J. Korean Soc. Appl. Biol. Chem. 53(1), 33-38 (2010)

Article

Antioxidant and Antimicrobial Activities of Extracts from a Medicinal Plant, Sea Buckthorn

Jong Hyun Jeong[1], Ji Won Lee[1], Kyoung Su Kim[3], Ju-Sung Kim[2], Sang No Han[4], Chang Yeon Yu[1], Ju Kyong Lee[1], Yong Soo Kwon[5], and Myong Jo Kim[1,2]*

[1]*College of Bioscience & Biotechnology, Kangwon National University, Chuncheon 200-701, Republic of Korea*

[2]*Oriental Bio-herb Research Institute, Kangwon National University, Chuncheon 200-701, Republic of Korea*

[3]*Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea*

[4]*Samsung Herb Medicine Co., Ltd, Chuncheon 200-160, Republic of Korea*

[5]*College of Pharmacy, Kangwon National University, Chuncheon 200-701, Republic of Korea*

Received August 7, 2009; Accepted December 10, 2009

This study was performed to evaluate the antioxidant and antimicrobial activities from the methanol extracts of the root and stem of *Hippophae rhamnoides* and their further partitioned fractions including hexane, ethyl acetate, butanol, and water. Antioxidant activities of the plant parts were measured by 1,1-dephenyl-2-picryl-hydrazyl (DPPH), superoxide dismutase (SOD)-like activity, and ferric thiocyanate (FTC) assays, and compared to standard antioxidants of butylated hydroxyl anisole, butylated hydroxyl toluene, α-tocopherol, and ascorbic acid. Antimicrobial activity of the plant extracts and fractions were evaluated by determining the minimum inhibitory concentration values. DPPH assay showed that the overall strong antioxidant activities from the methanol extracts and fractions. The values of SOD-like activities in hexane fractions of the root and stem were close to the measurement of a reference, ascorbic acid at 1,000 ppm. The methanol extract of the root in FTC assay showed a remarkable antioxidant and free radical scavenging activity. The extracts and fractions of the root and stem showed better antimicrobial activity than compared antimicrobial agents, (+)-catechin, ketoconazol, and mycostantin. This study indicates that the plant root and stem contain a variety of compounds contributing to antioxidant and antimicrobial activity, which could be used for food additives and the development of useful natural compounds.

Key words: antimicrobial activity, antioxidant activity, DPPH, FTC, *Hippophae rhamnoides*, ROS, SOD

Reactive oxygen species (ROS) such as superoxide anion radical (O_2•-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (•OH) are produced during physiological process in aerobic organisms. ROS are very toxic and reactive, causing severe damage to proteins, lipids (membranes), and nucleic acids. The inappropriate regulation of ROS has dire consequences to an organism, which is highly implicated with diseases. These include cancer, diabetes, atherosclerosis, liver chirrhosis, and aging process [Brownlee, 2001; Serafini *et al.*, 2002; Di Matteo and Esposito, 2003; Behera *et al.*, 2006]. Evidence shows that patients with markers of ROS-induced damage also reveal a reduction in their antioxidant defense systems [Coppey *et al.*, 2003; Martin-Gallan *et al.*, 2003]. In addition to harmful effects of abnormal ROS production in organisms, ROS can be also produced in food products via their auto oxidation or microbial spoilage during prolonged storage [Yildirim *et al.*, 2000]. Consequently, ROS-induced lipid peroxidation leads not only to a loss in food quality, but also to associated heath risks [Ladikos and Lougovois, 1990; Heo *et al.*, 2005]. Thus, synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), or antimicrobial agents (calcium benzoate, sodium benzoate, sorbate) can be used as food additives to protect the human body against oxidative damage by

*Corresponding author
Phone: +82-33-250-6413; Fax: +82-33-253-6413
E-mail: kimmjo@kangwon.ac.kr

doi:10.3839/jksabc.2010.006

free radicals and ROS [Sherwin, 1990]. However, the fact that the synthetic agents can cause liver damage or carcinogenesis, drives search for biological compounds from natural resources, which are more effective and safer [Branen, 1975; Grice, 1988].

