African Journal of Biotechnology Vol. 11(39), pp. 9457-9467, 15 May, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2988
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Antioxidative, anticholinesterase and antityrosinase activities of the red alga *Grateloupia lancifolia* extracts

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Accepted 17 January, 2012

The solvent extracts of a red alga *Grateloupia lancifolia* were used to evaluate biofunctional activities. The diethyl ether extract showed the highest total phenolic content and biological activities. The IC_{50} value of the diethyl ether extract on 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was 113.8 μg/ml. In the reducing power test, the half maximal effective concentration (EC_{50}) values of the diethyl ether, water and methanol extracts were 1.21, 1.74 and 3.96 mg/ml, respectively. In the linoleic acid system, all the extracts had significantly higher inhibitory activity than α-tocopherol ($p < 0.05$). The diethyl ether extract had the highest tyrosinase, acetylcholinesterase and butyrylcholinesterase inhibitory activities with the IC_{50} values of 47.8, 99.8 and 23.7 μg/ml, respectively. Therefore, the *G. lancifolia* extract could be used as a potential source for the development of dietary foods, food products and additives.

Key words: *Grateloupia lancifolia*, red alga, anticholinesterase, antioxidant, antityrosinase.

INTRODUCTION

Antioxidants found in food and supplements support human intrinsic antioxidative protection to maintain the internal oxidative status by various processes such as *in situ* regeneration of antioxidant molecules (vitamins and enzymes) or direct neutralization of oxidatives (Mamelona et al., 2007). Epidemiological studies demonstrated a positive relationship between a high intake of fruits or vegetables and reduced risk of cardiovascular and other chronic diseases, which may be attributed to their antioxidant activities (Nakamura et al., 2008). On the other hand, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are suspected to have some side effects such as liver damage and carcinogenesis (Grice, 1986; Wichi, 1988). Thus, a lot of attention has been paid to the identification of new sources for safe natural antioxidants.

Tyrosinase (EC. 1.14.18.1) is a copper containing enzyme widely distributed in microorganisms, plants and animals (Whitaker, 1995). It catalyzes the oxidation of phenolic substrates to o-quinones, which are then polymerized to brown, red or black pigments (Friedman, 1996). In addition to undesirable color and flavor, the quinine compounds produced in the browning reaction may irreversibly react with amino and sulfhydryl groups of protein. This reaction decreases the digestibility of protein and bioavailability of essential amino acid, including lysine and cysteine (Kim and Uyama, 2005). Therefore, tyrosinase inhibitors have become increasingly important in cosmetic and medicinal products, primarily to control hyperpigmentation (Parvez et al., 2007). Several synthetic tyrosinase inhibitors have been developed, but exhibited lack of efficiency or adverse side effects (Hamon and Criton, 2009). For this reason, it is still necessary to search and develop novel tyrosinase inhibitors with potent activities and fewer side effects.

Dementia is a chronic progressive mental disorder, which adversely affects memory, thinking, comprehension, calculation and language. The most common forms of dementia are Alzheimer's disease (AD), Parkinsonism, Dementia with Lewy Bodies and Myasthenia gravis. All these disorders are related to abnormalities in the central cholinergic system, which shows a decline in acetylcholine levels (Natarajan et al.,

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2009). The brain of mammals contains two major forms of cholinesterases; acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). These two enzymes differ in substrate specificity, kinetics and activity in different brain regions. Cholinesterases (ChE) inhibitors are currently the main pharmacological approach to treatment, offering a rational evidence-based approach to symptom management (Giacobini, 2003). However, the anti-ChE agent such as physostigmine has been shown to have a small, short-term positive effect on cognitive functions (Schwarz et al., 1995).

Edible seaweeds have been known to contain antibacterial (Xu et al., 2003; Sandsdalen et al., 2003), antidiabetic (Ohta et al., 2002; Iwai, 2008), antioxidant (Kuda et al., 2005; Kumar et al., 2008), α-glucosidase and β-glucuronidase inhibitory activities (Kim et al., 2008a, b), and tyrosinase inhibitory activity (Yoon et al., 2009). In addition, few reports are available on the anticholinesterases of seaweeds (Natarajan et al., 2009). *Grateloupia lancifolia*, a red alga belonging to Halymeniaceae family, has been reported on α–glucosidase inhibitory activity in our previous study (Kim et al., 2010). However, no information is available on antioxidative properties, as well as cholinesterase and tyrosinase inhibitory activities of *G. lancifolia*. Therefore, the objectives of this study were to investigate the antioxidant, cholinesterase and tyrosinase inhibitory activities of the solvent extracts of *G. lancifolia*.

MATERIALS AND METHODS

Reagents

Folin Ciocalteu's phenol reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), linoleic acid, ammonium thiocyanate, ascorbic acid, butylated hydroxyanisole (BHA), α-tocopherol, mushroom tyrosinase, L-3,4-dihydroxyphenylalanine (L-DOPA), acetylthiocholine iodide (ATCI), 5,5′-dithiobis[2-nitrobenzoic-acid] (DTNB), physostigmine, acetylcholinesterase from *Electrophorus electricus* (EC 3.1.1.7, Type VI-S) and butyrylcholinesterase from equine serum (EC 3.1.1.8) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used in this study were of analytical grade.

Algal samples

G. lancifolia was harvested in the eastern coastal area of the Korean peninsula in July and August, 2008. For identification, the seaweed was fixed with 10% formalin-seawater (v/v) immediately after collected and transported to laboratory, which was then identified by microscope according to De Clerck et al. (2005). Fresh alga was individually washed with tap water and air-dried in the shade at room temperature. Dried samples were individually cut into small pieces (2 × 3 cm), homogenized, sifted with a 500 μm sieve, and stored at -40°C until used.

