Free radical Scavenging Activity and Total Phenolic Compounds of *Gracilaria changii*

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Abstract

The antioxidant activity of the marine algae *G. changii* from Malaysia was determined. The free radical scavenging activity of the 80% methanolic extract of *G. changii* was assessed with the aid of the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. The crude extract showed a low free radical scavenging capacities in comparison with commercial antioxidant BHT (butylated hydroxytoluene), and vitamin E. The IC_{50} values of the extracts of algae on DPPH scavenging activity was 14.70 mg/ml. The TLC-DPPH study, exhibited only one DPPH radical scavenging bands out 7 major band of the extract and detected with R_f value 0.63. In order to assess the role of polyphenolic components in the relevant activity, phenolic contents of the extract also determined and the value was 5.0 mg (gallic acid equivalent/1 g extract). This finding indicated that crude extract of *G. changii* was a starting material for the isolation of compound(s) with effective activities as radical's scavengers.

Key words: Antioxidant activity, DPPH radical scavenging assay, *Gracilaria changii*

INTRODUCTION

There has been interest in the contribution of free radical reaction participating in reactive oxygen species to the overall metabolic perturbation that result in tissue injury and disease. Reactive oxygen such as superoxide anion, hydrogen peroxide, and hydroxyradical are generated in specific organelles of cells (Mitochondria and Microsomes) under normal physiological condition. These reactive oxygen species can damage DNA, so as to cause mutation and chromosomal damage, oxidize cellular thiols and abstract hydrogen atoms from unsaturated fatty acids to initiate the peroxidation of membrane lipids [1-2]. Recently, various phytochemicals and their effect on health, especially the suppression of active oxygen species by natural antioxidant from tea, spices and herbs, have been intensively studied [3-5].

The most commonly used antioxidant at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate (PG), and *tert*-butylhydroxytoluene (TBHQ) [6]. However there are suspected of being responsible for liver damage and carcinogenesis in laboratory animals [7-8]. Therefore, the development and utilization of more effective antioxidant of natural origin are desire [9].

Malaysia is endowed naturally with a very rich algae life such as the *Gracilaria changii.* There are documentations about the use of these algae as a food source but there is no report about the antioxidant activities of the algae. Therefore the aim of the present study was to investigate and to evaluate *G. changii* crude extract antioxidant and radical scavenging activities by DPPH free radical scavenging assay, which is widely used for screening bioactive compounds.

MATERIALS AND METHODS

Algae sample and preparation of the extract

Fresh *G. changii* sample was collected from Pantai Morib, Selangor Malaysia in January 2003 and authenticated by Prof. Phang Siew Moi (Institute of Biological Sciences, Faculty of Science University Malaya, Malaysia). The fresh algae sample was rinsed with seawater to remove debris and epiphyte before further washed in fresh water and brushed with soft brush and sun dried. Approximately, 100 g of dried algae sample was added to 200 ml of methanol and let soaked for 4 days. Removal of dried algae sample from extract by filtration through cheesecloth, and the filtrate was further concentrated using a rotary evaporator. The dried fractions were then re-dissolved in 80% methanol (v/v) to yield solution containing 1.0, 2.5, 5.0, 10.0, 15.0, 20.0 and 25.0 mg of extract per ml.

Free-radical Scavenging Activity

Quantitative measurement of radical scavenging properties was carried out in a universal bottle. The reaction mixture contained 50 µl of test samples (or 80% MeOH as a blank) and 5 ml of a 0.04% (w/v) solution of DPPH in methanol. Different known antioxidants, vitamin E, and butylated hydroxytoluene (BHT, Sigma) were used for comparison or as a positive control.

Discoloration was measured at 517 nm after incubation for 30 min. Measurements was performed at least in triplicate. DPPH radical's concentration was calculated using the following equation:

DPPH scavenging effect (%) = $A_o - A_1 / A_o$ X100

Where A_o was the absorbance of the control and A_1 was the absorbance in the presence of the sample [9] (crude extract of *G. changii*). The actual decrease in absorption induced by the

test compounds was compared with the positive controls. IC_{50} value was calculated use the dose inhibition curve.

TLC-DPPH separation and determination of radical scavenging activity

Crude extract of *G. changi* was subjected to thin layer chromatography study. The solvent system optimized for *G. changii crude* extract was methanol and: Chloroform (85:15 v/v). The plates were developed in an unsaturated chamber to the distance of 75 mm. After 15 min air-drying, the plates were sprayed by 0.04% DPPH solution for 5 seconds and images were observed under visible light at exactly 2 min after spraying. The area of bright yellow bands against the purple background then determined radical scavenging activity.

