

Antioxidative Properties of Brown Algae Polyphenolics and Their Perspectives as Chemopreventive Agents Against Vascular Risk Factors

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Several polyphenolic compounds and complex mixtures were isolated from brown algae species. The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) of these compounds were evaluated to determine their physiological usefulness as antioxidants for vascular protection. The antioxidative protection of low-density lipoprotein (LDL) was also evaluated and compared with that of catechin, because the generation of oxidized LDL is one of the most active and specific risk factors contributing to atherogenesis. Oral administration to rats of a commercially available sample (VNP™) containing 30% of these polyphenolic compounds and 70% dietary fiber revealed that the serum reducing capacity measured in terms of FRAP value was significantly elevated 30 min after the treatment, but declined rather quickly thereafter, indicating the good oral absorption of the compounds and their fast binding to the luminal surface of the blood vessels. An eight-week, human, clinical trial (n=31) of VNP™ showed significant improvement in erectile function measured by IIEF (international index of erectile function) score. These results collectively demonstrated the usefulness of these polyphenolic compounds as fundamental chemopreventive agents against vascular risk factors originating from oxidative stress.

Key words: Brown algae polyphenolics, Antioxidative, Oxidized LDL, Endothelial, Vascular protection, Erectile function

INTRODUCTION

The vascular system, even though it is not generally recognized as a separate organ, can be considered as a delocalized organ with numerous tubular branches, a functional capillary area as large as 700 m², and a responsibility for fundamental aspects of every cell in the body at every minute (Fawcett, 1994). However, the modern lifestyle characterized by a calorie-rich diet, lack of physical exercise and mental stress due to excessive competition constantly puts our blood vessels in a very destructive environment (Kris-Etherton, 1999; Massey, 2001).

Reactive oxygen species (ROS) of either exogenous or

endogenous (from normal metabolism and inflammation) origin constantly attack endothelial cells, leading to endothelial dysfunction which breaks the homeostasis of nitric oxide (NO) level and allows arteriosclerotic progress (Harrison, 1997). Subsequent hypercoagulative states of blood synergistically aggravate the situation by increasing the blood viscosity. More specifically, oxidative modification of low-density lipoproteins (LDLs) by ROS is strongly involved in the pathogenesis of endothelial dysfunction (Parthasarathy *et al.*, 1998; Heinecke, 1998; Steinberg, 1997). Oxidized LDL is known to alter endothelial cell functions, thereby modifying their capacity to prevent cell adhesion and their fibrinolytic properties. Recent studies have emphasized the role of oxidized LDLs (OxLDLs) in the pathogenesis of atherosclerosis, which is characterized by intimal thickening and lipid accumulation in the arteries (Steinberg, 1997). Endothelial dysfunction or activation elicited by OxLDLs is the key step in the initiation of atherosclerosis. In response to stimulation by OxLDLs,

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endothelial cells reduce the release of NO, express adhesion molecules, and secrete chemoattractant and growth factors. Subsequently, OxLDLs are avidly ingested by macrophages, resulting in foam cell formation which eventually dumps cholesterol inside the arterial wall. OxLDLs are also involved in inducing smooth muscle cell (SMC) migration, proliferation, and transformation (Ross, 1999).

In normal vessels, endothelial cells play a crucial role in maintaining vascular homeostasis by neutralizing various risk factors. However, prolonged oxidative stress to these cells exhausts them, leading to endothelial dysfunction which often generates a degenerative vicious cycle toward ischemic catastrophe such as heart attack and stroke (Usui *et al.*, 1999; Wever *et al.*, 1998; Steinbrecher, 1999; Sawamura *et al.*, 1997).

Many investigators have found several types of antioxidants from leaf, bark, root and fruit of various plant species. Recently, as polyphenolic compounds become known as safe and non-toxic antioxidants, the physiological effects of polyphenolic compounds have been receiving a lot of attention as chemopreventive or prophylactic agents to reduce the risk of heart and vascular diseases (Diaz *et al.*, 1997). Many epidemiological and *in vivo* studies have accumulated evidence showing that these antioxidants can reduce lipid peroxidation which is involved in atherosclerosis, thrombosis, and erectile dysfunction (ED).