Preparation of the extracts

Powder of *G. lancifolia* (20 g) was extracted for 5 h with 400 ml of diethyl ether, methanol, or water in a glass conical flask on a shaker at ambient temperature, respectively. The extracts were filtered using an Advantec No. 5C filter paper (Tokyo Roshi Kaisha Ltd., Japan). The residues were then extracted twice more with 400 ml of the same solvent and the extracts were combined. The water extract was lyophilized, and the diethyl ether and methanolic extracts were evaporated to absolute dryness under vacuum at < 40°C (Buchi RE121 Rotavapor, Swizland).

Determination of the total phenolic contents

The total concentration of phenolics in the extracts was determined according to a procedure described by Singleton and Rossi (1965). A 0.1 ml of the extract solution was mixed with 7.9 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent. After 3 min of incubation, 1.5 ml of 12% Na_2CO_3 solution was added and allowed to stand at room temperature for 30 min in the dark. The absorbance was measured at 760 nm using a spectrophotometer (V-530 Jasco Co., Japan). The concentration of phenolic compounds was estimated using a calibration curve with a coefficient correlation of $R^2 = 0.9998$ obtained from various diluted concentrations ranging between 0 and 0.25 mg/ml of gallic acid (GA). Total phenolic content was expressed as mg GA equivalents per g of the extract (mg GA/g extract).

DPPH radical scavenging activity

Scavenging activity on DPPH radicals was determined according to the method of Blois (1958) with a slight modification. Briefly, 1 ml of the extract at different concentrations was mixed with 3 ml of 0.1 mM DPPH solution in ethanol. The mixture was then vortexed vigorously and incubated at room temperature for 30 min in the dark. The absorbance of each sample solution was measured at 517 nm using a spectrophotometer (V-530 Jasco Co., Japan). Ascorbic acid and BHA were used as positive controls. A blank experiment was also carried out applying the same procedure to a solution without the test material. The ability to scavenge the DPPH radical was calculated as percent DPPH scavenging using Equation 1:

$$\text{DPPH radical scavenging activity (\%)} = [A_0 - A_1]/A_0 \times 100 \quad (1)$$

Where, A_0 is the absorbance of the control and A_1 is the absorbance of the sample.
Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

Antioxidant activity in the linoleic acid system with ferrothiocyanate reagent (FTC)

Total antioxidant activity was measured according to the ferric thiocyanate (FTC) method (Kumar et al., 2008). The extract (4 mg) was dissolved in ethanol and mixed with 2.5% linoleic acid in the solution of ethanol (4.1 ml), 0.05 M phosphate buffer (8 ml, pH 7) and distilled water (3.9 ml), and then kept in screw-cap containers under dark conditions at 40°C. Aliquots (0.1 ml) was drawn and mixed with 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. On exactly 3 min after the addition of 0.1 ml ferrous chloride (0.02 M) in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured at 500 nm every 24 h until the absorbance of the control reached maximum. The control and standard were subjected to the same procedure except that for the control, there was no addition of sample and, for the standard, 4 mg

of sample was replaced with 4 mg of α-tocopherol. The percent of inhibition of linoleic acid peroxidation was calculated as Equation 2:

Inhibition (%) = $[A_0 – A_1]/A_0 \times 100$ (2)

Where, A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Hydrogen peroxide scavenging activity

Hydrogen peroxide scavenging activity was measured according to the method of Lee et al. (2008). The test sample (0.5 mg/ml) in ethanol was mixed with 100 µl of phosphate buffer (0.1 M, pH 5) and 20 µl of hydrogen peroxide (10 mM) in a 96-well microplate and then incubated at 37°C for 5 min. ABTS (30 µl, 1.25 mM) and peroxidase (30 µl, 1 Unit/ml) were added to the mixture and then incubated at 37°C for 10 min. The absorbance was read with microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA) at 405 mn to determine the extent of hydrogen peroxide scavenging activity. The hydrogen peroxide scavenging activity was calculated according to Equation 3:

Hydrogen peroxide scavenging activity (%) = $[A_0– A_1]/ A_0 \times 100$ (3)

Where, A_0 is the absorbance of the control and A_1 is the absorbance of the tested extract.

Reducing power assay

The reducing power of the extracts was determined according to the method of Lee et al. (2007). Each extract in ethanol (2.5 ml) was mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% postassium ferricyanide, and the mixture was then incubated at 50°C for 20 min. The reaction was stopped by adding 2.5 ml of 10% trichloroacetic acid (w/v). The mixture was then centrifuged at 200 × g for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride, and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Tyrosinase inhibitory activity assay

Tyrosinase inhibitory activity was determined according to the modified method of Chang et al. (2007). A 20 µl of tyrosinase (1000 U/ml in 50 mM phosphate buffer, pH 6.8) was mixed with 100 µl of the extract (dissolved in DMSO) and 1.9 ml of 50 mM phosphate buffer (pH 6.8). The reaction mixture was incubated at 25°C for 5 min. Then, 880 µl of L-DOPA as a substrate in the same buffer was added to start the reaction. The increase in absorbance at 475 nm was monitored with the spectrophotometer (V-530 Jasco Co., Japan). Kojic acid was used as a positive control. The tyrosinase inhibitory activity was calculated as Equation 4:

Tyrosinase inhibitory activity (%) = $[(A_0 - A_1)/A_0] \times 100$ (4)

Where, A_0 is the absorbance at 475 nm with DMSO instead of the tested sample and A_1 is the absorbance at 475 nm with the tested sample.

