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# Efficiency of Antioxidant Compounds from Macro Edible Algae

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**Abstract:** Antioxidant efficiency was studied in fresh edible algae: *Cladophora*, *Microspara* and *Nostochopsis* spp. from Nan River, Nan province. The samples were collected from Pua and Tha-Wang-Pha district, studied antioxidant activity and compared the antioxidant efficacy of the extracts from the three fresh edible algae with a standard solution, 3-tert-butyl-4-hydroxyanisole (BHA). This study found that an extract of *Nostochopsis* spp. was the highest antioxidant activity to DPPH free radical between 32.1 and 87.5, the highest amount of beta-carotene was 4.71-7.09 mg/g and the highest amount of phenol in fresh edible algae extracts from *Microspora* was between 9.37 and 37.02 mg/g. The highest vitamin C in *Microspora* was between 110.41-138.53 mg/100 g.

**Key words:** Efficiency of antioxidant, macro algae, edible algae.

## 1. Introduction

There are currently attempting to find natural substances that have antioxidant properties, because nowadays, most people pay attention to keep healthy even more. The study of medical science showed that cells accumulate free radical damage over times, which are substances that contain atoms or molecules with a single or unpaired valency electron. Free radicals are unstable and high sensitive chemical reaction towards other substances. For chemical reaction, the unpaired or single electron is resolved by addition of an electron from neighbor atoms or molecules to normally exist in pair electrons. So it continues in process to be a chain reaction and damage to those molecules [1, 2]. Although, the body creates antioxidants to inhibit free radicals agent both enzymes and non-enzyme but when older the ability to create an antioxidant is reduced to lack of balance between the amount of free radicals and antioxidants. So that, it is necessity to have a source of antioxidants: vitamins, minerals and other types of antioxidants to balance of antioxidants in the body and prevent a variety of diseases that are caused by free radicals [1]. Internal and external factors such as diet and pollution of the environment effect on the body that generate free radicals in a higher than normal situation, and over to body used and dispose of it. The process of generating cellular energy in body used oxygen that has free radicals of oxygen ($O_2$). This substance can be combined with certain substances in the body and produce a toxin that destroys tissue, or change the genetic information within cells. Excess free radicals can oxidize the components of the body quickly and form continuous as a chain reaction. When the body exposure to such toxic that react oxidation within the body, and free radicals which cause many diseases. To prevent the free radicals, therefore, body should create or get antioxidants that can prevent or slow the

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oxidative process. The antioxidants are substances that might come from diet or the chemical synthesis that must consider the safety in utilization because of their toxic. So the natural antioxidants which are safe and non-toxic were high desired [3]. Algae organisms have been reported that they have amount of antioxidants different in species. They had a mechanism to produce antioxidant that can prevent the free radicals harmful itself [2]. This research aimed to study the efficiency of antioxidant from fresh edible algae by comparing the performance antioxidant activity of the extract from fresh edible algae with a standard solution, 3-tert-butyl-4-hydroxyanisole (BHA) and determinate the indicator of total phenol compound content, beta-carotene (b-carotene) and vitamin C (ascorbic acid).

## 2. Methodology

*2.1 Algae Samples*

Fresh edible algae samples were collected from 5 study sites at Nan River, Tha Wang Pha District, Nan province and identified in *Cladophora*, *Microspora* and *Nostochopsis* spp. [4]. Samples were used at least 10 kg, rinsed thoroughly fresh water and freezed-dry at −20 °C.

*2.2 The Study of Comparison the Efficiency of Antioxidants from Fresh Edible Algae and an Indicator of Antioxidants*

(1) Prepared a solution of DPPH (2,2-diphenyl-1-picrylhydrazyl) in ethanol to concentrate 0.2 mM. Then DPPH solution was scanned for maximum wave length at 517 nm by a UV spectrophotometer.

(2) The extracts of algae samples were prepared 1 g with ethanol in a 250 mL Erlenmeyer flask, stirred well, and covered with aluminum foil. Then set at room temperature for 24 hrs, strained through filter paper No. 1, dried the solution by using rotary evaporator. Dissolved extract again with ethanol 50%, adjusted to 50 mL and bring sample extracts in the refrigerator at a temperature of 4 °C in the dark to use further analysis.

(3) 1 mL extract samples were mixed with 2 mL DPPH solution in a brown bottle and set aside about 30 minutes.

(4) Preparation of BHA standard solutions at concentration 0.6667 mg/mL in methanol, mixed 1 mL BHA standard solution with 2 mL DPPH solution in a test tube and set in the dark for 30 minutes.