Plants possess a variety of beneficial factors that can be used to control human diseases. For examples, evidence supports that polyphenols derived from plants have potential health benefits with anticancer, antimicrobial, and antioxidant activities [Yamamoto and Gaynor, 2001; Cushnie and Lamb, 2005]. Sea buckthorn, *Hippophae rhamnoides* (*H. rhamnoides*) is a medicinal plant, grown in wide areas of Europe and Asia [Rousi, 1971]. Different parts of the plant have long been used for the treatments of many health problems including colds, pain, and rheumatoid arthritis [Yao and Tigerstedt, 1992; Guliyeva *et al.,* 2004]. *H. rhamnoides* possesses a wide variety of phytochemicals, many of which have potential applications for human health [Beveridge *et al.,* 1999; Zeb, 2004a]. Consistent with the importance of *H. rhamnoides*, several studies revealed that its fruits, seeds and leaves contain high amounts of beneficial compounds such as vitamins, carotenoids, flavonides, and phenolic compounds with antioxidant and antimicrobial activities [Shipulina *et al.,* 1995; Zeb, 2004b; Chauhan *et al.,* 2007]. However, there is limited information on the activities of biological compounds from *H. rhamnoides* stems and roots against oxidative stress and microbe. Hence, this study investigates antioxidant and antimicrobial activities of extracts and fractions of *H. rhamnoides* stems and roots.

Materials and Methods

Plant materials and extraction. The root and stem *of H. rhamnoides* were obtained from Samsung herb medicine Co. (Chuncheon, Korea) and dried at room temperature for 7 days. The ground materials were used for methanol extraction. After the evaporation of MeOH using a rotary vacuum evaporator (NE-2001 & AC-1112A, Eyela Co., Tokyo, Japan), the crude extracts of the root and stem were partitioned using purified water and organic solvents (Sigma-Aldrich, Strinheim, Germany) including *n*-hexane, EtOAc, and BuOH. All the processes were triplicated.

DPPH assay. Radical scavenging activity was measured using DPPH as previously described [Xiong *et al.*, 1996]. The methanolic extracts were resolved in MeOH and various concentrations of each extract were used. The assay mixture contained in total volume of 5 mL, 3 mL MeOH, 1 mL extract, and 1 mL DPPH (0.15 mM in MeOH). After 30 min incubation at 25°C, the decrease in absorbance was spectrophotometrically measured at 517 nm. BHA, BHT, and α-tocopherol were used as references. The RC_{50} value indicates the concentration of tested sample required for 50% reduction of the free radical concentration. The experiment was performed in triplicate.

Assay for SOD-like activity. SOD-like activity was measured by the method of Marklund and Marklund [1974]. The reaction mixture contained 0.2 mL of the sample, 2.6 mL of tris buffer (50 mM tris, 10 mM EDTA, pH 8.5), and 0.2 mL of 7.2 mM pyrogallol, and incubated at 25°C for 10 min. After adding 0.1 mL of 1 N HCl, the oxidized pyrogallol was measured at 420 nm using a spectrophotometer (V530, Jasco Co., Tokyo, Japan). The SOD-like activity was expressed as reduction rate of absorbance according to the following equation: SOD-like activity (%)=[1−(absorbance value of sample/absorbance value of control)]×100. The experiment was performed in triplicate.

Ferric thiocyanate (FTC) method. The test for antioxidant activity was performed using FTC as previously described [Inatani *et al.*, 1983]. The reaction mixture containing 0.5 mg of the methanol extract in 4 mL of ethanol, 2 mL of 2.5% linolenic acid in ethanol, 4 mL of 0.05 M phosphate buffer (pH 7.0), 1.9 mL of water, and 0.1 mL of 10% tween 20 were prepared in a vial and placed in a dark incubator at 40°C. To 0.1 mL of this mixture, 9.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate were added. Three minutes after the addition of 0.1 mL of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance was measured at 500 nm every 48 h. BHA was used as a positive control and a mixture without a plant extract was used as a negative control. The experiment was performed in triplicate.

Antimicrobial assay. Microorganisms used for antimicrobial assay contain bacteria, *Bacillus subtilis* (*B. subtilis*), *Escherichia coli* (*E. coli*), *Salmonella typhimurium* (*S. typhimurium*), and *Staphylococcus aureus* (*S. aureus*), and yeasts, *Candida albicans* (*C. albicans*) and *Pichia jadinii* (*P. jadinii*). Bacteria and yeasts were grown in yeast and malt extract broth (Difco, Franklin Lakes, NJ) at 37 and 30°C, respectively. The MIC values were determined using a 96-well microtiter plate [Kobayashi *et al.*, 1996]. The minimum inhibitory concentration (MIC) was defined as the lowest concentration able to inhibit the visible growth of microorganisms. The inoculum suspensions were freshly prepared from overnight cultures. Serial two-fold dilutions of the extract, sterilized by 0.45-μm Millipore filters were added to an inoculum suspension in the broth medium containing a microbe, and incubated for 24 h. MIC values for (+)-catechin, ketoconazol, mycostantin, and tetracycline were determined.