Anticholinesterase inhibitory activity assay

The anticholinesterase assay was performed according to the modified colorimetric Ellman's method (Loizoo et al., 2009), which is based on the reaction of released thiocoline to give a colored product with chromogenic reagent. *E. electricus* (EC 3.1.1.7, Type VI-S) AChE and equine serum (EC 3.1.1.8) BChE were used as the source of both cholinesterases, while acetylthiocholine iodide (ATCI) and butyrylthiocholine chloride (BTCC) were used as the substrates of the reaction. A 20 µl of AChE or BChE (0.36 U/ml in 10 mM phosphate buffer, pH 8) and the extract (100 µl) at different concentrations were added to 2.36 ml of 10 mM phosphate buffer (pH 8) and then incubated at 30°C for 5 min. The reaction was started by adding 5,5′-dithiobis[2-nitrobenzoic-acid] (DTNB) solution (20 µl of 1.5 mM in 10 mM phosphate buffer, pH 7) and ATCI or BTCI (20 µl of 15 mM in 10 mM phosphate buffer, pH 7) and tubes were allowed to stand in a water bath at 30°C for 20 min. The reaction was stopped by adding physostigmine (30 µl of 0.1 mM in 10 mM phosphate buffer, pH 7). The hydrolysis of acetylthiocholine or butyrylthiocholine was monitored by the formation of the yellow 5-thio-2-nitro-benzoate, and immediately recorded on the spectrophotometer (V-530 Jasco Co., Japan) at 405 nm. The inhibition (%) was calculated as Equation 5:

Inhibition (%)= $[(A_0 - A_1)/A_0] \times 100$ (5)

Where, A_0 is the absorbance of the control (without extract) and A_1 is the absorbance of the tested extract.

Statistical analysis

Microsoft Excel 2007 was used to calculate the mean and standard deviations for all of the multiple measurements and to generate graphs. SPSS for windows (version 11.5, SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Each value is expressed as the mean ± standard deviation (SD) of triplicate parallel measurements. Differences among groups were subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. A value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Extraction yield and total phenolic content

The extraction yield and total phenolic content of the *G. lancifolia* extracts are shown in Table 1. The total phenolic content was highest in the diethyl ether extract (55.5 mg GA/g extract), followed by the methanolic extract (37.8 mg GA/g extract), and the water extract (36.5 mg GA/g extract). No correlation was found between the yield and the content of phenolic. For example, although the diethyl ether extract had the highest total phenolic content, it had the lower yield than the water extract. Therefore, the total phenolic content of the *G. lancifolia* extracts depended on the type of extractive solvents. The total phenolic content of the diethyl ether extract from *G. lancifolia* (55.0 mg GA/g dried extract) was higher than that of the methanol extracts from two brown seaweeds, *Sargassum baccularia* (5.6 mg GA/g extract) and *Cladophora patentiramea* (15.6 mg GA/g extract) (Sheikh et al., 2009), and a red alga, *Kappaphycus alvarezii* (6.8 to 20.5 mg/g extract) (Kumar et al., 2008), which were in consistent with the results in this study. On the other

Table 1. Extraction yield and total phenolic content of diethyl ether, methanol and water extracts from *G. lancifolia.*

Extract	Extraction yield (g/100 g dry sample)	Total phenolic content (mg GA/g extract)
Diethyl ether	6.06 ± 0.08[1, b]	55.0 ± 0.3[a]
Methanol	4.66 ±0.05[c]	37.8 ± 0.3[b]
Water	12.50 ± 0.19[a]	36.5 ±0.4[b]

[1] Values are expressed as means ± SD (n = 3). [a-c] Values in the same column with different superscripts are significantly different ($p < 0.05$).

hand, Thitilertdecha et al. (2008) reported that both extraction yield and total phenolic content of the *Nephelium. lappaceum* methanolic extract were higher than those of the diethyl ether and water extracts, which was different from this study. The phenolic compounds in edible plants are currently recognized as a natural antioxidant because of the importance for human health (Özcan et al., 2009; Han et al., 2007). The high total phenolic content of the *G. lancifolia* extracts could be contributed to its high antioxidant and enzymatic inhibitory activities.

Scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH)

The DPPH method is described as a simple, rapid and convenient due to independent of sample polarity for screening many samples for radical scavenging activity (Marxen et al., 2007). The dose-response curve of DPPH scavenging activities of the extracts is presented in Figure 1. At 500 μg/ml, the scavenging abilities on DPPH radicals were 92.3, 89.6 and 63.6% for the diethyl ether, methanolic and water extracts, respectively while those of ascorbic acid and BHA, the well-known antioxidants, were 96.6 and 83.5%, respectively. In order to compare the DPPH radical scavenging potencies among the extracts, the concentration required to scavenge 50% of the DPPH free radicals (IC_{50}) was determined from the results of a series of concentrations tested. The scavenging activity of the extracts on DPPH was in the order of the diethyl ether (113.8 μg/ml) > water extract (118.4 μg/ml) > methanolic extract (194.9 μg/ml). However, the DPPH radical scavenging activity of *G. lancifolia* extracts was weaker than those of ascorbic acid and BHA (IC_{50} values < 10 μg/ml). The DPPH radical scavenging activity is known to have a strong correlation with phenolic compounds (Kim et al., 2006; Thitilertdecha et al., 2008). Hence, the higher DPPH radical scavenging activity of the diethyl ether extract could be attributed of its high total phenolic contents (Table 1). However, although the total phenolic content of methanolic extract was similar to that of the water extract, it exhibited lower DPPH radical scavenging activity than the water extract. This result could be explained by the facts that the DPPH radical scavenging ability of the *G. lancifolia* extracts depends on not only total phenolic content but also individual phenolic units such as protocatechuic, ferulic, coumaric, caffeic and gallic acids (Demiray et al., 2009), which may act as efficient antioxidants rather than contributing to high total phenolic contents. In addition, total phenolic contents were determined by using Folin-Ciocalteu reagent that reacts nonspecifically to phenolic compounds. Hence, total phenolic contents determined by this method could not reflect the exact amount of phenolic antioxidants (Sultana et al., 2007). Based on the IC_{50} values, the DPPH radical scavenging activity of the *G. lancifolia* extract (IC_{50} values ranging from 113.8 to 194.9 μg/ml) was stronger than that of a red alga, *K. alvarezii* (3.03 and 4.16 mg/ml for ethanol and water extracts, respectively), and a brown alga, *P. antillarum* (0.337 mg/ml) and a green alga, *C. racemosa* (14.3 mg/ml) (Chew et al., 2008; Kumar et al., 2008). Hence, the *G. lancifolia* extracts are a good source of antioxidants with high DPPH radical scavenging ability.