Total phenolic compound analysis

The amount of total phenolics in *G. changii* extracts was determined with the Folin-Ciocalteu reagent using the method of Spanos and Wrolstad [10], as modified by Lister and Wilson [11]. To 50 ml of each sample (three replicates), 2.5 ml 1/10 dilution of Folin-Ciocalteau's reagent and 2 ml of Na2CO3 (7.5%, w/v) were added and incubated at 45 ^{o}C for 15 min. The absorbance of all samples was measured at 765 nm using a UV–vis spectrophotometer (GAT UV-9100). Results were expressed as milligrammes of gallic acid (20-50µg/ml; R^2=0.963) equivalent per gram of dry weight (mg GAE/g dw).

RESULTS AND DISCUSSION

The crude extract of *G. changii* was screened for DPPH radical scavenging activity according to the method described and the results of the screening are shown in Figure 1 as comparable with known antioxidant.

The methanolic extract tested showed a lower reducing power activity than BHT and vitamin E. BHT showed the highest reducing power activity (66.0%) followed by Vitamin E (40.1%) and the methanolic extract (4.3%) respectively at the concentration tested (1 mg/ml). We therefore suggest that at proper concentration of the crude extracts of *G. changii* may act as free radicals scavenger and may react with free radicals to convert them to more stable products and terminate radical chain reaction [12]. Our result demonstrated that crude extract of *G. changii* thereby has moderate free radical scavenging activity.

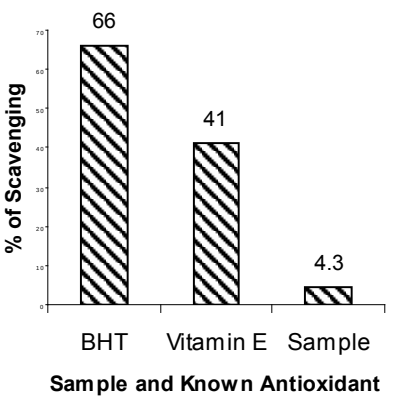

Figure 1. Scavenging effect (%) of crude extract of *G. changii* and known antioxidant at 1.0 mg/ ml

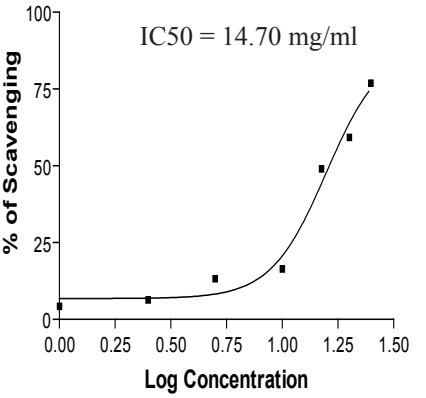

Figure 2. Dose inhibition curve and IC50 values of the crude extract of *G. changii*

All these properties are involved in the overall anti-oxidant activity, which makes it suitable for the prevention of human disease in which free radicals play an important role [13-14].

Dose inhibition curve and IC_{50} values of the crude extract are shown in Fig 2. In the dose-response experiment it could be observed that total inhibition of the enzymes was never achieved. Maximal inhibition was in the range of 75-85%, in the presence of 25 mg/ml extract. With the addition of larger amount of extract to DPPH assay mixture, the degree of inhibition decreased, indicating a pro-oxidant effect. The explanation for the higher IC_{50} (IC_{50} = 14.70 mg/ml) value found in the experiment was because the sample used was a crude extract with the compound(s) react as antioxidant.

Result of the TLC-DPPH separation and determination of radical scavenging activity showed in Figure 3. Our thin layer chromatography study showed that the crude extract of *G. changii* contained 7 major bands. A major part of compounds in *G. changii* crude extract was found to be inactive based on DPPH scavenging area. Only one DPPH radical scavenging band was separated and detected with R_f value 0.63 (Figure. 3). In such situation, the real concentration for the compound(s), which reacts as an antioxidant, was very low in the crude extract.