(5) Prepare blank, mixed 1 mL methanol 50% with 2 ml DPPH solution in test tube, set in the dark for 30 minutes and measured the absorbance of the sample extract, BHA standard solution and blank at a wavelength in 1. With UV-visible spectrophotometer for 3 times repeated. Calculated the percentage of radical scavenging activity

$$\%\ \text{radical scavenging} = [(1 - \text{A sample})/\text{A blank}] \times 100$$

where, A sample = absorbance of the sample, A blank = absorbance of the blank.

*2.3 The Volume of Antioxidant Activity Indicator*

2.3.1 Beta-Carotene (β-Carotene)

(1) Measurement of maximum wavelength ($\lambda_{max}$), lead β-carotene standard solution at the concentration 2, 4, 6 and 8 mg/g in chloroform and measured the maximum absorption at wavelength 200-800 nm by UV-visible spectrophotometer.

(2) Created the standard curve, lead standard solution of β-carotene at concentration 2, 4, 6 and 8 mg/g to measure the maximum absorption.

(3) Algae samples extract to measure the maximum absorption. Then it obtained absorbance values to determine the concentration of β-carotene solution from the standard curve for 3 times repeated.

2.3.2 Total Phenol Compound Content

Test according to Ref. [5], sample was reacted with Folin-Ciocalteu reagent that consists of phospho molybdic-phosphotungstic acid reagents. This substance will be reduced by the phenolic hydroxyl groups of total polyphenols, emerged as tungsten and

molybdenum blue which is blue and absorbance at maximum wavelength 765 nm. Total phenolic compound is determined by comparing the amount of gallic acid, follow this method. Measurement of the maximum absorption ($\lambda_{max}$), leads to a standard solution of tannic acid at concentrations of 20, 40, 60 and 80 mg/g in methanol to measure the maximum absorption at wavelength 765 nm by UV-visible spectrophotometer.

2.3.3 Determination of Vitamin C (Ascorbic Acid)

Test according to Ref. [6], vitamin C has antioxidant activity so the quantification of antioxidants in the same group as vitamin C or vitamin C in vegetables or fruits is used. Isolated these compounds from the samples with trichloroacetic acid then reacted with dinitrophenyl hydrazine reagent, the production from this method is color and absorbance at a wavelength 520 nm by UV-visible spectrophotometer.

(1) Measurement of the maximum absorption ($\lambda_{max}$), lead vitamin C standard solution at 2, 4, 6 and 8 ppm to measure the maximum absorption at wavelength 200-800 nm by UV-visible spectrophotometer.

(2) Created standard curve, pipetted 300 ppm each concentration of vitamin C standard solution at 2, 4, 6 and 8 ppm, to fill with 100 µL dinitrophenyl hydrazine reagent, heated the mixture at 60 °C for 1 hr and then cooled in an ice bath, filled 400 µL Sulfuric acid, set in the dark for 20 minutes, then measured absorption by UV-visible spectrophotometer to determinate the maximum wavelength. Then the absorbance is found the concentration of vitamin C from a standard curve and calculated the amount of vitamin C. Bring 200 µL samples solution to fill 300 µL trichloroacetic acid, mixed well and centrifuged to collect 300 µL fluid to fill 100 µL dinitrophenyl hydrazine reagent. Then, heated the mixture at 60 °C for 1 hrs and cooled in an ice bath. Filled 400 µL sulfuric acid, set in the dark for 20 mins, and measured the absorption by UV-visible spectrophotometer at a wavelength 520 nm for 3 times repeated.

## 3. Results

Antioxidant efficiency was studied in fresh edible algae: *Cladophora*, *Microspara* and *Nostochopsis* spp. from Nan River, Nan province, Thailand (Figs. 1-4). The studied antioxidant activity and compared the antioxidant efficacy of the extracts from the three fresh edible algae with a standard solution, 3-tert-butyl-4-hydroxyanisole (BHA). This study found that the extract of *Cladophora* exhibited highest DPPH free radical scavenging activity activity was found in the extract at site 4, 37.6 to 45.6. *Microspora* was activated with highest DPPH antioxidant 23 to 49.6 and *Notochopsis* spp. was activated with highest DPPH antioxidant 87.5. The β-carotene of algae in