Antioxidant activity in the linoleic acid system with ferrothiocynate reagent (FTC)

The FTC method measures the amount of peroxide value in the beginning of the lipid peroxidation which is the primary products of oxidation. The peroxide reacts with ferrous chloride to form a reddish ferric chloride pigment. The amount of peroxide formed was determined by reading absorbance at 500 nm (Kumar et al., 2008). High absorbance is an indication of high concentrations of formed peroxides. In the control, the absorbance increased up to 1.13 at 96 h of incubation (Figure 2) and then decreased. This is due to oxidation of linoleic acid generating linoleic acid hydroperoxides which decompose to many secondary oxidation products. The oxidized products react with ferrous sulphate to form ferric sulphate and then to ferric thiocyanate of blood-red color (Jayaprakasha et al., 2001). After the incubation period (120 h), the formation of peroxides will be stopped due to non-availability of linoleic acid.The oxidative activity of linoleic acid was markedly inhibited by all the extracts tested compared to α-tocopherol (Table 2). The antioxidant effects of the diethyl ether, methanolic and

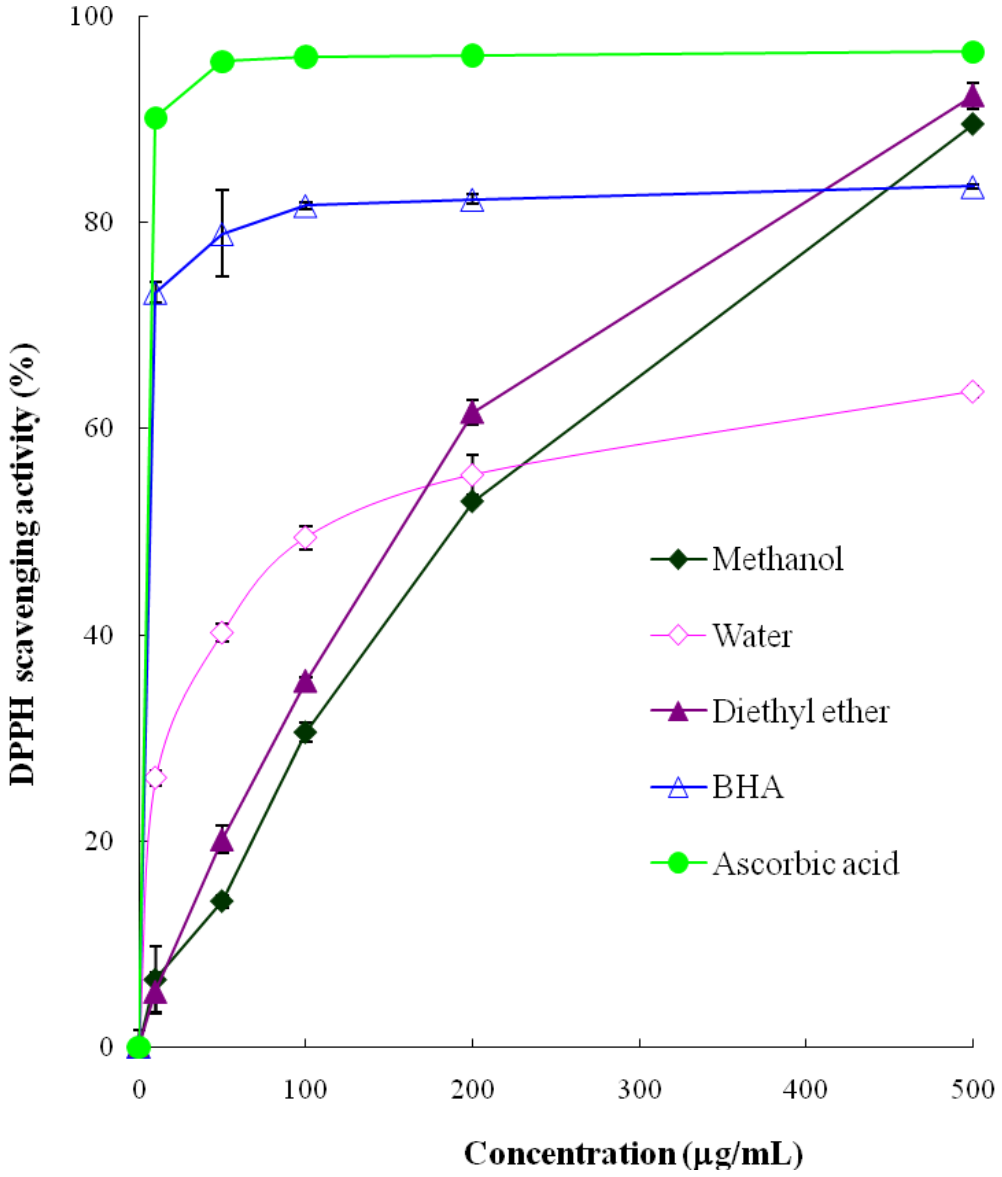

Figure 1. DPPH radical scavenging activity of diethyl ether, methanol and water extracts from *G. lancifolia*. BHA and ascorbic acid were used as possible controls. Each value is expressed as mean ± SD (n = 3).

water extracts of *G. lancifolia* incubated for 96 h with linoleic acid were 54.7, 53.6 and 49.6%, respectively. Interestingly, all the extracts of *G. lancifolia* possessed significantly ($p < 0.05$) higher antioxidant activity than α-tocopherol (45%) (Table 2).