Even though many studies have been performed on polyphenolic antioxidants derived from terrestrial plants, only very limited information has been available for such compounds from marine plants (Ng *et al.*, 2000; Deighton *et al.*, 2000; Velioglu *et al.*, 1998; Proteggente *et al.*, 2002; Pietta *et al.*, 1998; Cao *et al.*, 1996; Burns *et al.*, 2000). The only significant class of polyphenolic compounds from marine plants is "Phlorotannins" or algal polyphenolic compounds, which are only known from brown algae (*Phaeophyta*) (Swanson and Druehl, 2002). They have a wide range of molecular sizes (400 to 400,000 Da) and can occur in variable concentrations (0.5-20% of the dry weight) in brown algae. Phlorotannins are commonly believed to have defensive or protective functions, e.g., against herbivores, bacteria, fungi, fouling organisms and UV-B radiation, and to function in wound healing processes or in cell-wall construction

In this paper, we report the application of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and ferric ion reducing assays to assess the free radical scavenging capacities and the reducing potentials of the antioxidant constituents of several polyphenolic compounds and mixtures from brown algae in comparison with other well-known antioxidants (Benzie and Strain, 1996; Benzie and Szeto, 1999; Schlesier *et al.*, 2002).

As well as general antioxidative protection in support of

cardiovascular protection, more specific antioxidative protection of LDL by these compounds is also reported since OxLDL has been known as a key risk factor directly contributing to atherosclerosis. We also describe here the *in vivo* enhancement of reducing ability by oral administration of these compounds.

Since it is well accepted that deterioration of erectile function is usually accompanied by vascular degeneration, erectile function is often termed as a "barometer of cardiovascular health" (Caterina, 2000; Azadzo and Goldstein, 1992; Azadzo and Tejada, 1992; Buvat *et al.*, 1985). This concept is also supported by the findings at a molecular level that erection occurs by the vasodilation of the penile artery due to large NO release from endothelial cells under circumstances of sexual stimulation (Hurt *et al.*, 2002). Therefore the systemic assessment of erectile function can be considered as an appropriate and comprehensive *in vivo* indication of general vascular function, as well as specifically that of the penile artery. Therefore, in this paper, an eight-week human trial of a standardized edible form of brown-algae polyphenolics and the corresponding improvement of erectile function is also reported to provide a future perspective for using these polyphenolics as chemopreventive agents against the risk factors of vascular diseases.

MATERIALS AND METHODS

All the reagent grade chemicals, purchased from Sigma, Aldrich and Merck chemical companies, were used without further purification. Thin-layer chromatography was performed on Merck silica gel GF-254 and an RP-18F-254 precoated plate. Identification was done with UV light and typical TLC indicating solution (*p*-anisaldehyde/ sulfuric acid/ acetic acid mixture). Flash column chromatography was performed with Merck silica gel (230-400 mesh). UV spectra (in EtOH) were recorded on an HP 8453 UV spectrophotometer and IR spectra were measured on a Bomem MB-100 FT-IR spectrometer. The NMR spectra were recorded on a JEOL ECP-500 FT NMR at 500 MHz for ¹H and 125 MHz for ¹³C, using TMS as an internal standard. HPLC was performed on an HP 5980A. FABMS was obtained with a VG Autospec Ultima mass spectrometer.

Pretreatment of algae material

Eisenia bicyclis was freshly collected from Ulung Island, *Ecklonia Stolonifera* from Kijang, and *Ecklonia cava*, *Ecklonia kurome* and *Hizikia fusiformis* from Cheju Island, all in Korea. Their leaves were washed with tap water immediately after collection, air-dried at room temperature in a darkened room until a water content of 15-25 wt% was reached. The dried algae leaves were cut into small pieces and kept at -40°C until use. The samples of each

species prepared in this manner *Hizikia fusiformis*, *Ecklonia kurome*, *Ecklonia Stolonifera*, *Eisenia bicyclis* and *Ecklonia cava*, were designated as A1, A2, A3, A4 and A5, respectively.

