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Total phenolics, flavonoids and antioxidant activity of macroalgae fermented with lactic acid bacteria

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ABSTRACT

α,α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging activity, antioxidant capacity, total phenolic and flavonoids contents of lactic acid bacteria fermented-fresh water macroalgae extracts were studied. Three treatments of macroalgae; 1) *Spirogyra* spp. Ubon Ratchathani Province, 2) Khai Ubon Ratchathani Province *(Cladophora* spp. and *Microspora* spp.) and 3) Khai Nan Province (*Cladophora* spp. and *Microspora* spp.) were studied. The fermented macroalgae were extracted with ethanol. The percentage of DPPH free radical scavenging activity of the ethanolic extracts of the lactic acid bacteria fermented macroalgae of the treatment 1, 2 and 3 were 84.77, 0.67 and 77.06, respectively. In addition, the antioxidant capacities (10 g/L) were 63.45, 36.50 and 31.75 mg AE/g of extract as ascorbic acid equivalent, respectively. Total phenolic contents of the treatment 1, 2 and 3 were 29.54±0.93, 37.23±1.92 and 2.55±1.06 mg/g as gallic acid equivalent of extract, respectively. The total flavonoid contents of the treatment 1, 2 and 3 were 21.48, 11.55 and 13.48 mg/g as quercetin equivalent of extract, respectively. Extracts of *Spirogyra* sp. fermented with lactic acid bacteria (treatment 1) show the highest flavonoid contents, antioxidant capacity and DPPH free radical scavenging activity.

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INTRODUCTION

Spirogyra spp. or Thao in Thai common name are the macroalgae in the Division of Chlorophyta. They are found in a wide range of habitats, including small stagnant water bodies, ditches as well as the littorals of lakes and streams. Distribution of *Spirogyra* sp. in northern and northeastern of Thailand is cosmopolitan with reaching abundance during hot dry and before entering rainy season. *Cladophora* spp. and *Microspora* spp. are also the macroalgae in Division Chlorophyta and the Thai common name is "Khai". Their identity characters are the light rough. The different characteristic of two species of Khai is the branching filament. It was found that *Cladophora* spp. have the branching filament but *Microspora* spp. do not produce any branching filament. The extract of *Spirogyra neglecta* can inhibit gastric ulcer formation induced by physical and chemical stresses in rats. It also showed hypolipidemic and hypoglycemic abilities in type 2 diabetic rats induced by streptozotocin and high fat diet (Lailerd *et al.,* 2009). *Spirogyra neglecta* (Hassall) Kützing from northern of Thailand produced antioxidant activity in the liver of rat. (Thumvijit *et al.,* 2013).

It has been reported that antioxidant property of lactic acid bacteria is essential for their optimum growth (Konings *et al*., 1997; Mistry *et al*., 1987) and this could be useful not only for several food manufacturing industries as the dietary source of antioxidants, but also for providing probiotic bacteria with the potential of producing antioxidants in the intestinal tract (Phadtare, 2004; Saide and Gillil, 2005). Interestingly, some species of *Lactobacillus* (*Lb.*) and *Streptococcus* (*Strep*.) also produce antioxidative activity (Carroll, *et al*., 2007; Peran, *et al*., 2006).

In Japan, several algae are commonly used as foods such as marine algae extract can be used as a healthy food supplement to enhance efficiency of the digestion system. In addition, some kinds of algae were extracted and used in the cosmetics like moisturizer, anti-aging and anti-wrinkle products. As mentioned above, Thailand is a great source of macroalgae and some species have been consumed in local family as well as submitted to produce local products like snacks for sale. To obtain the value added macroalgae products and to study the biological activity of macroalgae harvested from Ubon Ratchathani, the aims of this research were: 1) to study the local macroalgae from natural water resources and terrestrial ecosystem in Ubon Ratchathani, Thailand; 2) to evaluate the chemical contents, total phenolic and flavonoids contents; and 3) to examine the anti-oxidant capacity and DPPH radical scavenging activity. The results of this study could be supported the use of macroalgae in anti-aging and anti-wrinkle cosmetics.

MATERIALS AND METHODS

Chemicals and reagents

1,1-diphenyl-2-picryl hydrazyl (DPPH), folin–ciocaleteau reagent, gallic acid and quercetin were obtained from Sigma Chemical Co. (St. Louis, MO). Methanol, ethanol, ethyl acetate, hexane, aluminium chloride, potassium acetate, ammonium molybdate, sodium phosphate and sodium carbonate were obtained from BDH, Poole, England. Visible spectra measurements were done using Genesys-5 visible spectrometer.