Results and Discussion

DPPH free radical scavenging activity. The scavenging activity of DPPH free radicals by the plant extracts was determined. The extracts and fractions obtained from the plant root and stem overall exhibited a remarkable antioxidant effect at low concentrations, compared to the commercial antioxidants BHA, BHT, and α-tocopherol (Table 1). The lower the RC_{50}, higher the antioxidant potential. The EtOAc fraction both from the root and stem showed the highest antioxidant activity among the tested extracts and fractions. Next higher activity was found in the BuOH fractions. Although the water fractions exhibited the weakest activity among the samples, they showed higher activities than the controls. This study suggests that the plant roots and stems possess various phytochemical components with different properties, which have a strong antioxidant activity. Polyphenolic components including flavonoids are very important plant constituents because of their scavenging activity of ROS [Puangpronpitag *et al.*, 2008]. Polyphenolic antioxidants are rich in EtOAc and BuOH fractions of plant extracts [Pyo *et al.*, 2004; Rao *et al.*, 2008]. This suggests that polyphenolic constituents in the EtOAc and BuOH fractions from *H. rhamnoides* root and stem may contribute to the highest antioxidant activities in the DPPH assay.

Table 1. DPPH radical scavenging activity of the extracts of *H. rhamnoides*

Plant extract & fractions	RC_{50} (μg/mL)	
	Root	Stem
MeOH extract	5.0±0.50	2.7±0.50
Hexane fraction	3.0±1.00	3.2±0.23
EtOAc fraction	1.5±0.29	1.0±0.35
BuOH fraction	2.5±0.58	1.4±0.20
Water fraction	9.5±0.20	3.0±0.20
BHT	34±0.58	
BHA	14±0.50	
α-Tocopherol	12±1.15	

The RC_{50} value indicates the concentration of tested sample required for 50% reduction of the free radical concentration. Data were represented as the mean ± SD of triplicates.

SOD-like activity. To investigate whether the plant extracts have superoxide-scavenging factors, we measured the ability of plant extracts to scavenge superoxide radicals that lead to pyrogallol autoxidation, as previously studied [Kim *et al.*, 1995]. As concentration of the root and stem extracts increases, SOD-like activity were also increased (Table 2). Considerable increase in SOD-like activity was particularly found from the hexane fraction of the root and stems at 1,000 ppm, which is 65.7±0.12% and 73.4±1.0%, respectively. This is a similar value to ascorbic acid used as a reference. Relatively higher SOD-like activity was shown in hexane fractions reaching over 92% at 5,000 ppm, compared to other fractions. The SOD-like activities of root and stem at 5,000 ppm followed the order hexane>EtOAc>MeOH>BuOH> water, and hexane>MeOH>BuOH>EtOAc>water, respectively. This study supports that the plant root and stem have active compounds scavenging superoxide radicals, dominantly present in the hexane fractions. Antioxidant defense mechanisms in cells against oxidative damage include various enzymatic and non-enzymatic antioxidants. SOD is a primary enzyme in enzymatic antioxidant defenses. Removal of superoxide anion radicals is considered important because they serve as precursors of other activated oxygen including H_2O_2 and •OH. Therefore, the presence of factors having strong SOD-like activity in *H. rhamnoides* could effectively eliminate the toxic effects of ROS.