Hydrogen peroxide scavenging activity

The reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-), hydrogen peroxide (H_2O_2), peroxyl radical ($ROO^•$), singlet oxygen ($^1O_2$), and peroxynitrite ($ONOO^-$) are known to cause oxidative damage, contributing to the development of chronic diseases such as cancer, heart disease, and cerebrovascular disease (Lee et al., 2008). The ability of *G. lancifolia* extracts to scavenge hydroxyl peroxide at 0.5 mg/ml concentration is presented in Figure 3. The highest hydrogen peroxide scavenging activity was observed in the diethyl ether extract (88.5%), with no significant difference between the methanolic and water extracts (49.7 and 47.6%, respectively). Hence, the order of hydrogen peroxide scavenging activity for the extracts was found to be similar to that of total phenolic contents, indicating that phenolic compounds of the *G. lancifolia* may contribute to the antioxidant activity in terms of hydrogen peroxide scavenging capacity. However, the

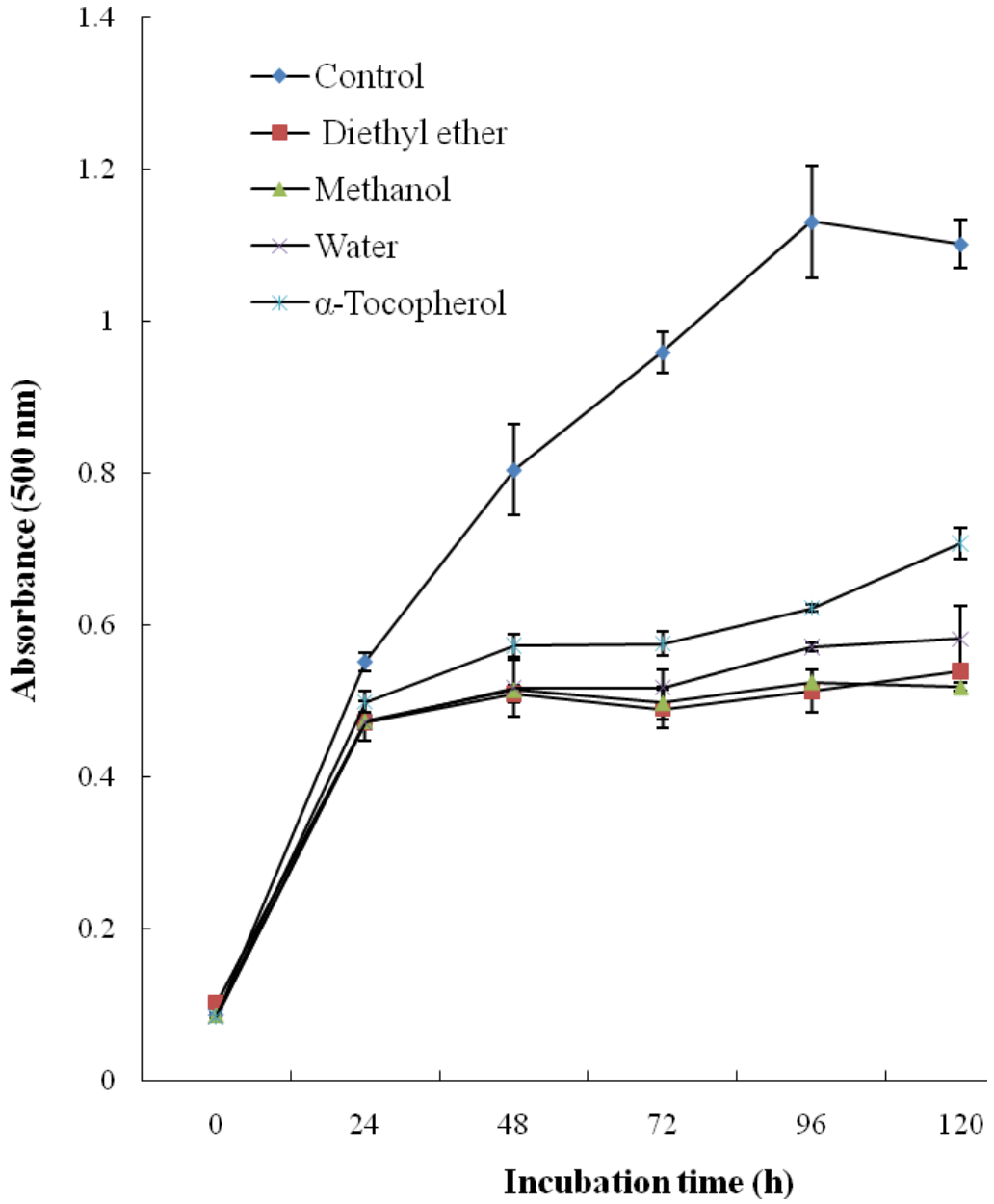

Figure 2. Anitoxidant activity of diethyl ether, methanol and water extracts from *G. lancifolia* as measured by the ferric thiocyanate method. α-Tocopherol was used as possible control. Each value is expressed as mean ± SD (n = 3).

abilities of ascorbic acid and BHA to scavenge hydrogen peroxide were 96.8 and 95.7%, respectively at the same concentration, which were significantly ($p < 0.05$) higher than those of the *G. lancifolia* extracts.

Reducing power

In the reducing power assay, the presence of anti-oxidants in the samples would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. A higher absorbance indicated a higher reducing power. The reducing power of the diethyl ether, methanolic and water extracts of *G. lancifolia* was a concentration dependent (Figure 4). The diethyl ether extract showed higher activity than the other extracts at all concentrations examined, which was similar to the result of DPPH radical scavenging activity. It is believed that the DPPH radical activity of these extracts is related to their reducing power. The reducing power of a compound may

Table 2. Antioxidant activity of diethyl ether, methanol and water extracts from *G. lancifolia* as measured by the thiocyanate method.

Extract	Absorbance at 500 nm	Inhibition[1] (%)
Control[2]	1.131 ±0.074[d 3]	0.00[d]
Diethyl ether	0.513 ±0.028[a]	54.67 ±1.78[a]
Methanol	0.525 ± 0.016[a]	53.61 ± 1.08[a]
Water	0.571 ± 0.005[b]	49.55 ± 0.34[b]
α-Tocopherol	0.620 ± 0.005[c]	45.03 ± 0.34[c]

[1] Inhibition % (capacity to inhibit the peroxide formation in linoleic acid) = [1 − (absorbance of sample at 500 nm)/(absorbance of control at 500 nm)] × 100. [2] Control was incubated with linoleic acid but without the samples. [3] Results are presented as means ± SD (n = 3). [a-d] Values in the same column with different superscripts are significantly different ($p < 0.05$).

