl radical which both, [18] and result in this finding may have similar mechanism.

Pearson correlation coefficient between antioxidant assays of dried and rehydrated seaweed were shown in Table 2. As indicated in Table 2, there was strong significant correlation between TPC with FRAP ($r = 0.728$, $p<0.01$), TEAC ($r = 0.755$, $p<0.01$), and ORAC ($r = 0.930$, $p<0.01$) value. Previous studies also showed that there was positive correlation between TPC with FRAP[19-21], TPC with TEAC[22] and TPC with ORAC[23] value of different plant extracts. This result indicated there is a relation between phenolic concentrations with FRAP, ABTS radical scavenging (TEAC), and peroxyl radical scavenging (ORAC) in seaweed extracts.

Table 2 Pearson's correlation coefficients between antioxidant assays of dried and rehydrated *Kappaphycus alvarezii* from Langkawi, Kedah and Semporna, Sabah.

Correlation coefficient (r)	TPC	DPPH	FRAP	TEAC	ORAC
TPC	1	0.433	0.728*	0.755*	0.930*
DPPH		1	0.751*	0.205	0.154
FRAP			1	0.650	0.515
TEAC				1	0.683
ORAC					1

* Correlation is significant at $p <0.01$ level

The example of phenolic compound that are abundant in algae is phlorotannins[24], phenolic acid and flavonoid compound[25] which also were contribute to antioxidant activity of seaweed extract[26] (Jimienez et al. 2001). While, the phenolic content and radical scavenging activities in DPPH analysis did not show any significant correlation (Table 2). This result was consistent with[27] who reported that there was no significant correlation between TPC and DPPH of brown seaweed extract. This indicated that the seaweed extract contained other compound which could involve in antioxidant activity.

A significant ($p<0.01$) strong positive correlation was also found between DPPH with FRAP assays ($r = 0.751$). The studied[28] reported similar significant correlation between DPPH with FRAP assay, in methanol extract of guava fruit. The correlation between DPPH and FRAP assay might be due to similar reaction of mechanism which is single electron transfer mechanism[29].

Conclusion

In conclusion, rehydrated seaweed possesses higher antioxidant activity compared to dried seaweed for both locations (Semporna Sabah and Langkawi, Kedah). The antioxidant activity in *K. alvarezii* was different according to location, except for FRAP and TEAC assay. Strong positive correlations between phenolic content and FRAP, TEAC, and ORAC value showed that, phenolic compounds were the contributors of antioxidant activity in *K. alvarezii.*

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