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ANTIOXIDANT ACTIVITY OF *Padina minor* YAMADA

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ABSTRACT

A brown alga, *Padina minor* Yamada (division Phaeophyta), collected from Naiyang beach, Phuket province, Thailand was examined for antioxidant properties. The aqueous extract of *P. minor* showed radical scavenging activity of 864.71 ± 19.75 mg gallic acid equivalent (GAE) and 0.567 ± 0.004 µM Trolox equivalent (TEAC) when tested by the DPPH (1-diphenyl-2-picrylhydrazyl) and $ABTS^{\bullet+}$ (2,2'-azino-bis 3-ethylbenzthiazoline -6-sulfonic acid cation) assays, respectively. Additionally, the extract inhibited lipid peroxidation of rat liver (0.052 ± 0.001 mM TEAC). Total phenolic content of the extract was found to be 217.40 ± 11.28 mg GAE. The antioxidant activities of *P. minor* indicate its potential role as nutraceutical and cosmeceutical products.

KEYWORDS: *Padina minor*, DPPH, $ABTS^{\bullet+}$, lipid peroxidation, phenolic content, anti-oxidant activity

1. INTRODUCTION

Marine algae have long been used as food and medicine in Asian countries such as Japan, China, and Korea. Most of the brown marine algae intake in the daily diet is of *Undaria* (wakame) and *Laminaria* (kombu) species. *Undaria* and *Laminaria* species are recommended for treating cancer in Chinese and Ayuverdic medicinal texts [1-2]. Consumption of the brown marine algae is thought to ameliorate some inflammatory disorders, breast cancer and high cholesterol level [3].

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Antioxidant activity has been proposed to play roles in various pharmacological activities such as anti-aging, anti-inflammatory, anti-atherosclerosis, anti-cancer activities [4-5]. Inhibition of free radical induced damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of diseases [6]. Among the activities claimed to be present in most of the nutraceuticals and cosmeceuticals is the antioxidant activity. Several synthetic antioxidants are available, but are quite unsafe and their toxicity is of concern [7]. Natural products with antioxidant activity may be used for human consumption because of their safety. Interestingly, some brown algae such as *Sargassum silliquastrum* and *Sargassum* spp. have been found to exhibit antioxidant activity [8–9]. The present study was undertaken to evaluate *Padina minor* Yamada, a brown marine alga abundant in the southern part of Thailand, for antioxidant activity**.** The alga has never been examined for medicinal properties including antioxidant activity.

2. MATERIALS AND METHODS

2.1 Preparation of aqueous extract of *Padina minor* Yamada

Fresh brown marine algae collected from Naiyang beach, Phuket province, Thailand were identified and authenticated as *P. minor* Yamada.

The algae were washed thoroughly in tap water and dried in an oven at 50 – 60 °C for 48 h. The dried algae (200 g) were heated with 1 L of distilled water at 50 °C for 24 h and then filtered through 4 layers of gauze. The filtrate was evaporated and lyophilized by means of a rotary evaporator at 70°C and a freeze dryer respectively, to obtain a dry extract. The yield was 20.1%. The extract was dissolved in distilled water at various concentrations before used.

2.2 DPPH (1-diphenyl-2-picrylhydrazyl) radical-scavenging assay

The aqueous extract of *P. minor* was assayed for DPPH radical-scavenging activity (DPPH assay) according to the method of Hou *et al.* [10]. Briefly, each 0.3 ml of the solution of the extract was added to 0.1 ml buffer (pH 7.9), and then mixed with 0.6 ml of 100 mM DPPH in methanol for 20 min, under light protection. The concentrations of the extract were in the range of 1–40 mg/ml. The absorbance at 517 nm was determined. Deionized water was used as blank. The DPPH radical-scavenging activity was calculated according to the following equation:

Scavenging activity (%) = 100 x (A517blank- A517sample)/ A517blank
where A517 was the absorbance at 517nm.

Gallic acid was used as a standard. The antioxidant activity of samples was expressed as gallic acid equivalent (GAE) which represented the concentration (mg) of the extract per 1 mg of gallic acid. All determinations were carried out in triplicate.

The EC50 which stands for the concentration required for 50% scavenging activity was calculated from dose-response curve.