Antioxidant activity of the MeOH extracts. Free radical formed under oxidative stress causes lipid oxidation, which can be prevented by natural compounds present in plants. Natural antioxidants are found in various parts of plants including root and stem [Pratt and

Table 2. SOD-like activity of the extracts of *H. rhamnoides*

Plant parts	Concentration (ppm)	SOD-like activities of various fractions (%)					
		MeOH	Hexane	EtOAc	BuOH	Water	Ascorbic acid
Root	100	3.4±0.1	7.3±0.2	2.9±0.3	1.1±0.3	0.5±0.3	32.5±0.5
	1,000	12.8±1.0	65.7±0.1	25.7±1.4	11.7±0.4	11.3±2.0	68.5±0.5
	5,000	44.3±2.0	92.4±0.3	67.9±2.0	41.1±2.0	24.1±1.5	>99.0
	10,000	66.6±0.1	95.1±0.1	80.4±2.0	58.7±2.0	33.7±2.0	>99.0
Stem	100	2.1±0.4	9.1±1.4	2.2±0.5	8.0±0.5	2.6±0.4	32.5±0.5
	1,000	25.4±1.5	73.4±1.0	31.4±1.5	21.3±2.0	21.8±0.4	68.5±0.5
	5,000	69.8±1.4	92.9±0.2.	54.0±0.3	58.5±0.5	39.7±1.7	>99.0
	10,000	72.6±1.4	94.6±0.3	58.0±2.0	61.2±0.5	48.5±1.2	>99.0

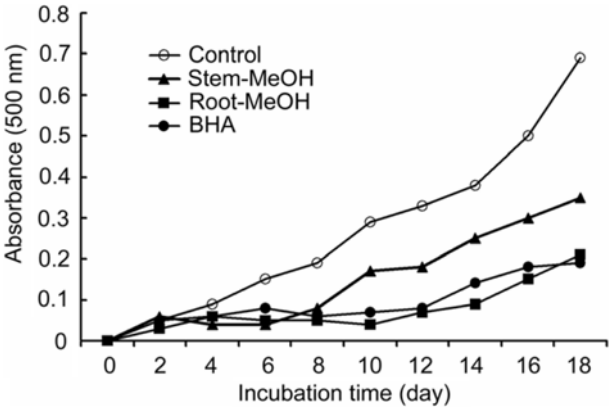

Fig. 1. Antioxidant activity of the methanol extract of the root and stem of *H. rhamnoides* as measured by the FTC method. Mean values from triplicates were shown; error bar was omitted for the clarity.

Hudson, 1992]. In this study, the antioxidant activity of the root and stem extracts of *H. rhamnoides* was measured every 2 days over a period of 18 days, using FTC. The FTC method was used to measure the amount of peroxide during the initial stage of lipid oxidation. Low absorbance values indicate high levels of antioxidant activity. The MeOH extracts of the root and stem showed higher antioxidant activities compared to the control (Fig. 1). There is a similarity between antioxidant activities of the root extract and BHA, while the antioxidant activities of the stem extract was up to 3-fold less than those of the root. The study suggests that the medicine plant contains constituents able to protect from lipid oxidation by scavenging free radicals.

Antimicrobial activity of *H. rhamnoides* extracts. There is a growing interest in searching for natural products as safer and effective alternatives to pesticides, and a source of new medicines. Plants produce a variety of antimicrobial compounds to protect themselves from biotic attack [Veluri *et al.*, 2004]. In this study, we studied the antimicrobial properties of *H. rhamnoides* extracts against microorganisms causing serious food poisoning or infections [Chickering and Park, 1919; Bodey, 1984; Pothakamurya *et al.*, 1995; Tsolis *et al.*, 1999; Hauge, 2008]. The antimicrobial activity of the extracts was assessed by determining the MIC as indicated in the materials and methods. The MeOH extracts and other fractions of the root and stem showed a higher antifungal activity against yeasts *C. albicans* and *P. jadinii*, compared to the antifungal drugs ketoconazol and mycostantin (Table 3). The MeOH extract of the stem showed 2-fold higher antimicriobial activity than that of the root against tested Gram (+) bacteria, *B. subtilis* and *S. aureus*, and yeast *C. albicans*. The root and stem extracts and fractions exhibited a higher antimicrobial activity than (+)-catechin that is a well-known plant bioflavonoid having marked antimicrobial and antioxidant activities, although they are much less effective than a microbe-driven antibacterial agent tetracycline. This result is consistent with previous reports that extract from the other parts of sea buckthorn plant, such as leaves, fruits, and seeds exhibits antioxidant and antimicrobial activities (Geetha *et al.*, 2002; Chauhan *et al.*, 2007). Taken