Preparation of polyphenolic fraction C1

A1 (100 g) was extracted with boiling water (2 L) for 1 h. The resulting solution was dried and milled to yield a brown powder (27 g), 10 g of which was dispersed to distilled water (50 mL) and the soluble portion was subjected to ODS column chromatography with 10% methyl alcohol. The eluted solution, which gave a single broad peak, was collected and evaporated to dryness in a rotary evaporator to give dark brown solid (2.3 g).

Preparation of crude samples for polyphenolic compounds C2, C3, C4 and C5

A2, A3, A4 and A5 (100 g each) were extracted with 95% ethanol (1 L) for 2 h in a water bath at 50°C. The extraction was repeated twice. The combined ethanolic extracts were evaporated under reduced pressure to give a yellowish to dark brown, gummy-solid and dissolved in distilled water. The aqueous suspension was partitioned in ethyl acetate. The ethyl acetate soluble portion was subjected to silica-gel column chromatography with ethyl acetate as an eluent. The eluted solutions were evaporated to dryness in a rotary evaporator to give the dark brown solids EA2, EA3, EA4 and EA5, respectively.

Preparation of C2, C3, C4 and C5

Final purification to obtain compounds C2, C3, C4 and C5 from the corresponding EA2, EA3, EA4 and EA5 fractions, respectively, was accomplished by HPLC [Waters Spherisorb S10 ODS2 column (20×250 mm); eluent, 30% MeOH; flow rate, 3.5 mL/min] to isolate the major peak from each fraction. Each fraction was evaporated to dryness in a rotary evaporator to give C2, C3, C4 and C5, respectively, as white solids.

C2: white solid; UV(EtOH) λ_{\max} 232 nm; $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) 6.04 (s, 2H) 6.09 (s, 2H), 8.63 (s, 2OH), 9.07 (s, 2OH), 9.13 (s, 2OH), 9.14 (s, 4OH), 9.26 (s, 2OH); $^{13}\text{C-NMR}$ (DMSO- d_6 , 125 MHz) 94.3, 96.7, 98.3, 98.4, 100.2, 122.5, 123.2, 124.1, 137.8, 141.9, 142.4, 145.0, 145.9, 151.8, 159.3, 161.0; FABMS m/z 743.1 (M+H) $^+$.

C3: white solid; UV(EtOH) λ_{\max} 235 nm; $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) 5.94 (s, 2H) 6.15 (s, 2H), 7.91 (s, 2OH), 8.75 (s, 2OH), 9.11 (s, 4OH), 9.16 (s, 2OH), 9.46 (s, 2OH); $^{13}\text{C-NMR}$ (DMSO- d_6 , 125 MHz) 94.3, 94.4, 96.7, 98.6, 104.8 122.9, 123.6, 123.8, 137.7, 141.8, 142.3, 145.1, 146.3, 152.2, 159.2, 160.9; FABMS m/z 743.1 (M+H) $^+$.

C4: white solid; UV(EtOH) λ_{\max} 225 nm; $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) 5.71 (d, $J=2.40$, 2H), 5.79 (t, $J=1.8$,

1H), 5.81 (d, $J=1.8$, 1H), 5.93 (s, 1H), 5.97 (d, $J=3.0$, 1H), 6.01 (d, $J=3.0$, 1H), 6.13 (s, 1H), 6.15 (s, 1H), 9.17 (s, OH), 9.24 (s, OH), 9.25 (s, OH), 9.29 (s, OH), 9.37 (s, OH), 9.45 (s, OH), 9.5 (s, OH), 9.62 (s, OH), 9.70 (s, OH); $^{13}\text{C-NMR}$ (DMSO- d_6 , 125 MHz) 93.6, 93.7, 94.1, 94.5, 96.2, 98.1, 98.2, 98.4, 98.5, 146.0, 146.1, 122.2, 122.3, 122.6, 123.2, 123.3, 124.0, 124.2, 137.1, 137.2, 141.9, 142.0, 142.6, 142.9 145.9, 146.1, 151.2, 153.1, 154.2, 155.9, 158.8, 160.3; FABMS m/z 743.1 (M+H) $^+$.