2.3 ABTS (2,2'-azino-bis 3-ethylbenzthiazoline -6-sulfonic) radical cation decolorization assay

The scavenging activity of $ABTS^{\bullet+}$ was measured ($ABTS^{\bullet+}$ assay) according to the method described by Re *et al.* [11] with some modifications. The ABTS reagent was prepared by mixing 5 ml of 7 mM ABTS with 88 µl of 140 mM $K_2S_2O_8$. After the mixture was kept in the dark at room temperature for 16 h to allow the completion of radical generation, it was diluted with 95% ethanol. To determine the scavenging activity, 1 ml ABTS reagent was mixed with 10 µl of sample or negative control (methanol) and the absorbance was measured at 734 nm 6 min after the initial mixing, using ethanol as blank. The concentrations of the extract were in the range of 2-50 mg/ml. The inhibition percentage of the sample was calculated by the following equation:

Scavenging activity (%) = {1– (A734 sample / A734 control)} x 100%
where A734 was the absorbance at 734 nm.

Trolox, a derivative of vitamin E, was used as a standard. The antioxidant activity of sample was expressed as Trolox equivalent antioxidant capacity (TEAC) which represented the concentration (µM) of Trolox, having the same activity as 1 mg of sample. All determinations were carried out in triplicate. The EC50 was calculated from dose-response curve.

2.4 Inhibition of lipid peroxidation

Lipid peroxide formation was measured (lipid peroxidation assay) by the method of Masao *et al*. [12]. Male Sprague-Dawley rats (weighing 200 - 250 g) purchased from the National Laboratory Animal Center (Salaya Mahidol University, Thailand) were used in the study. The rat was sacrificed by dislocation of the neck. The abdomen was opened, the liver was removed and then homogenized in 150 mM Tris–HCl buffer (pH 7.2). The reaction mixture was composed of 0.2 ml of rat liver homogenate in phosphate buffer, 30 mM KCl, 0.5 mM ferrous iron, 0.06 mM ascorbic acid, and various concentrations of the samples in a final volume of 1.0 ml. The concentrations of the extract were in the range of 0.3-25 mg /ml. After the mixture was incubated at 37 °C for 1 h. 0.4 ml of the reaction mixture was treated with 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 0.2 ml of 0.8% Thiobarbituric acid (TBA), 1.5 ml of 20% acetic acid and 0.4 ml of distilled water. Then, the mixture was incubated in a water bath at 100 °C for 1 h. After the mixture was cooled to 25 °C, 1 ml of distilled water and 5 ml of n -butanol were added, followed by vigorous shaking for 1 min. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured. The inhibition of lipid peroxide formation was calculated by the following equation:

Inhibition (%) = 100 x (A532control- A532sample)/ 532control
where A517 was the absorbance at 532 nm

Inhibition of lipid peroxidation was expressed as Trolox equivalent antioxidant capacity (TEAC). All determinations were carried out in triplicate. The EC50 was calculated from dose-response curve.

2.5 Determination of total phenolic content

Total phenolic content was determined according to the method of Hammerschmidt and Prat [13]. Briefly, 0.2 ml of the sample solution was mixed with 1.0 ml of 10% Folin –Ciocalteu solution and 0.8 ml of 7.5% sodium carbonate solution. The mixture was incubated for 1 h at room temperature. The absorbance at 760 nm was measured and converted to phenolic contents according to the calibration curve of gallic acid.

3. RESULTS AND DISCUSSION

3.1 The marine brown algae: *Padina minor* Yamada (Division Phaeophyta)

The algae was greenish brown to yellowish brown in color, and was found on rock fragments or pebbles in intertidal areas. It appeared as small thrall us, fan-shaped with 3-6 cm high. The blade was thin and composed of two layers.