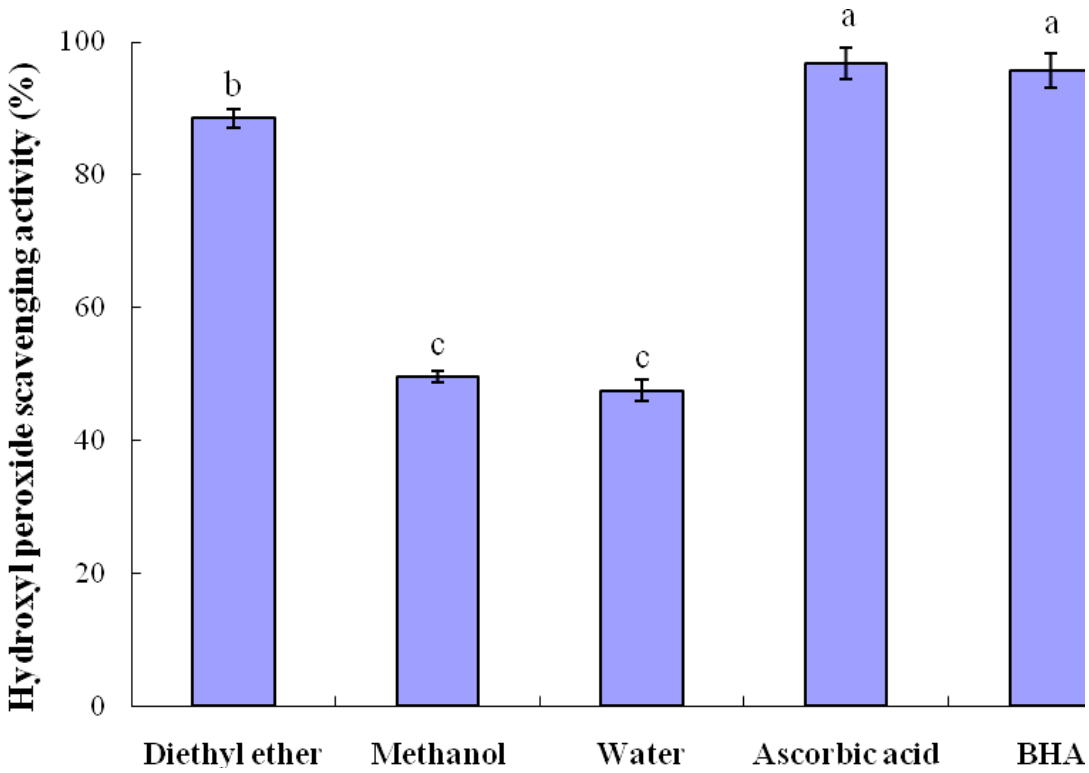

Figure 3. Hydrogen peroxide scavenging activity of diethyl ether, methanol and water extracts from *G. lancifolia*. Each value is expressed as means ± SD (n = 3). BHA and ascorbic acid were used as possible controls. [a-c] Different superscripts on the bars indicate significant difference ($p < 0.05$).

serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). At 4 mg/ml, the absorbance at 700 nm was 1.12 for the diethyl extract, whereas 1.08 and 0.49 for the water and methanolic extracts, respectively. With regards to the seaweed extracts, the absorbance at 700 nm of various extracts from a brown seaweed, *K. alvarezii* was in range of 0.16 to 0.74 at 5 mg/ml (Kumar et al., 2008), whereas at 1 mg/ml, those of other three brown seaweeds, *S. marginatum*, *P. tetrastomatica* and *T. conoides* were less than 0.1 (Chandini et al., 2008). These results suggest that the reducing potential of the diethyl ether and water extracts from *G. lancifolia* was much higher than those of *K. alvarezii, S. marginatum*, *P. tetrastomatica* and *T. conoides*.

Tyrosinase inhibitory activity

The *G. lancifolia* extracts inhibited tyrosinase activity in a Dose dependent manner (Figure 5). The diethyl ether extract had the highest tyrosinase inhibitory activity