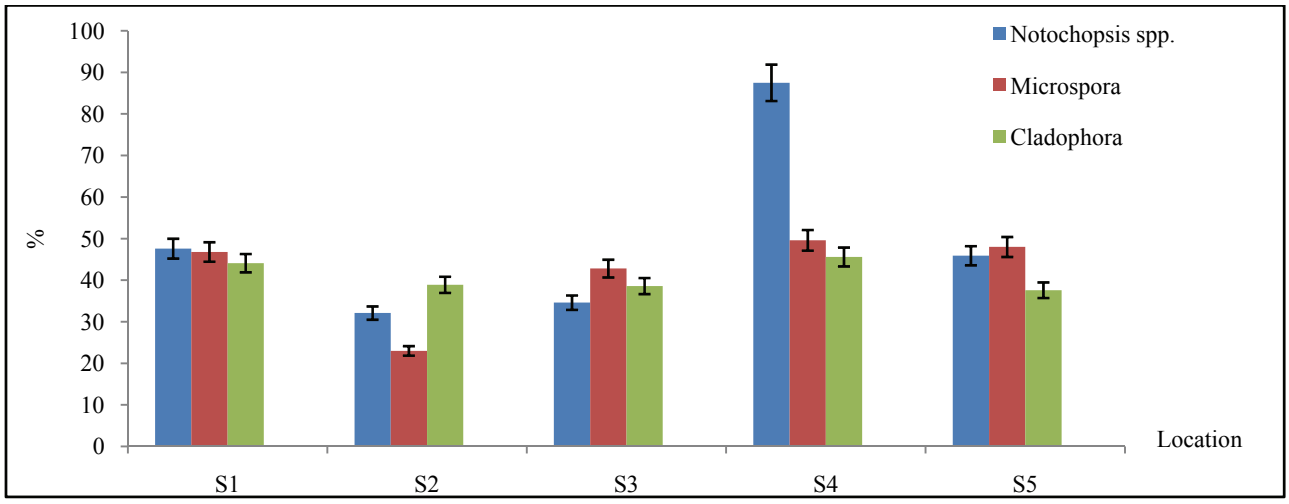


**Fig. 1 Efficiency of antioxidant from fresh edible algae.**

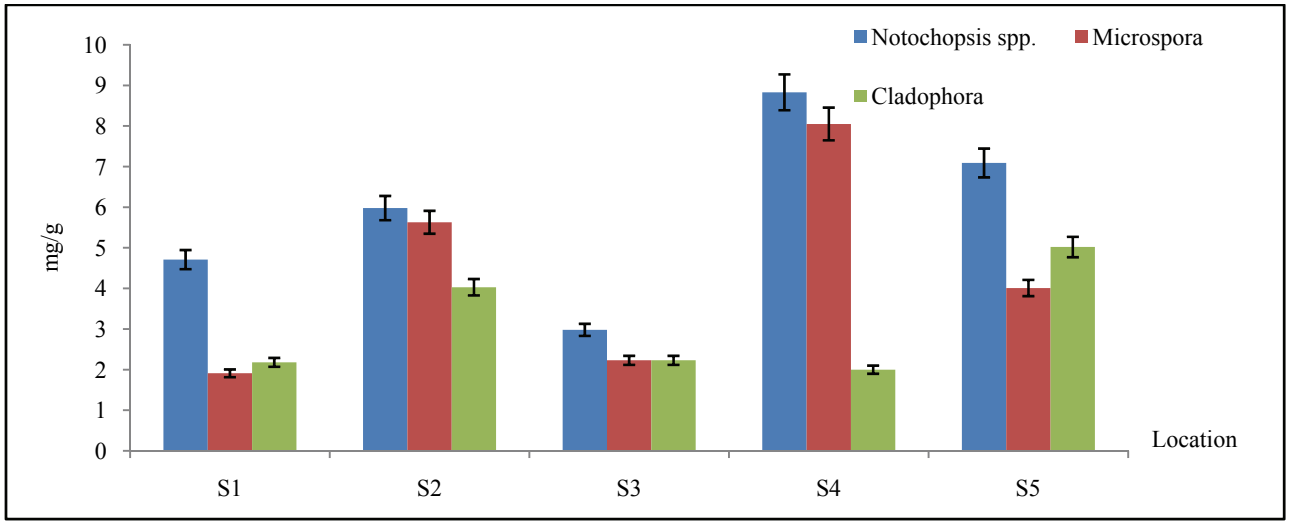


**Fig. 2 Beta-carotene (β-carotene) from fresh edible algae.**

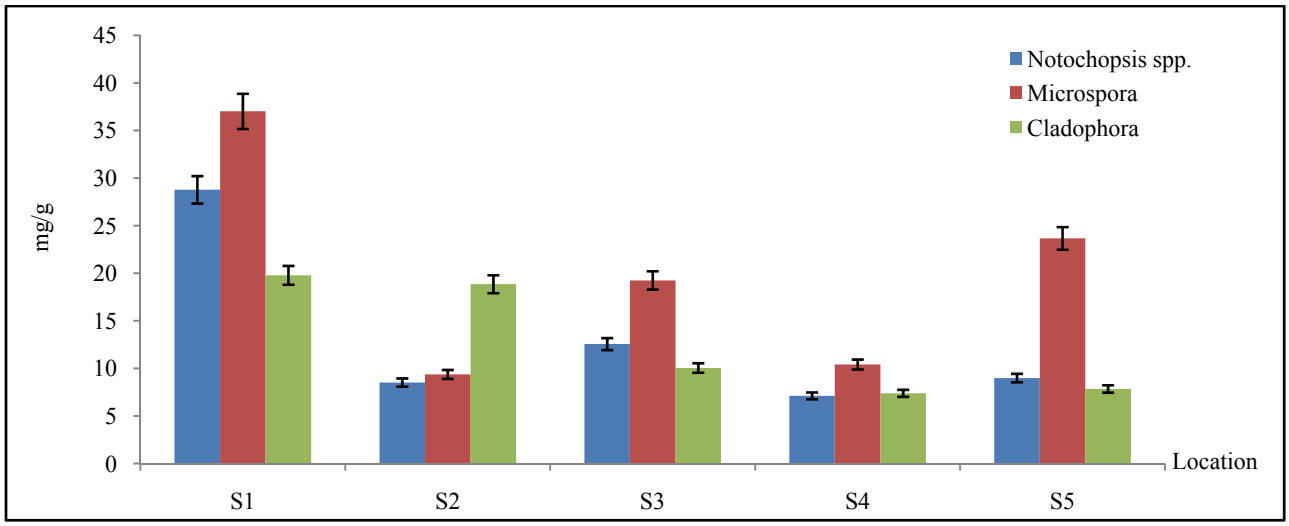


**Fig. 3 Total phenolic compounds from fresh edible algae.**

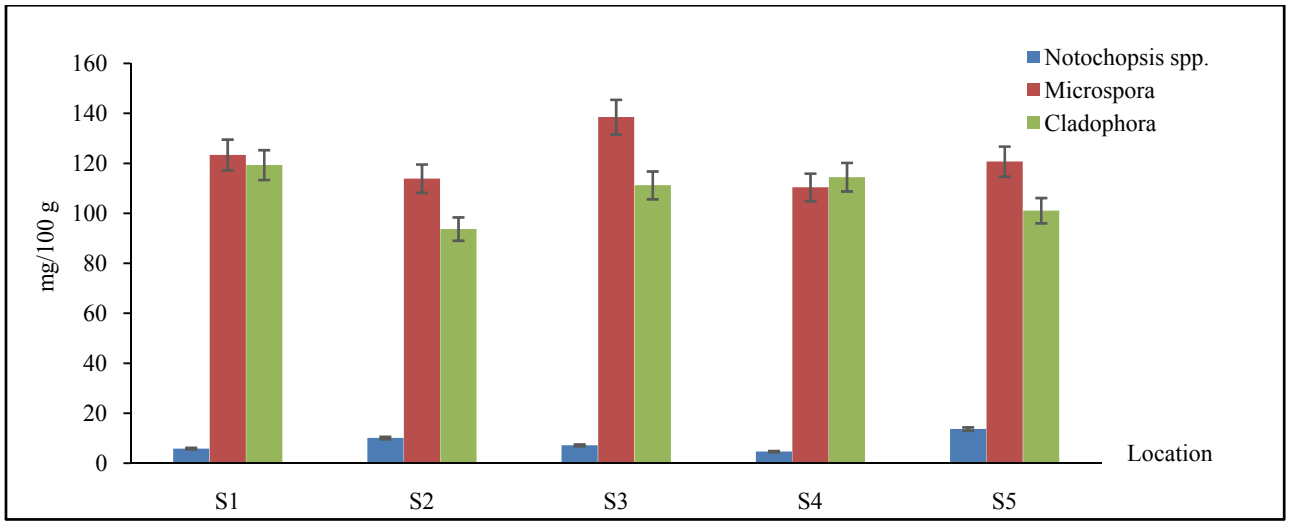


**Fig. 4 Vitamin C from fresh edible algae.**

the 4th sampling site had a higher than other sites consistent with the efficiency of antioxidants of algae in No.1, in the 4th sampling site had the highest efficiency, similarly. The β-carotene each freshwater species extracts found the β -carotene from *Cladophora*, *Microspora* and *Notochopsis* spp. were 7.39-19.79, 9.37-37.02 and 7.12-28.78 mg/g, respectively. The highest of phenol in extracts from *Cladophora*, *Microspora* and *Notochopsis* spp. were 7.39-19.79 mg/g, 9.37-37.02 mg, and 7.12-28.78 mg/g, respectively. The highest vitamin C in fresh water edible extracts *Cladophora*, *Microspora* and *Notochopsis* spp. were 93.72, 119.33, 110.41 mg/100 g of extracts.