C5: white solid; UV(EtOH) λ_{\max} 227 nm; $^1\text{H-NMR}$ (DMSO- d_6 , 500 MHz) 5.73 (d, $J=1.85$, 1H), 5.76 (d, $J=1.85$, 1H), 5.84 (t, $J=1.85$, 1H), 5.83 (t, $J=1.85$, 1H), 6.29 (s, 1H), 6.43 (s, 1H), 6.72 (s, 1H), 8.22 (s, OH), 9.19 (s, OH), 9.22 (s, OH), 9.45 (s, OH), 9.86 (s, OH), 9.88 (s, OH), 10.15 (s, OH); $^{13}\text{C-NMR}$ (DMSO- d_6 , 125 MHz) 94.3, 94.0, 95.3, 96.9, 97.0, 98.8, 99.6, 103.8, 103.9, 120.6, 123.0, 123.1, 126.9, 134.5, 137.4, 142.5, 145.3, 147.0, 147.5, 150.0, 150.9, 151.4, 159.4, 159.5, 160.5, 160.8; FABMS m/z 603.1 (M+H) $^+$.

Samples for animal and human

VNPTM, a standardized, commercial sample of an edible form of brown-algae polyphenolics, registered as a food ingredient in Korea, was kindly supplied by Ventree Co., Ltd. and used for animal and human tests. Polyphenolic composition of VNPTM was determined by extracting 10 g of VNPTM with absolute ethanol (100 mL) for 2 h in a water bath at 50°C, and repeating the extraction twice. The combined ethanolic extracts were evaporated under reduced pressure to give a dark brown solid C6 [2.5 g]. HPLC analysis showed that C6 contained C1 (38.7%), C2 (2.8%), C3 (3.2%), C4 (5.0%) and C5 (1.2%) with other unidentified polyphenolic peaks (49.1%).

Free radical scavenging activity

The DPPH radical scavenging effect was carried out according to the method first employed by Blois (1958). The sample solution (100 μL) was added to 900 μL of DPPH solution in ethanol (1.01×10^{-4} M). After incubation at room temperature for 30 min, the absorbance of this solution was determined at 518 nm using a spectrophotometer and the remaining DPPH was calculated. All experiments were carried out in triplicate and repeated at least three times. Results are expressed as the percentage decrease with respect to control values. Each fraction was evaluated at the final concentration of 100 $\mu\text{g/mL}$ in the assay mixture.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed as previously described by Benzie and Strain (1999). The experiment was conducted at 37°C under pH 3.6 condition with a blank sample in parallel. In this assay, reductants ("antioxidants") in the

sample reduce the Fe³⁺/TPTZ (tripirydyltriazine) complex present in stoichiometric excess, to the blue colored ferrous form, with an increase in absorbance at 593 nm. The difference in absorbance between the blank and each sample is proportional to the total ferric reducing antioxidant power (FRAP value) of the antioxidants in the sample. An aqueous solution of known ascorbic acid was used for calibration. The final results were expressed as micromolar ascorbic acid equivalent (μ M). The assay was evaluated at sample concentrations of 100 μ g/mL and at isolated compound concentrations of 500 μ M.

Prevention of LDL from oxidation

Oxidation of LDL (5 mg protein/mL) was carried out in a water bath at 37°C. A sample of 100 μ L of LDL (5 mg/mL) was incubated with 800 μ L of 30 μ M CuSO₄ (in phosphate buffer, pH 7.4) and 100 μ L of prepared sample (2 mg/mL) at 37°C for 4 h. After reaction, 300 μ L of 100 μ M EDTA was added to the mixture solution to stop the reaction. A mixture of 1 mL of the TBA-TCA-HCl reagent (0.375% thiobarbituric acid, 15% trichloroacetic acid, 0.25N hydrochloric acid) and 0.5 mL of the post-incubated sample mixture was placed in a test-tube with a screw cap. The tube was placed in boiling water for 30 min. After cooling, the reaction mixture was centrifuged at 3,000 rpm for 15 min. The absorbance of the supernatant was measured at 535 nm. The malondialdehyde concentration of the sample can be calculated using tetramethoxypropane as a standard (Buege and Aust, 1987).