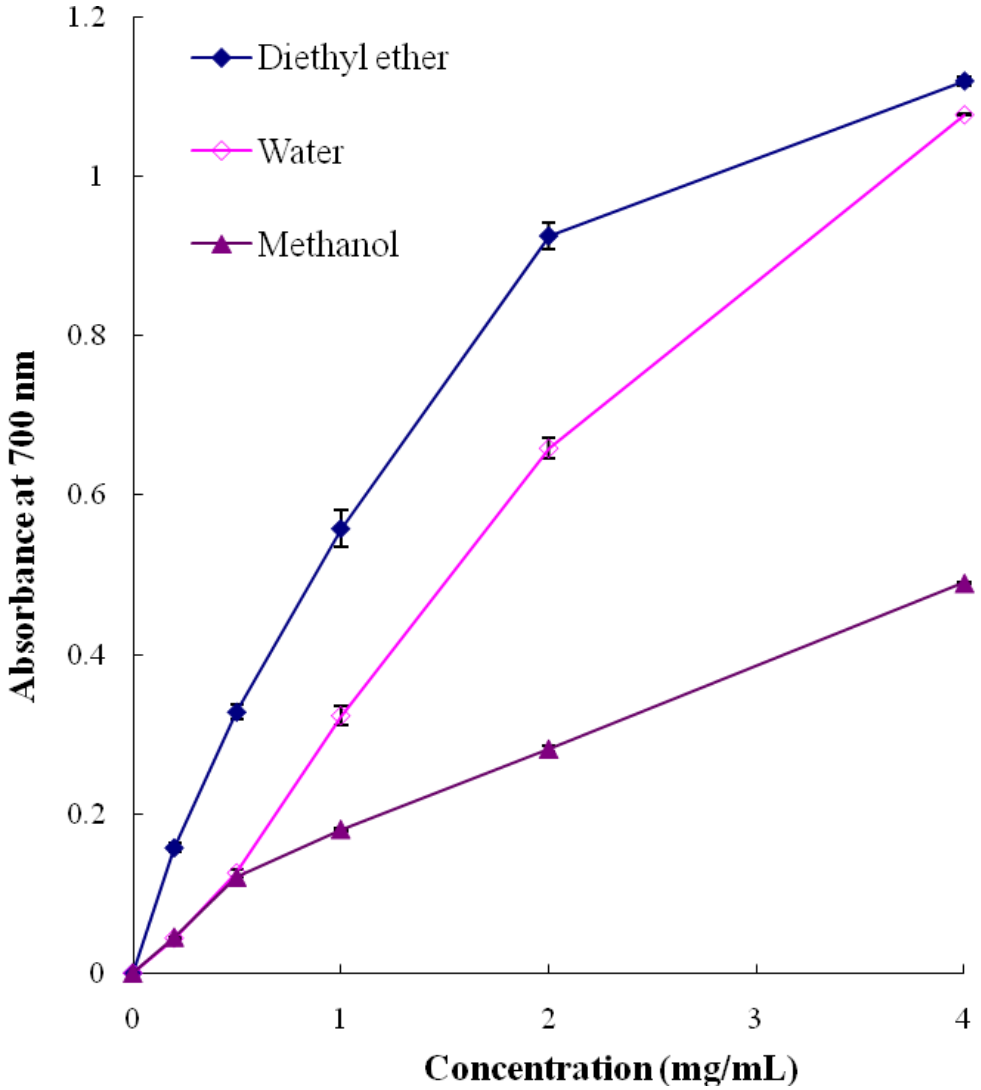

Figure 4. Reducing power of diethyl ether, methanol and water extracts from *G. lancifolia*. Each value is expressed as mean ± SD (n = 3).

among the solvent extracts. In order to compare the inhibitory potencies among the extracts, the half inhibition concentration (IC_{50}) values of the extracts were determined. The tyrosinase inhibitory activity of the diethyl ether extract at 256 μg/ml was 98.9%, which was significantly ($p < 0.05$) higher than that of the water extract (64.9%) and the methanolic extract (37.5%). At the same concentration, kojic acid as a positive control inhibited tyrosinase activity by 100% (data not shown). IC_{50} values of the *G. lancifolia* extracts were found to be 47.9, 85.9 and > 256 μg/ml for the diethyl ether, water and methanolic extracts, respectively which is higher than 5.1 μg/ml of kojic acid. Kang et al. (2004) reported that the IC_{50} values of the extracts from sixteen seaweeds against mushroom tyrosinase were higher than 500 μg/ml. Thus, the *G. lancifolia* extracts had stronger tyrosinase inhibitory activity than that of other seaweeds, but lower than that of kojic acid. The strong tyrosinase inhibitory activity of the *G. lancifolia* extracts could decrease the amount of melanin, the major factor responsible for the degree of coloration of skin (Kim et al., 2005; Yoon et al., 2009).

Acetyl- and butyrylcholinesterase inhibitory activities

BChE activity progressively increases in patients with AD, while AChE activity remains unchanged or declines. Both enzymes therefore represent legitimate therapeutic targets for ameliorating the cholinergic deficit considered to be responsible for the declines in cognitive, behavioral and global functioning characteristic of AD (Greig et al., 2002). Cholinesterase inhibitors represent the standard therapeutic approach to the treatment of AD. The anticholinesterase activity of the *G . lancifolia* extracts is