3.2 DPPH assay

The assay is the most widely used method for screening antioxidant activity, since it can accommodate many samples in a short period and detect active ingredients at low concentration [14–15]. The decrease in absorbance of the DPPH radical caused by antioxidant was due to the scavenging of the radical by hydrogen donation. It is visually noticeable as a color change from purple to yellow. Assay precision (coefficient of variation, CV) of this method was in the range of 1-3%. The aqueous extract of *P. minor* and gallic acid showed DPPH radical scavenging activity in a concentration–dependent manner (Figure 1), with the correlation coefficient values (r) of 0.9987 and 0.9964, respectively. The aqueous extract of *P. minor* exhibited maximum scavenging activity of 77% whereas that of gallic acid was 88%. The EC50 values and GAE are shown in Table 1.

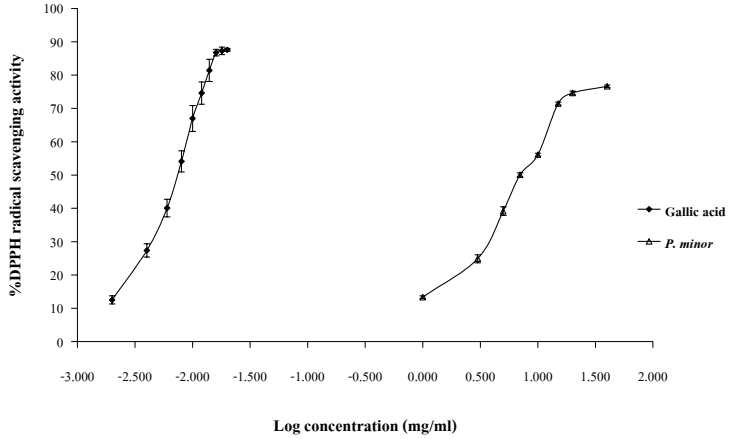


Figure 1 Concentration - response (scavenging activity of DPPH radicals) curves of the aqueous extract of *P. minor* and gallic acid

3.3 $ABTS^{\bullet+}$ assay

The assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants (scavengers of aqueous phase radicals) and of chain breaking antioxidants (scavenger of lipid peroxyl radicals) [16]. The CV of this assay method was in the range of 1-4%. The scavenging activity of the aqueous extract of *P. minor* and Trolox against $ABTS^{\bullet+}$ radicals are shown in Figure 2. A progressive response was observed when higher concentrations were used. The correlation (r) values of the aqueous extract of *P. minor* and Trolox were 0.9992 and 0.9989, respectively. The extract expressed the maximum scavenging activity of 98% which was approximately equal to that of Trolox (99%). The EC50 values and TEAC are shown in Table 1. Trolox was more effective than the aqueous extract of *P. minor*.

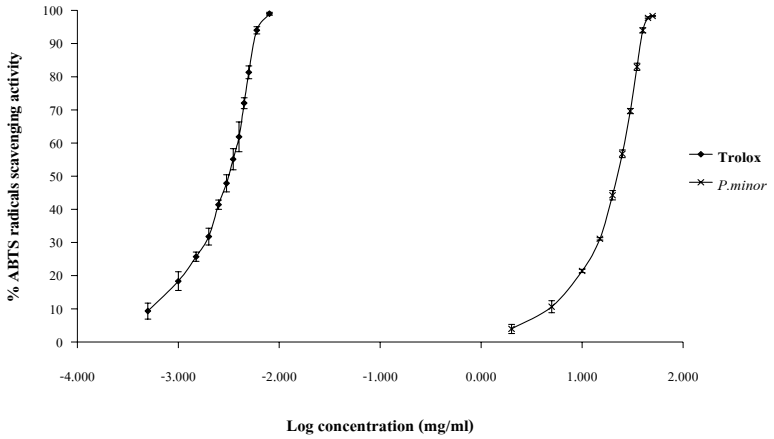


Figure 2 Concentration - response (scavenging activity of $ABTS^{\bullet+}$ radicals) curves of the aqueous extract of *P. minor* and Trolox

3.4 Lipid peroxidation assay

Lipid peroxidation is very important process in free radical pathology as it is so damaging to cells. The liver of rat was used as a source of polyunsaturated fatty acids for determining the extent of lipid peroxidation. Malondialdehyde, a lipid peroxidation product, is an indicator of reactive oxygen species (ROS) generation in the tissue [17]. The CV of this method was in the range of 2-4%. The inhibition of lipid peroxide formation of the aqueous extract of *P. minor* and Trolox are shown in Figure 3. The extract and Trolox illustrated concentration-dependent inhibition. The correlation (r) values of the extract and Trolox were 0.9989 and 0.9952, respectively. The maximal inhibition of the aqueous extract of *P. minor* was 93% whereas that of Trolox was 91%. The EC50 values and TEAC are shown in Table 1. Trolox was more effective than the aqueous extract of *P. minor*.

