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Total phenolics, flavonoids and antioxidant activity of fresh water macroalgae from Ubon Ratchathani, Thailand

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ABSTRACT

α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging activity, antioxidant capacity, total phenolics and flavonoids contents of the extracts of fresh water macroalgae were studied. Three types of macroalgal ; 1) *Spirogyra* sp., 2) *Cladophora glomerata* and *Microspora* sp. and 3) *Nostoc commune*, were harvested from Ubon Ratchathani, Thailand. The macroalgaes were extracted with 95% ethanol. The ethanolic extracts showed high the DPPH free radical scavenging activity and antioxidant capacity. The percentage of DPPH free radical scavenging activity of the ethanolic extracts of type 1 (*Spirogyra* sp.) was 52.33 while type 2 *(Cladophora glomerata* and *Microspora* sp.) and type 3 (*Nostoc commune*) were lower. The antioxidant capacities of ethanolic extract of type 1, 2 and 3 algae were 178.89, 150.67 and 173.28 mg AE/g of extract, respectively. Total phenolic contents of ethanolic extract of type 1, 2 and 3 algae were 155.82, 12.12 and 18.22 mg GAE/g of extract, respectively. The total flavonoid contents of ethanolic extract of type 1, 2 and 3 algae were 2.64, 3.83 and 0.52 mg QE/g of extract, respectively. The *Spirogyra* sp. extract showed the highest in total phenolic contents, total flavonoid contents, antioxidant capacity and DPPH free radical scavenging activity. They are appropriate for applying to produce anti-aging cosmetics.

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INTRODUCTION

Macroalgaes such as *Spirogyra* sp., *Cladophora* sp., *Microspora* sp. and *Nostoc commune*, are the most easily recognized green freshwater due to its spirally coiled chloroplasts. It is found in a wide range of habitats, including small stagnant water bodies, ditches as well as the littorals of lakes and streams. Distribution of macroalgaes in the Northern and Northeastern of Thailand is commonly found with high yield during hot and dry season unite early raining season. Because of its different geographical distributions, which may induce the emerging of variation at genetic level. Macroalgaes can respond to the environmental condition through the expression of different filament type group (morphotypes), cell length/width and number of chloroplast spirals which are related to physico-chemical parameters of water resource. By using morphological observation, light and SEM have been introduced to investigate the ultra morphology including morphotype characters as described before which can provide useful information and significant taxonomic evidence.

It has been reported that the extract of *Spirogya neglecta* can inhibit gastric ulcer formation induced by physical and chemical stresses in rats, showed hypolipidemic and hypoglycemic abilities. In addition, *Spirogyra neglecta* from Northern of Thailand produced antioxidant activity in the liver of rat (Thumvijit *et al.,* 2013). The species of *Lactobacillus* and *Streptococcus* also produce antioxidative activity (Carroll *et al.,* 2007; Peran *et al.,* 2006)

Phenolic compounds are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups. The phenolic and flavonoid 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 *et al.,* 1998).

In Japan, several algae are used as foods. The Japanese use marine algal extract as a healthy food supplement to enhance efficiency of the digestion system. In addition, the Japanese also use algal extracts for cosmetic purpose such as moisturizer, anti-aging and anti-winkle products. Thus this research will study local macroalgae in Northeastern of Thailand from natural water resources and terrestrial ecosystem in Ubon Ratchathani province. Chemical contents in alga were extracted by using ethanol. Total phenolic contents, total flavonoid contents, evaluation of antioxidant capacity by phosphomolybdenum method and DPPH radical scavenging activity using DPPH method were investigated for evaluation as to whether they were good candidates to be used in anti-aging and anti-winkle cosmetics.

MATERIALS AND METHODS

Chemicals and Instrument

α, α-diphenyl-β-picrylhydrazyl(DPPH), folin–ciocalteu reagent, gallic acid and quercetin were obtained from Sigma Chemical Co. (St. Louis, MO). Ethanol, 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 (type 1 *Spirogyra* sp., type 2 *Cladophora glomerata* and *Microspora* sp. and type 3 *Nostoc commune*), were harvested from Moon and Khong river in Ubonratchathani, Thailand. The soil and other impurities were removed manually. The samples were cleaned by tap water and then dried using hot air oven at 60°C until the moisture content was constant. The yield of each dried material was 1 kg and packed in plastic bag, stored at 4°C before further analysis.

Extraction

The dried algae was successively extracted in a maceration tang with 1,000 mL of ethanol under a room temperate for 3 days. All of the extract were kept without the sunlight. All of the ethanolic extracts were filtered, evaporated and concentrated to dryness by rotary evaporator. The dried algae was extracted for 3 times in the same experiments. The ethanolic extracts of algae were obtained. The yield of ethanolic extracts was calculated using the following formular, %yield = weight of dried extract x 100/weight of dried sample.

Determination of total phenolic contents

The contents of total phenolic compounds in the extracts were determined as modified by Kumaran and Karunakaran (2006) and the results were expressed as mg of gallic acid equivalent. The dried extracts (1 g) were dissolved in ethanol and the standard of gallic acid solution at the different concentrations at 10, 25, 50, 100, 150, 250 and 500 mg/L were prepared. The 0.5 mL of extract and standard solutions were transferred into a 15-mL 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 incubation at room temperature, the absorbance was measured at 730 nm using Genesys-5 visible spectrometer. The ethanol was used as blank solution for the experiments. The estimations of total phenolic contents in all the extracts were carried out in triplicate and the mean results were presented.