Materials

Algae (treatment 1: Spirogyra spp., treatment 2: Cladophora spp. and Microspora spp. and treatment 3: Cladophora spp. and Microspora spp.) (Figure 1, 2 and 3) were studied. Treatment 1 and 2 were harvested from Ubon Ratchathani Province, Thailand and treatment 3 was harvested from Nan River in Nan Province, Thailand. All treatments were fermented with lactic acid bacteria. The soil and other impurities were removed manually, cleaned by a tap water and dried using hot air oven at 60°C until dried. The yield of each dried material obtained was 1,000 grams and stored at 4°C until further use.

Extraction

The samples of algae were macerated with 1,000 mL of ethanol at a room temperate without the sunlight for 3 days. The extracts were then filtered, evaporated and concentrated to dryness by rotary evaporator. The algae extracts were extracted for 3 times in the same experiments. The resultant samples were stored at 4°C until further use.

Determination of total phenolic content

The concentration of total phenolic compounds in each extract was determined as previously described by Kumaran and Karunakaran (2006) and the results were expressed as gallic acid equivalents. The extracts (1 g) were dissolved in ethanol and the different concentrations (10, 25, 50, 100, 150, 250 and 500 mg/L) of gallic acid standard solution were prepared. The 0.5 mL of extract and standard solution were taken into a vial (contained the 5-mL distilled water). The 0.5 mL of Folin–Ciocalteu reagent and 2 mL of 10% sodium carbonate solution were added to all the vials, mixed the mixture well by a vortex mixer. After 10 min of incubation at the room temperature, the absorbance was measured at 730 nm using a spectrometer. The ethanol was used as blank solution for the experiments. The estimation of total phenolic contents in all the extracts was carried out in triplicate and the mean results were represented as mean.

Determination of total flavonoid contents

Total flavonoid contents (TFC) of the algae extracts were determined by using the aluminium chloride colorimetric method. The algae extracts (0.5 mL), 10% aluminium chloride (0.1 mL), 1 M potassium acetate (0.1 mL) and distilled water (4.3 mL) were mixed after incubation at room temperature for 30 min. The absorbance was measured at 415 nm using a spectrometer. In this experiment, quercetin was used to make the calibration curve. The estimation of total flavonoids was carried out in triplicate and the results were represented as mean.

Radical scavenging activity using DPPH method

The extracts (0.1 g) were dissolved in a 2 mL of ethanol. The 0.1mL of samples were added into the 15 mL vial contained the 2 mL of methanolic solution of DPPH (1.0 mM) and shaken vigorously. All of the vials were kept into a dark condition at a room temperature for 4 min (Jung et al., 2006). A control was prepared as described above without samples or standards. Ethanol was used for the baseline correction. The changes in the absorbance of the all the samples and standards were measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage (Kumaran and Karunakaran, 2006) and was calculated using the following formula, % radical scavenging = $[(A_0-A_1) \times 100/ A_0]$, A_0 as Abs (control), A_1 as Abs (sample)

Evaluation of antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of the ethanolic extracts of algae was evaluated. An aliquot of 0.1 mL of sample solution (100 µg/mL) was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 90°C for 60 min. After the samples had cooled down to a room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against blank. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as the rest of the sample. For samples of unknown composition, water soluble antioxidant capacity was expressed as equivalents of ascorbic acid (mg/g of extract).

RESULTS AND DISCUSSION

The yields of ethanolic algae extracts of the treatment 1, 2 and 3 were 40.544, 45.736 and 45.880%, respectively.

Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups. The phenolic contents may contribute directly to the antioxidative action (Duh, *et al.*, 1999). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans (Tanaka, Kuei, Nagashima, & Taguchi, 1998). The total phenolic contents (TPC) of the extracts determined by Folin–Ciocalteu method were reported as gallic acid equivalents (Table 1). TPC of the ethanolic extracts of the algae treatment 1, 2 and 3 were 29.54, 37.23 and 2.55 mg GAE/ g of extract, respectively. The phenolic compounds are the dominant antioxidants that exhibit scavenging efficiency on free radicals and reactive oxygen species are numerous and widely distributed in the plant kingdom (Prior & Cao, 2000).

In this present study, the relative antioxidant abilities of algal extracts were investigated by some *in vitro* models such as antioxidant capacity by Phosphomolybdenum method and radical scavenging activity using DPPH method and the results were given in Table 1.

The total flavonoids contents found in the treatment 1, 2 and 3 were 21.48, 11.55 and 13.48 mg quercetin equivalent (QE)/g of extract, respectively. These variations may be due to environmental conditions affecting the components of the algae.

The antioxidant capacity of the extracts was measured spectrometrically through phosphomolybdenum method based on the reduction of Mo (IV) to Mo (V) by the sample analyze and the subsequent formation of green phosphate/Mo (V) compounds with a maximum absorption at 695 nm. The antioxidant capacities of the ethanolic extracts of the algae treatment 1, 2 and 3 were 63.45, 36.50 and 31.75 mg/g of extract as ascorbic acid equivalent at the level of 10 mg/ml, respectively.