Table 3. Antimicrobial activity of the extracts of *Hippophae rhamnoides*

Plant parts	Extract & fractions	MIC (μg/mL)					
		Bacteria				Fungi yeasts	
		B. subtilis	*S. aureus*	*E. coli*	*S. typhimurium*	*P. jadinii*	*C. albicans*
Root	MeOH	1000	500	500	1000	125	125
	Hexane	1000	1000	500	1000	125	125
	EtOAc	500	250	250	500	125	125
	BuOH	500	125	500	500	125	125
	Water	500	1000	500	500	125	125
Stem	MeOH	500	250	500	1000	125	62
	Hexane	1000	250	500	500	62	62
	EtOAc	1000	125	250	500	62	62
	BuOH	125	500	500	500	125	125
	Water	500	500	500	500	125	125
(+)-Catechin		>1000	>1000	>1000	>1000	500	500
Ketoconazol		-	-	-	-	250	250
Mycostantin		-	-	-	-	500	500
Tetracycline		8	8	8	8	-	-

The MIC was defined as the lowest concentration able to inhibit the visible growth of microorganisms.

together, our results indicate the potential high-value of *H. rhamnoides* in the development of natural antimicrobial and antioxidant agents.

Acknowledgments. This work was supported by the Oriental Bio-herb Research Institute, Kangwon National University, South Korea, and by a Research Grant from Kangwon National University.

References

Behera BC, Verma N, Sonone A, and Makhija U (2006) Determination of antioxidative potential of lichen *Usnea ghattensis* in vitro. *Food Sci Technol Leb* **39**, 80-85.

Beveridge T, Li TS, Oomah BD, and Smith A (1999) Sea buckthorn products: manufacture and composition. *J Agri Food Chem* **47**, 3480-3488.

Bodey GP (1984) Candidiasis: A growing concern. *Am J Med* **77**, 1-48.

Branen AL (1975) Toxicology and biochemistry of butylated hydroxyanisole and butylated hydroxytoluene. *J Am Oil Chem Soc* **52**, 59-63.

Brownlee M (2001) Biochemistry and molecular cell biology of diabetic complications. *Nature* **414**, 813-820.

Chauhan AS, Negi PS, and Ranteke RS (2007) Antioxidant and antibacterial activities of aqueous extract of sea buckthorn (*Hippophae rhamnoides*) seeds. *Fitoterapia* **78**, 590-592.

Chickering HT and Park JH (1919) *Staphylococcus aureus* pneumonia. *JAMA* **72**, 617-626.

Coppey LJ, Gellett JS, Davidson EP, and Yorek MA (2003) Preventing superoxide formation in epineurial arterioles of the sciatic nerve from diabetic rats restores endothelium-dependent vasodilation. *Free Radical Res* **37**, 33-40.

Cushnie TP and Lamb AJ (2005) Antimicrobial activity of flavonoids. *Int J Antimicrob Agents* **26**, 343-356.

Di Matteo V and Esposito E (2003) Biochemical and therapeutic effects of antioxidants in the treatment of alzheimer's disease, parkinson's disease, and amyotrophic lateral sclerosis. *Curr Drug Target CNS Neurolog Disord* **2**, 95-107.

Geetha S, Sai Ram M, Singh V, Ilavazhagan G, and Sawhney RC (2002) Anti-oxidant and immunomodulatory properties of sea buckthorn (*Hippophae rhamnoides*). An in vitro study. *J Ethnopharmacol* **79**, 373-378.

Grice HC (1988) Safety evaluation of butylated hydroxyanisole from the perspective of effects on forestomach and oesophageal squamous epithelium. *Food Chem Toxicol* **26**, 717-723.

Guliyeva VB, Gul M, and Yildirim A (2004) *Hippophae rhamnoides* L.: chromatographic methods to determine chemical composition, use in traditional medicine and pharmacological effects. *J Chromatogr B* **812**, 291-307.

Hauge S (2008) Food poisoning caused by aerobic spore-forming bacilli. *J Applied Microbiol* **18**, 591-595.

Heo S-J, Park E-J, Lee K-W, and Jeon Y-J (2005) Antioxidant activities of enzymatic extracts from brown seedweeds. *Bioresour Technol* **96**, 1613-1623.

Inatani R, Nakatani N, and Fuwa H (1983) Antioxidative effect of the constituents of rosemary (*Rosmarinus officinalis* l.) and their derivatives. *Agric Biol Chem* **47**, 521-528.

Kim SJ, Han D, Moon KD, and Rhee JS (1995) Measurement of superoxide dismutase-like activity of natural antioxidants. *Biosci Biotechnol Biochem* **59**, 822-826.