## 4. Discussion

*4.1 DPPH Antioxidant Activity of the Fresh Edible Algae Extracts*

The extracts from three species of fresh edible algae have DPPH antioxidant. The extract of Cladophora was activated highest DPPH antioxidant between 37.6 and 45.6, *Microspora* was activated highest DPPH antioxidant between 23 and 49.6 and *Notochopsis* spp. was activated highest DPPH antioxidant between 32.1 to 87.5, respectively. From the research studies of Panyoyai [7] and Peerapornpisal [8] found that Cladophoraglomerata and Nostochopsislobatus Woodem Geitler capable to inhibit free radicals, as well as fresh edible algae extracts in this study. According to the study of Puntip [9] it is reported that Lobophoravariegata extracts have DPPH antioxidant with IC50 values of 35.33 ± 5.03 and 61.33 ± 8.32 mg/ml and Ulvaintestinalis has the best antioxidant properties by measuring the DPPH free radical scavenging assay used 65.18 mg/ml water as a solvent. In addition, the study sites affected the efficiency of antioxidants, the 4th sampling site all species had higher antioxidants than any areas because the algae can be growth well in good quality water and flow evenly over time. Corresponding with Peerapornpisal et al. [8], Panyoyai [7] found that Cladophoraglomerata and Nostochopsislobatus Woodem Geitler can be inhibited DPPH free radicals, as well as algae spirogyra.

*4.2 The Volume of Antioxidant Activity Indicator*

4.2.1 The Amount of Beta-Carotene (β-Carotene)

The amount of β-carotene each freshwater species extracts found that the highest amount of β-carotene from *Cladophora*, *Microspora* and *Notochopsis* spp. was between 7.39-19.79 mg/g, 9.37-37.02 mg/g and 7.12-28.78 mg/g, respectively. Peerapornpisal et al. [8] found that β-carotene and xanthophylls was in spirogyra spp., as the same freshwater algae. The amount of β-carotene of algae in the 4th sampling site had a tendency higher than other sites consistent with the efficiency of antioxidants of algae in No. 1, in the 4th sampling site had the highest efficiency, similarly.

4.2.2 The Amount of Phenol from Fresh Edible Algae Extracts

The highest amount of phenol in fresh edible algae extracts from *Cladophora*, *Microspora* and *Notochopsis* spp. was between 7.39-19.79 mg/g, 9.37-37.02 mg, and 7.12-28.78 mg/g, respectively. Phenolic compounds that have antioxidant activity in freshwater algae have found in genus *Spirogyra* spp. [8]. The compounds in algae play an important role to be antioxidant was Phenol [3, 10-12]. In ecology, believed that algae produce such compounds to prevent UV, each species has different antioxidant mechanism and varies ways.

4.2.3 Vitamin C Content of Fresh Edible Algae Extracts

The highest vitamin C in fresh water edible extracts *Cladophora*, *Microspora* and *Notochopsis* spp. was between 93.72-119.33 mg/100 g of extracts, 110.41-138.53 mg/100 g of extract and 4.68-13.69 mg/100 g of the extracts, respectively. There have been reported that vitamin C was found in freshwater algae (*Spirogyra* spp.) [8]. Vitamin C in algae plays an important role to inhibit free radical as well.

## 5. Conclusions

Fresh edible algae extracts from *Cladophora*, *Microspora* and *Notochopsis* spp. have the potential to develop as an antioxidant by extraction from the three algae. They can activate inhibiting DPPH free radical, high amount of phenol content and β-carotene. In this study, found that *Nostochopsis* spp. can highest activate inhibition of DPPH free radicals at 87.5 and the highest amount of β-carotene was 8.83 mg/g. The highest amount of phenol and vitamin C from *Microspora* were 37.02 mg/g and 138.53 mg/g, respectively. Three algae have the potential to develop as antioxidants to replace synthetic substances that are toxic to cell and body.

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