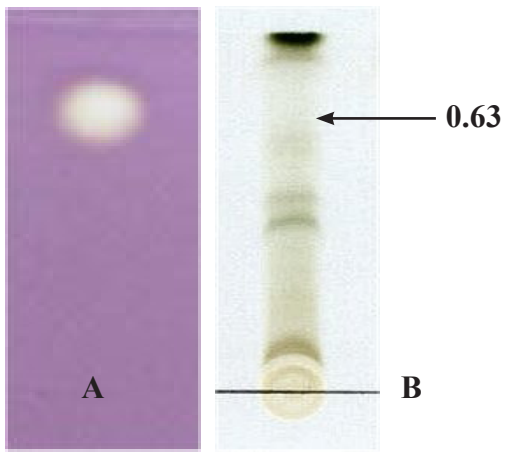

Figure 3. TLC-DPPH separation and determination one DPPH radical scavenging band with Rf 0.63 value (A) compared with control (B)

The phenolic content of the sample was determined using Folin-Ciocalteu reagent and was 5.0 mg/g dry weight expressed as gallic acid equivalents (GAE). Typical phenolics that possess antioxidant activity have been characterized as phenolic acids and flavonoids [15]. Phenolic acids have repeatedly been implicated as natural antioxidants in fruits, vegetables, and other plants. For example, caffeic acid, ferulic acid, and vanillic acid are widely distributed in the plant kingdom [16].

From these results it can concluded that the crude extract of *G. changii* are promising starting material for the isolation of compound(s) with antioxidant activities. There is lack of information available on the chemical composition of *G. changii,* which exhibit antioxidant activity. Further phytochemical work need to be done on these extracts including fractionation to isolate active constituent and subsequent pharmacological evaluation.

REFERENCES

[1] Halliwell B, Gutteridge JMC. 1985. The chemistry of oxygen radicals and other oxygen-derived species. In: Free Radicals in Biology and Medicine. pp. 20-64. Oxford University Press, New York.

[2] Ames BN, Shigenaga MK, Hagen TM. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Sciences. 90: 7915-7922.

[3] Ho CT, Ferraro T, Chen Q, Rosen RT. 1994. Phytochemicals in teas and rosemary and their cancer-preventing properties. In: Foods Phytochemicals for cancer prevention. II. Tea, Spices, and Herbs (eds. Ho CT, Osawa T, Huang MT, Rosen RT), ACS Symposium Series 547. pp. 2-9. American Chemical Society, Washington, DC.

[4] Sies H, Stahl W. 1995. Vitamins E and C, b-carotene and other carotenoids as antioxidants. American Journal of Clinical Nutrition. 62:1315S-1321S.

[5] Elmastas M, Gulcin I, Beydemir S, Kufrevioglu OI, Aboul-Enein HY. 2006. A study on the in vitro antioxidant activity of Juniper (*Juniperus communis* L.) fruit extracts. Analytical Letters. 39: 47–65.

[6] Sherwin FR. 1990. Antioxidant. In: Food Additive (ed. Branen R), pp. 139-193. Marcel Dekker, New York.

[7] Grice HC. 1986. Safety evaluation of butylated hydroxytoluene (BHT) in the liver, lung and gastrointestinal tract. Food Chemistry and Toxicologi. 24**:** 1127-1130.

[8] Wichi HP. 1988. Enhanced tumor development by butylated hydroxyanisole (BHA)from the prospective of effect on forestomach and oesophageal squamous epithelium. Food Chemistry and Toxicology. 26: 717 – 723.

[9] Oktay M, Gulcin I, Kufrevioglu OI. 2003. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extract. Lebensmittel-Wissenchaft und Technologie. 36**:** 263-271.

[10] Spanos GA, Wrolstad RE. 1990. Influence of processing and storage on the phenolic composition of Thompson seedless grape juice. Journal of Agricultural and Food Chemistry. 38: 1565–1571.

[11] Lister E, Wilson P. 2001. Measurement of total phenolics and ABTS assay for antioxidant activity (personal communication). Crop Research Institute, Lincoln, New Zealand.

[12] Duh PD, Yen GC. 1997. Antioxidative activity of three herbal water extracts. Food Chemistry. 60: 639–645.

[13] Lugasi A, Dworschak E, Horvahovich P. 1999. Additional information to the in vitro antioxidant activity of *Ginkgo biloba.* Phytotherapy Research. 13**:** 160-162.

[14] Karbownik M, Lewinski A. 2003. The role of oxidative stress in physiological and pathological processes in the thyroid gland; possible involvement in pineal-thyroid interactions. Neuroendocrinology Letters. 24(5): 293-303.

[15] Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry. 47(10): 3954–3962.

[16] Larson RA. 1988. The antioxidants of higher plants. Phytochemistry. 27: 969–978.