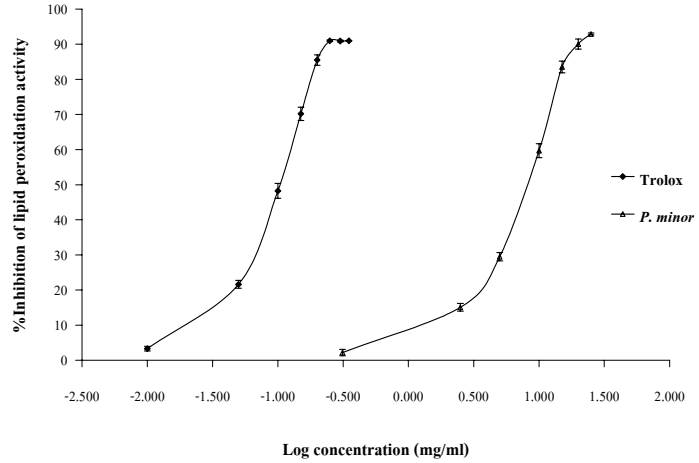


Figure 3 Concentration - response (inhibition of lipid peroxidation) curves of the aqueous extract of *P. minor* and Trolox

3.5 Phenolic content

The phenolic substances are known to possess the ability to reduce oxidative damage and act as antioxidants [18]. They can trap the free radicals directly or scavenge them through a series of coupled reactions with antioxidant enzymes [19]. In addition, it was reported that phenolic substances were associated with antioxidant activity and played important role in stabilizing lipid peroxidation [20]. It was found that total phenolic content of the aqueous extract of *P. minor* extract was 217.40 ± 11.28 g GAE.

Table 1 Comparison of the scavenging activity of the aqueous extract of *P. minor* and standard drugs in DPPH, $ABTS^{\bullet+}$ and lipid peroxidation assays

Assay	*P. minor*	Gallic acid or Trolox
DPPH		
EC50 (mg/ml)	6.92 ± 0.16	864.71 ± 19.75 (Gallic acid)
GAE	0.008 ± 0.56	
$ABTS^{\bullet+}$		
EC50 (mg/ml)	21.86 ± 0.17	0.003 ± 0.0001 (Trolox)
TEAC	0.567 ± 0.004 uM	
Lipid peroxidation		
EC50 (mg/ml)	8.71 ± 0.23	0.11 ± 0.002 (Trolox)
TEAC	0.052 ± 0.001 mM	

Data expressed as mean ± S.D. of triplicate measurements.
GAE expressed as mg sample per mg of gallic acid.
TEAC, expressed as µM Trolox per mg of sample.
Each value is the mean ± SD of triplicate measurements.

4. CONCLUSIONS

The results of the three assays: DPPH, $ABTS^{\bullet+}$, and lipid peroxidation of rat liver are in agreement in that the aqueous extract of *P. minor* showed an ability to reduce free radicals or oxidative damage. Interestingly, although the concentrations of the extract of *P. minor* for scavenging activity were much higher than that of Trolox and gallic acid when tested in DPPH, $ABTS^{\bullet+}$ and lipid peroxidation assays, their maximal effects (efficacy) were approximately the same. However, Trolox is a synthetic substance whereas *P. minor* from a natural source, is abundant in the southern part of Thailand. The antioxidant activity of the aqueous extract of *P. minor* is likely to be due to its phenolic contents. Further studies are needed to provide evidence to support the development of *P. minor* as a anutraceutical and cosmeceutical product. The studies for antioxidant activity include scavenging activity for hydroxyl and superoxide radicals, reducing power and metal chelating activity. For use as a cosmeceutical agent, test for anti-tyrosinase activity, and test for skin irritation should be carried out. Toxicity testing should be performed in order to show its safety.

5. ACKNOWLEDGEMENTS

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