Kobayashi A, Kim MJ, and Kawaz K (1996) Uptake and exudation of phenolic compounds by wheat and antimicrobial components of the root exudate. *Z Naturforsch* **51**, 527-533.

Ladikos D and Lougovois V (1990) Lipid oxidation of muscle foods: A review. *Food Chem* **52**, 295-314.

Marklund S and Marklund G (1974) Involvement of superoxide anion radical in the oxidation of pyrogallol and a convenient assay for superoxide dismutase. *Euro J Biochem* **47**, 468-474.

Martin-Gallan P, Carrascosa A, Gussinye M, and Dominguez C (2003) Biomarkers of diabetes-associated oxidative stress and antioxidant status in young diabetic patients with or without subclinical complications. *Free Radical Bio Med* **34**, 1563-1574.

Pothakamurya UR, Monsalve-Gonzàleza A, Barbosa-Cánovasa GV, and Swansonb BG (1995) Inactivation of *Escherichia coli* and *Staphylococcus aureus* in model foods by pulsed electric field technology. *Food Res Int* **28**, 167-171.

Pratt DE and Hudson BJF (1992) Natural antioxidants not exploited commercially. In *Food Antioxidants*, Hudson BJF (ed.), pp. 171-192. Elsevier Applied Science, London, UK

Puangpronpitag D, Areejitranusorn P, Boonsiri P, Suttajit M, and Yongvant P (2008) Antioxidnat activities of polyphenolic compounds isolated from *Antidesma thwaitesianum* Müll Arg. seeds and marcs. *J Food Sci* **73**, 648-653.

Pyo YH, Lee TC, Logendra L, and Rogen RT (2004) Antioxidant activity and phenolic compounds of swiss chard (*Beta vularis* subspecies cycla) extracts. *Food Chem* **85**, 19-26.

Rao YK, Fang S-H, and Tzeng Y-M (2008) Antiinflammatory activities of flavonoids and a triterpene caffeate isolated from *Bauhinia variegata*. *Phytother Res* **22**, 957-962.

Rousi A (1971) The genus *Hippophae* L.: A taxonomy study. *Ann Bot Fenn* **8**, 177-227.

Serafini M, Bellocco R, Wolk A, and Ekstrom AM (2002) Total antioxidant potential of fruit and vegetables and risk of gastric cancer. *Gastroentero* **123**, 985-991.

Sherwin FR (1990) Antioxidants. In *Food Additives*, Branen R (ed.), pp. 139-193. Marcel Dekker, NY, U.S.A.

Shipulina LD, Vichkanova SA, Fateeva TV, Krutikova NM,

Sheichenko OP, Scheichenko VI, Okhotnikova VF, Zagorii VA, and Tolkaachev ON (1995) Antiviral drug hiporamin from *Hippophae rhamnoides*: medico-biological aspects. Materials of International Scientific Practical Conference 'Food Ecology Man' 4-6 December.

Tsolis RM, Adams LG, Ficht TA, and Bäumler AJ (1999) Contribution of *Salmonella typhimurium* virulence factors to diarrheal disease in calves. *Infect Immun* **67**, 4876-4885.

Veluri R, Weir TL, Bais HP, Stermitz FR, and Vivanco JM (2004) Phytotoxic and antimicrobial activities of catechin derivatives. *J Agric Food Chem* **52**, 1077-1082.

Xiong Q, Kadota S, Tadata T, and Namba T (1996) Antioxidative effects of phenylethanoids from *Cistanche desertiola*. *Biol Pham Bull* **19**, 1580-1585.

Yamamoto Y and Gaynor RB (2001) Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. *J Clin Invest* **107**, 135-142.

Yao Y and Tigerstedt P (1992) Variation of vitamin C concentration and character correlation between and within natural sea buckthorn (*Hippophae rhamnoides* L.) populations. *Acta Agric Scand* **42**, 12-17.

Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, and Bilaloglu V (2000) Comparison of antioxidant and antimicrobial activities of tilia (*Tilia argenta* Desf Ex DC), sage (*Salvia triloba* l.) and black tea (*Camellia sinesis*) extracts. *J Agric Food Chem* **48**, 5030-5034.

Zeb A (2004a) Chemical and nutritional constituents of sea buckthorn juice. *Pak J Nutr* **3**, 99-106.

Zeb A (2004b) Important therapeutic uses of sea buckthorn (*Hippophae*): a review. *J Biol Sci* **4**, 687-693.