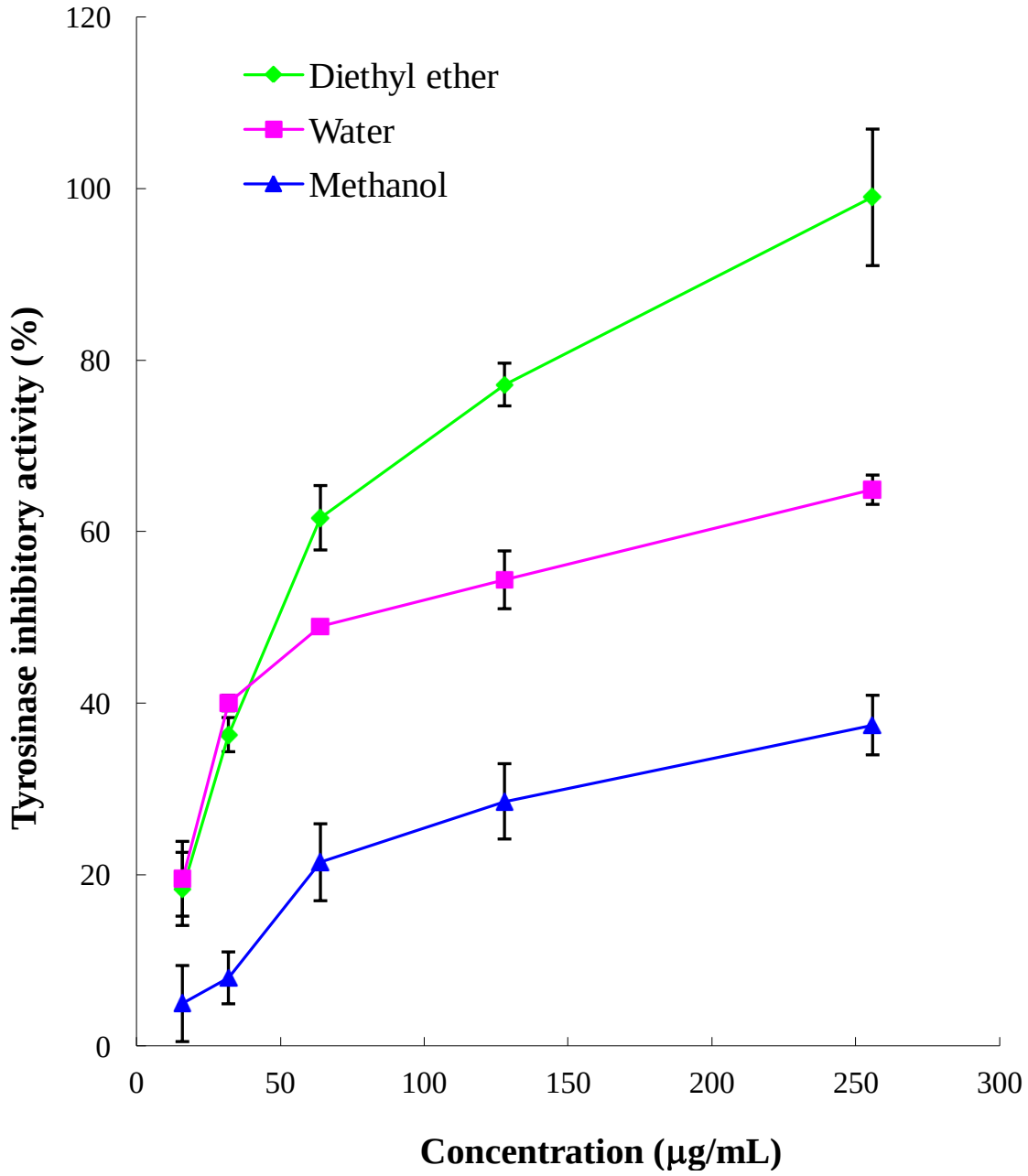

Figure 5. Tyrosinase inhibitory activity of diethyl ether, methanol and water extracts from *G. lancifolia*. Each value is expressed as mean ± SD (n = 3).

shown in Table 3. The methanolic and water extracts had moderate AChE and BChE inhibitory activities at 160 μg/ml. On the contrary, the diethyl ether extract showed strong inhibitory activity against both cholinesterases with IC_{50} of 99.8 and 23.6 μg/ml for AChE and BChE, respectively. Natajaran et al. (2009) reported that the IC_{50} values of the extracts from three brown seaweeds (*Gracilaria gracilis, Sargassum, Cladophora fasicularis*) inhabiting in South Indian coastal areas against AChE and BChE were approximately 2 mg/ml. Apparently, the anticholineterase activity of the *G. lancifolia* extracts was much stronger than that of *G. gracilis, Sargassum* and *C. fasicularis*. On the other hand, the IC_{50} values of physostigmine as a positive control against AChE and BChE were 1.37 and 1.42 μg/ml, respectively. The major classes of compound with anticholinesterase activity are alkaloids, terpenoids, glycosides and coumarins (Mukherjee et al., 2007). However, the clinical use of the cholinesterase inhibitors from the plants extracts for AD therapy are limited. Therefore, clinical efficacy and potential toxicity of active plants and compounds in larger trials requires further assessment before recommenda-

Table 3. Acetyl- and butyrylcholinesterase inhibitory activities of diethyl ether, methanol and water extracts from *G. lancifolia.*

Extract	AChE		BChE	
	Inhibitory activity (%) (160 μg/ml)	IC_{50} (μg/ml)	Inhibitory activity (%) (160 μg/ml)	IC_{50} (μg/ml)
Diethyl ether	91.1 ± 2.15[1, b]	99.8 ± 0.91	100 ±0.00[a]	23.6 ± 0.99
Methanol	34.5 ± 1.89[c]	□ 160	45.2 ±1.15[b]	□ 160
Water	27.1 ± 3.31[d]	□ 160	17.9 ± 0.50[c]	□ 160
Physostigmine	100 ± 0.00[a]	1.37 ± 0.01	100 ± 0.00[a]	1.42 ± 0.01

[1] Values are expressed as means ± SD (n = 3). [a-d] Values in the same column with different superscripts are significantly different ($p < 0.05$).

tions concerning their routine use can be identified (Mukherjee et al., 2007).

There are many reports (Luo et al., 2004; Williamson, 2001; Rasoanaivo et al., 2011) where the purified compounds resulted in the lower biological activities than the crude extracts because of the synergistic interaction or multi-factorial effects between these compounds. In many cases, it has not been possible to purify active components from crude extracts. Several proposed explanations have been given for this, such as the poor quality ethnopharmacological studies, plant material processing, different preclinical laboratory protocols from local practices, degradation of active compounds during fractionation and poor biological models to demonstrate activities (Rasoanaivo et al., 2011). Furthermore, the crude extract will be better to be commercialized than the purified compounds. Regardless of this fact, in order to know the compounds responsible for antioxidant and enzymatic inhibitory activities, the identification and characterization of active compounds of *G. lancifolia* are currently underway in our laboratory.

Conclusions

The results of the present study indicated that among the solvent extracts derived from *G. lancifolia*, the diethyl ether extract possessed the highest antioxidant, anticholinesterase and antityrosinase inhibitory activities. Phenolic compounds in the extracts might be responsible for their biological activities. These results suggest that the *G. lancifolia* extracts might be a new potential of antioxidant, cholinesterase and tyrosinase inhibitors, and could be developed as a new dietary supplement and functional foods. Finally, since the results reported herein were obtained *in vitro*, further studies are needed to confirm *in vivo*.

ACKNOWLEDGMENTS

This research was supported by the grant (No. RTI05-01-02) of the Regional Technology Innovation Program of the Ministry of Knowledge Economy (MKE), and the Regional Core Research Program funded by the Ministry of Education, Science and Technology (Medical & Biomaterial Research Center), Korea. The Han Nguyen was the recipient of a graduate fellowship provided by the Brain Korea (BK21) program sponsored by the Ministry of Education, Science and Technology, Republic of Korea.

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