In vivo reducing capacity

VNPTM was suspended with 0.5% CMC-Na (carboxymethyl-cellulose sodium) solution to give 200 mg/mL of solution. The formulated solution was well suspended by tube mixer and subsequently administered orally by intubating the solution directly into the stomach of three 6-week-old male Sprague Dawley rats. Administration volume of the solution was adjusted according to the body weight of each rat to yield a 2000 mg/kg (600 mg/kg of the polyphenolics) dose of the sample. Blood samples (1 mL/rat) were withdrawn at four time points: just before administration (control), and 30 min, 1 h and 6 h after administration. The plasma of the sample was isolated and kept at -40°C until use. The FRAP value of each sample was measured according to the method described above.

Human trial for erectile function

Patient screening: The study included the first 40 men who selected to receive VNPTM for the improvement of ED at the Urology Department, Anam Hospital, Korea University, Korea, and who met the study criteria, and agreed to participate in an 8-week, non-comparative trial. Inclusion criteria were: (1) ED duration of at least 6 months;

(2) only one, consistent, heterosexual partner for at least the past 6 months.

Dosage and administration: Included participants were given 360 capsules of VNPTM (400mg VNPTM per capsule) and instructed to ingest 6 capsules daily, three in the morning and three in the evening. A follow-up visit for assessment was scheduled for 8 weeks later.

Evaluations: Erectile function was assessed using the International Index of Erectile Function (IIEF), a 15-item, self-administered questionnaire (Rosen *et al.*, 1997). The maximum of the total IIEF score is 75 and the minimum is 5, with higher scores indicating greater erectile function. The 15 questions can be grouped into five response domains that address key elements of sexual dysfunction: erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction. Patients completed the IIEF at the start of the study and after 8 weeks.

Statistical analysis: All *in vitro* experimental results are expressed as mean \pm SD of three different measurements, those of *in vivo* as mean \pm SD of three different rat samples. IIEF scores were expressed as mean \pm SD of the test groups. IIEF scores before and after treatment were compared using the paired t-test. Statistical significance was set at P<0.05.

RESULTS AND DISCUSSION

Polyphenolic fraction C1 turned out to be a complex of polyphenol called phlorotannin, with a molecular weight of 10,000-400,000 dalton. ¹H-NMR data showed chemical shifts only on the range of phenyl and phenolic protons. The C2, C3, C4 and C5 fractions turned out to be single molecules, whose structures were identified by comparing the corresponding spectral data with those of the literature. Spectral data for C2, C3, C4 and C5 were well matched with 6,6-bieckol, 8,8-bieckol, dieckol and phlorofurofukoekol, respectively (Fig. 1) (Fukuyama *et al.*, 1989; 1989; 1990).

Since it is becoming clear that measurement of the antioxidant activities of a sample through a single *in vitro* or *in vivo* method may not provide a good prediction of the samples efficacy in human subjects, the antioxidant activity of each fraction in this study was analyzed using both FRAP and the radical scavenging activity on DPPH. Regardless of the structure or molecular weight, the brown-algae polyphenolic compounds used in this study showed excellent free-radical scavenging activity; better in fact than that of well-known antioxidants such as catechin, tocopherol, *t*-butylhydroxyanisole (BHA) and *t*-butylhydroxytoluene (BHT) (Table I). The high FRAP values confirmed their antioxidative power in terms of the reducing ability of the medium which contains them. This activity can boost the antioxidant capacity of the blood stream which is in direct contact with the endothelial cells of the blood vessels which are