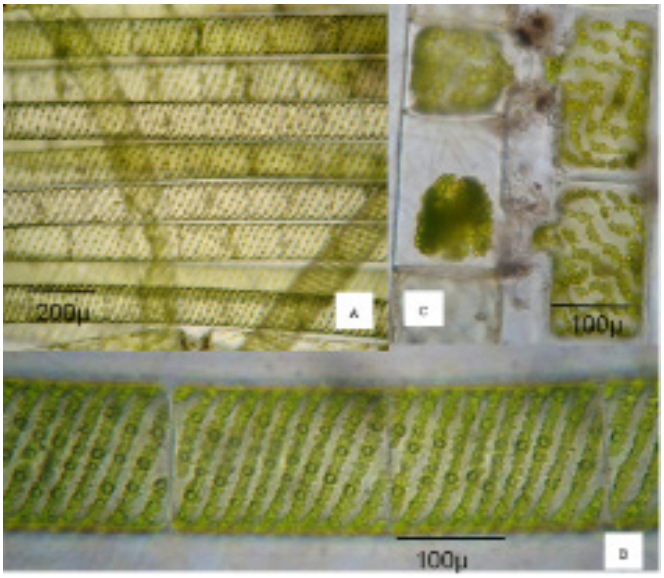

Figure 1 Treatment 1 A) *Spirogyra* spp. B) The spiral arrangement of *Spirogyra* sp. chloroplast and C) Conjugation reproduction of *Spirogyra* sp. Bar presented in the figure is 100 µm

Table 1 TPC, TFC, total antioxidant capacity and DPPH free radical scavenging activity of algal extracts

Algae extract	TPC (mgGAE/g)	TFC (mgQE/g)	antioxidant capacity (mg/g)	DPPH scavenging activity (%)
Treatment 1	29.54	21.48	63.45	84.77
Treatment 2	37.23	11.55	36.50	0.67
Treatment 3	2.55	13.48	31.75	77.06

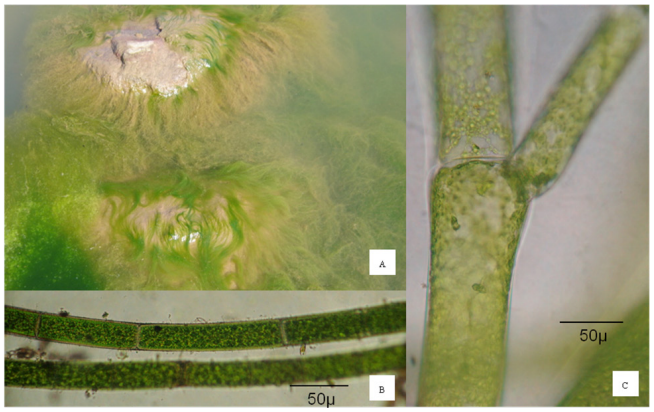

Figure 2 Treatment 2 A) the natural growth of *Cladophora* spp. and *Microspora* spp. in Mekong river, Ubon Ratchathani, Thailand B) *Microspora* spp. and C) *Cladophora* spp. Bar presented in the figure is 50 µm.

DPPH is a stable free radical that can accept electron or hydrogen radical to become a stable diamagnetic molecule (Soares, *et al.*, 1997). A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. This

Figure 3 Treatment 3 A) Dried *Microspora* spp. and *Cladophora* spp. obtained from Nan river, Nan, Thailand B) the structure of *Microspora* sp.

the medium. Thus, antioxidant molecules can quench DPPH (by providing hydrogen atom or by electron donation) and convert it to a colorless product, resulting in a decrease in absorbance at 517 nm (Yamaguchi, Takamura, Matoba, & Terao, 1998). In brief, the reduction capacity of DPPH was determined by the decrease in its absorbance at 517 nm, which is reduced by antioxidants (Duh et al., 1999). A significant decrease in the concentration of DPPH in this present study could be due to the scavenging ability of the extracts. The extracts caused an increase in free radical scavenging activity in a dose-dependent manner. Table 1 showed the test of DPPH scavenging activity assay. The ethanolic extracts showed high DPPH scavenging activities because ethanol, a polar solvent, can dissolve many active compounds including phenolic, flavonoid and terpenoid compounds, especially triterpine (glycoside form). The percentages of DPPH free radical scavenging activity of the ethanolic extracts of treatment 1, 2 and 3 were 84.77, 0.67 and 77.06, respectively.

CONCLUSION

It can be concluded that the extract of *Spirogyra* spp., *Microspora* spp. and *Cladophora* spp. fermented with lactic acid bacteria showed antioxidant property. In addition, it was found that *Spirogyra* spp., obtained from Ubon Ratchathani produced the highest total phenolic contents, flavonoid contents, antioxidant capacity and DPPH free radical scavenging activity when compared to other species of macroalgae. Thus, the results of this experiment provided the scientific data regarding the antioxidant activity of the algae extract that could be submitted to produce anti-aging cosmetics in the future.

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