Determination of total flavonoid contents

Total flavonoid contents (TFC) of the algae extract were determined by using the aluminium chloride colorimetric method. The results were expressed as mg of quercetin equivalent. The ethanolic extract solution (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. Quercetin was used to make the calibration curve with the different concentrations at 12.5, 25, 50, 75, 100, 150 and 250 mg/L. The estimations of total flavonoids were carried out in triplicate and the results were averaged.

Radical scavenging activity using DPPH method

The dried extracts (0.1 g) were dissolved in a 2 mL of ethanol. The 0.1-mL samples were added into the 15-mL vial contained the two milliliter 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 and was calculated using the following formula, % DPPH free radical scavenging activity = $[(A_0-A_1) \times 100/ A_0]$, A_0 as Abs (control), A_1 as Abs (sample)

Antioxidant capacity using phosphomolybdenum method

The antioxidant capacity of the extracts was measured spectrometrically through phosphomolybdenum method as modified by Adum and Magdalena (2006). The total antioxidant capacity of the dried extract 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 15-mL vials were capped and incubated in a boiling water bath at 90°C for 60 min. After the samples had cooled down to 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 mg of ascorbic acid equivalent. The different concentrations (1, 5, 25, 50, 100, 200, 250, 500 and 1,000 µg/mL) of ascorbic acid standard solutions were prepared to make the calibration curve.

RESULTS AND DISCUSSION

The yields of ethanolic extracts of the algae type 1 (*Spirogyra* sp.), type 2 (*Cladophora glomerata* and *Microspora* sp.) and type 3 (*Nostoc commune*), were 4.53%, 3.86% and 0.70%, respectively.

The total phenolic contents (TPC) of the extracts as determined by Folin–Ciocalteu method, were reported as gallic acid equivalents (Table 1). TPC of the ethanolic extracts of the algae type 1 (*Spirogyra* sp.), type 2 (*Cladophora glomerata* and *Microspora* sp.) and type 3 (*Nostoc commune*), were 155.82, 12.12 and 18.22 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 (Hossain and Rahman, 2011).

The relative antioxidant ability of *Spirogyra* sp. extracts was investigated through some in *vitro* models such as antioxidant capacity by phosphomolybdenum method, and radical scavenging activity using DPPH method. The results of total flavonoid contents of the algae type 1 (*Spirogyra* sp.), type 2 (*Cladophora glomerata* and *Microspora* sp.) and type 3 (*Nostoc commune*), were showed in Table 1. The total flavonoid contents were 2.64, 3.83 and 0.52 mg quercetin equivalent (QE)/g of extract, respectively. The variation may be due environmental conditions, which can modify the constituents of the algae. The antioxidant capacity of the extracts was measured spectrometrically through phosphomolybdenum method, which is based on the reduction of Mo (IV) to Mo (V) by the sample analyte and the subsequent formation of green phosphate /Mo (V) compounds with a maximum absorption at 695 nm. The antioxidant capacity of the ethanolic extracts of the algae type 1 (*Spirogyra* sp.), type 2 (*Cladophora glomerata* and *Microspora* sp.) and type 3 (*Nostoc commune*), were 178.89, 150.67 and 173.28 mg ascorbic acid equivalent (AE) /g of extract at the concentration level of 10 mg/mL, respectively.

DPPH is a stable free radical and accepts electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al*., 1997; Hatano *et al.,* 1989). A freshly prepared DPPH solution exhibits a deep purple color with an absorption maximum at 517 nm. This purple color generally fades when an antioxidant is present in the medium.

Figure 1. The algae type 1 (*Spirogyra* sp.)

Figure 2. The algae type 2 (*Cladophora glomerata* and *Microspora* sp.)

Figure 3. The algae type 3 (*Nostoc commune*)

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 *et al*., 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 due to the scavenging ability of the extracts. The extracts showed increased free radical scavenging activity with

Table 1 TPC, TFC, antioxidant capacity and DPPH free radical scavenging activity of the ethanolic extracts of fresh water macroalgae

macroalgae extracts	TPC (mg GAE/ g of extract)	TFC (mg QE/ g of extract)	antioxidant capacity (mg AE/ g of extract)	DPPH free radical scavenging activity (%)
Type 1	155.82 ±3.28	2.64±0.36	178.89±1.98	52.33±1.84
Type 2	12.12 ± 1.92	3.83±0.90	150.67±0.84	<0.1
Type 3	18.22 ± 0.53	0.52±0.15	173.28±2.08	<0.1

the increased concentration of the extract. Table 1 showed the testing of DPPH scavenging activity assay. The ethanolic extracts showed high DPPH free radical scavenging activities because ethanol as a polar solvent that dissolve many the active compound including phenolic compound, flavonoids and terpenoid compounds, especially triterpine as glycoside form (Adam and Magdalena, 2006). The percentage of DPPH free radical scavenging activity of the ethanolic extracts of type 1 (*Spirogyra* sp.) was 52.33 while type 2 *(Cladophora glomerata* and *Microspora* sp.) and type 3 (*Nostoc commune*) were lower at the concentration level of 10 mg/mL.

CONCLUSIONS

It can be conclude that the extract of 1) *Spirogyra* sp., 2) *Cladophora glomerata* and *Microspora* sp. and 3) *Nostoc commune* showed antioxidant property. In addition, it was found that the *Spirogyra* sp. extracts have a high total phenolic contents, total flavonoid contents, antioxidant capacity and DPPH free radical scavenging activity when compared to other species of fresh water macroalgae. Thus, the results provided the scientific data regarding the antioxidant activity of the algae extracts. They are appropriate for use in the production of anti-aging and anti-winkle cosmetics such as face mask, nourishing cream, serum and others.

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