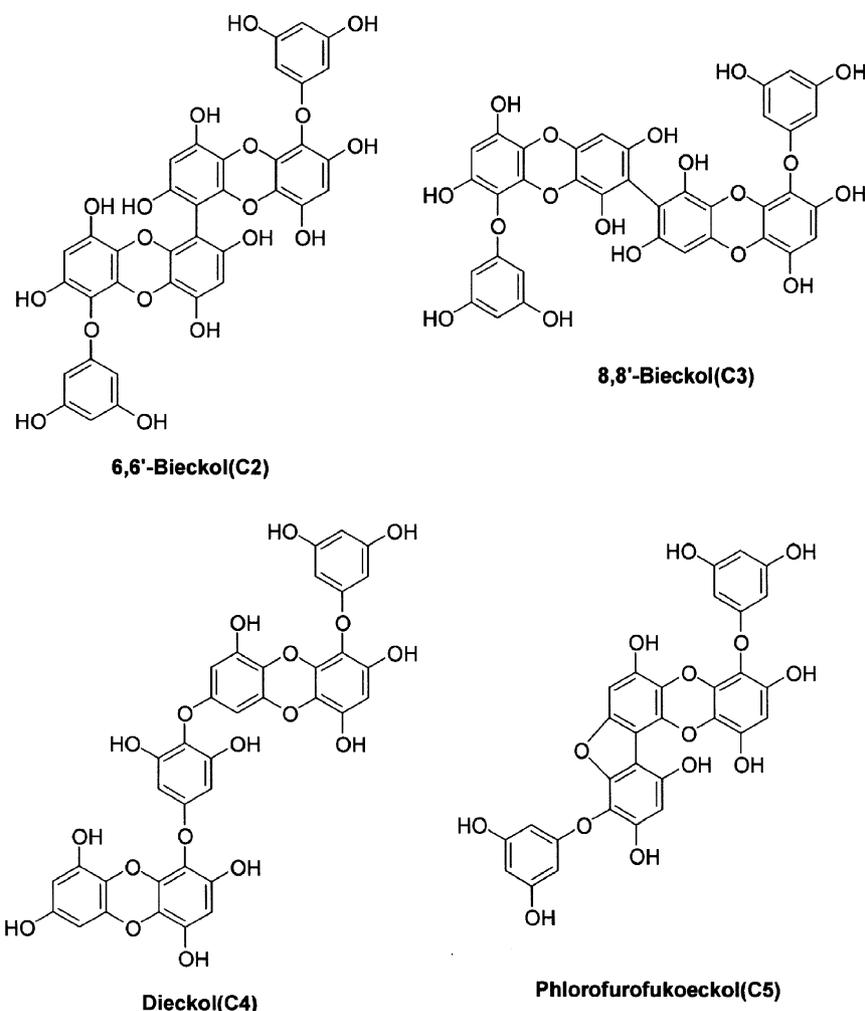


Fig. 1. Structures of **C2**, **C3**, **C4**, **C5**

Table I. Free Radical (DPPH) Scavenging Activity and Reducing Power (FRAP)

sample	DPPH scavenging, EC ₅₀ (μM)	FRAP value (μM)	
		At 500 μM	At 100 μg/mL
C1	8.9 μg/mL	*N/A	166 ± 9
C2	8.0	648 ± 16	175 ± 3
C3	6.8	764 ± 16	207 ± 3
C4	7.5	543 ± 7	146 ± 3
C5	8.0	427 ± 19	142 ± 7
C6	10.3 μg/mL	*N/A	120 ± 9
Catechin	10	502 ± 7	305 ± 7
BHA	34	515 ± 16	572 ± 14
BHT	144	126 ± 9	123 ± 9
tocopherol	17	155 ± 14	72 ± 9

*Data are not available because C1 and C6 are mixtures.

susceptible to oxidative destruction. While radical scavenging activity and reducing power are good *in vitro* indications

of the general antioxidative capacity of the substances, their ability (either direct or indirect), to prevent the oxidative generation of secondary risk factors such as the oxidized form of LDL may be more important and useful for cardiovascular protection. Oxidation of LDL can take place by transition metals and by oxidative attack from oxygen free radicals produced by macrophages or vascular cells (especially HO· and O₂⁻) (Diaz *et al.*, 1997; Halliwell and Gutteridge, 1992; Stohs and Bagchi, 1995). It is well known that the high serum level of LDL-cholesterol is a serious risk factor for atherogenesis. However, in a physiological sense, the danger of high LDL cholesterol comes mainly from its oxidized form, OxLDL. Therefore it is very important to suppress the formation of OxLDL to prevent endothelial dysfunction and atherogenesis.

The brown-algae polyphenolic C6 showed better protection than catechin, whose cardiovascular benefit has long been recognized (Table II). The edible form of these polyphenolics, VNP™ which contained about 30% polyphenols, showed comparable activity with catechin. Further studies

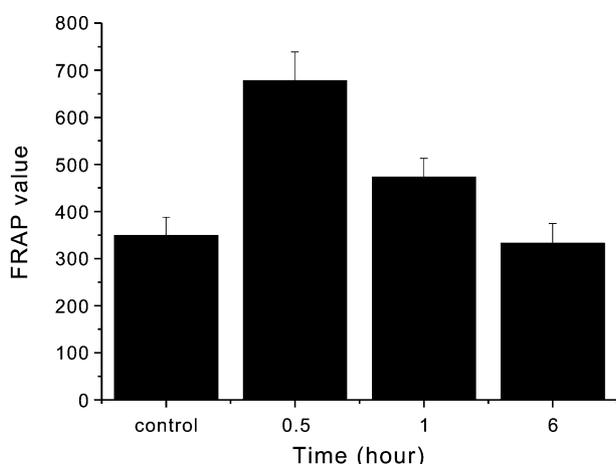
Table II. Protection of LDL from oxidation

Sample ^a	Inhibition of LDL oxidation (%)
VNP TM	18.7 ± 1.4
C6	45.3 ± 2.1
Catechin	24.7 ± 1.6
BHA	60.1 ± 3.1

^aConcentration of each sample during measurement was 0.2 mg/mL

on the activity of individual molecules are in progress and will reveal more about the structure-activity relationship. Polyphenolic compounds have been known to have good affinity toward some types of peptides or proteins depending on their three-dimensional structures (Haslam, 1996). Therefore their potent protection activity toward LDL may result from their unique affinity toward specific apoproteins on the LDL surface. Since OxLDLs influence cellular properties principally by attaching to specific receptors, the so-called scavenger receptors (SRs) (Steinbrecher, 1999; Sawamura *et al.*, 1997), natural agents which specifically suppress LDL oxidation and have general antioxidative capacity will be very promising as prophylactic agents for cardiovascular health.

As an *in vivo* evaluation of the antioxidative ability of the brown-algae polyphenolics in vascular circulation, the reducing ability of the plasma was measured upon oral administration of the edible form of brown-algae polyphenolics. In a study with rats fed with 600 mg/kg of brown algae polyphenolics, the antioxidant capacity of the blood extract, as represented by FRAP in equivalent of Vitamin C, rose to a maximum at 30 min after oral ingestion, and then declined slowly after 1-6 h (Fig. 2). Comparing this result with the kinetic study using radiolabeled procyanidins (a type of polyphenol with comparable molecular skeleton and OH groups), which revealed that absorption began 10 min after oral ingestion by mice and slowly declined after 3-7 h and that the distribution of the radioactivity tended to

**Fig. 2.** Rat Plasma level of FRAP after oral administration of VNPTM

be localized at proline-rich tissues such as aorta (Laparra *et al.*, 1977), brown-algae polyphenolics showed a similar kinetic pattern with procyanidins. Further studies will reveal a more accurate estimate of the physiologically feasible concentrations of polyphenolic compounds in humans, as well as a greater understanding of which components are the most absorbed, and how they are absorbed, transported through the blood, taken up by various tissues, and metabolized.

In a human trial for the oral administration of the edible form of brown-algae polyphenolics (VNPTM), erectile function was evaluated by comparing the IIEF scores at the start of the study and after 8 weeks. Thirty-one of the 40 patients finished the 8-week test. Average age of the patients (n=31) who finished the test was 52.7±7.7 (age range 38-65). Mean responses to IIEF questions are shown in Table III. The responses at the start of the study and 8th week were evaluated according to the following domains: erectile function (EF: score range 1-30), orgasmic function (OF: score range 0-10), sexual desire (SD: score range 2-10), intercourse satisfaction (IS: score range 0-15), and overall satisfaction (OS: score range 2-10) (Rosen, *et al.*, 1997; Gil, *et al.*, 2001; Lee, *et al.*, 2001).

Total IIEF score significantly increased from 29.1±13.1 to 47.0±14.5, an improvement of 62%. When the IIEF scores were grouped into their five separate domains, mean IIEF scores at the 8th week were significantly greater than those at the start of the study for all domains (all p<0.01). The degree of improvement was ranked in the following order: OF (87%), IS (74%), EF (66%), OS (62%), and SD (20%). Scores on key questions 3 (frequency of penetration) and 4 (frequency of maintaining an erection after penetration), which directly indicate the ability to

Table III. Patient IIEF Responses

Score Type	0 week (mean±SD)	8 week (mean±SD)	Change (%)	p Value
Total score	29.1 ± 13.1	47.0 ± 14.5	62	<0.01
Erectile Function (EF)	11.6 ± 6.5	19.3 ± 6.7	66	<0.01
Question 3*	1.9 ± 1.4	3.3 ± 1.5	74	<0.01
Question 4**	1.8 ± 1.4	3.2 ± 1.5	77	<0.01
Orgasmic Function (OF)	3.8 ± 2.7	7.1 ± 2.6	87	<0.01
Sexual Desire (SD)	5.0 ± 1.9	6.0 ± 1.6	20	<0.01
Intercourse Satisfaction (IS)	4.7 ± 3.0	8.2 ± 2.9	74	<0.01
Overall Satisfaction (OS)	4.0 ± 1.8	6.5 ± 1.9	62	<0.01

*When you attempted sexual intercourse, how often were you able to penetrate (enter) your partner? Rated on a scale of 1 (almost never or never) to 5 (almost always or always); score of 0=no attempt made.

**During sexual intercourse, how often were you able to maintain your erection after you had penetrated (entered) your partner? Rated on a scale of 1 (almost never or never) to 5 (almost always or always); score of 0=no attempt made.

achieve and maintain an erection sufficient for sexual activity, were improved by 74% and 77%, respectively ($p < 0.01$). It is very important to note that despite the marginal improvement in sexual desire (20%) which is of a psychological nature, great improvements were reported in the domains directly related with erection which is of a physical nature and dependent on normal vascular function of the penile artery. This suggests that the 8-week oral administration of the brown-algae polyphenolics significantly improved the function of the penile artery which physically controls erection. It is also noteworthy that the great increases of the scores in orgasmic function (87%), intercourse satisfaction (74%) and overall satisfaction (62%), as well as erectile function (66%), in comparison with the results for sildenafil reported by Marks *et al.* (Marks, *et al.*, 1999) (27%, 44%, 39% and 66%, respectively), indicate that the brown-algae polyphenolics significantly contributed to the normalization of the general vascular conditions around the penis. In other words, it strongly indicates that the long-term administration of these polyphenolics should significantly contribute to the neutralization of oxidative risk factors, thereby improving peripheral blood circulation around the muscles and nerves involved in the sexual function as well as the penile artery.

Since no side effects were reported, it is very promising to use brown-algae polyphenolics as a chemopreventive agent for general protection of the vascular system as well as improvement of erectile function.

In summary, brown-algae polyphenolics showed excellent antioxidative activities, both in the free-radical scavenging experiment and FRAP measurement. They also showed significant protection of LDL from oxidation. *In vivo* measurement of their reducing power showed that their absorption into the blood stream and clearance kinetics were for polyphenolic compounds. The affinity of polyphenolic compounds toward the exposed connective tissue of damaged blood vessels is thought to further potentiate their effectiveness since these damaged areas are the most susceptible sites for oxidative stress due to inflammatory action. An 8-week oral administration of these compounds showed a significant improvement in IIEF scores in all domains, supporting their physiologically significant antioxidative performance. This finding suggests the possibility for further development of a more refined and potent chemopreventive agent against the oxidative risk factors of vascular health.

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