OPEM
www.opem.org

Oriental Pharmacy and Experimental Medicine 2007 **7(1)**, 65-73

KHUP

Antioxidant activity of water and alcohol extracts of *Thuja orientalis* leaves

Iram Nizam* and M Mushfiq

Department of Chemistry, Aligarh Muslim University, Aligarh - 202 002, India

SUMMARY

Water and alcohol extracts were prepared from dried and powdered leaves of *Thuja orientalis (T. orientalis)*. The reducing power, total phenolic content, the 1,1-diphenyl-2-picrylhydrazyl scavenging activity, inhibitory effect on Fe (II)-EDTA-H_2O_2 (Fenton reaction system) induced DNA damage and inhibitory effect on human red blood cell (RBC) hemolysis were evaluated in the present study. At a concentration of 200 mg, water and alcohol extracts of *T. orientalis* inhibited the hydrolysis of DNA by 72.859% and 65.312%, respectively. Water and alcohol extracts of *T. orientalis* also inhibited 2,2'-Azobis(2-amidinopropane) dihydrochloride induced RBC hemolysis to the extent of 69.30% and 54.55%, respectively. The reducing power and antioxidative activity of water extract was found to be more than that of alcohol extract. This is attributable to the presence of higher amount of phenolic compounds in water extract. The present results indicate that the *T. orientalis* extracts are rich sources of natural antioxidants and can protect DNA and human red blood cells against free radical induced oxidative damage.

Key words: *Thuja orientalis*; Reducing power; 1,1-diphenyl-2-picrylhydrazyl; Scavenging activity; Antioxidative activity; DNA damage; RBC hemolysis

INTRODUCTION

The reactive oxygen species (ROS), superoxide ion ($\cdot O_2^-$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($\cdot OH$) are involved in various human diseases, such as, Alzheimer's disease, aging, cancer, inflammation, rheumatoid arthritis and atherosclerosis (Freeman, 1984; Singh, 1989; Squadrito and Pryor, 1998; Pryor, 2000). Several studies have demonstrated a correlation between the antioxidant properties of phytoconstituents with oxidative stress defense (Aruoma, 2003; Amarowicz *et al.*, 2004). Recently, there is an impetus for search of powerful and non-toxic antioxidants from natural sources, especially crude drugs derived from medicinal plants (Ramarathnam *et al.*, 1997). The plant phenolics have the ability to scavenge free radicals by single-electron transfer (Hirano *et al.*, 2001). Such natural antioxidants could prevent the formation of ROS-related disorders in human beings, making them safer than synthetic antioxidants, which may be carcinogenic and harmful to the lungs and liver (Branen, 1975).

Thuja orientalis L. [syn. *Platycladis stricta* Spach; *Platycladis orientalis* (L.) Franco; *Biota orientalis* (L.) Endl.], commonly known as Oriental Thuja, Oriental Arbor Vitae and Chinese Arbor Vitae, is an ornamental conifer of the cypress family. The name 'Arbor vitae' is from Latin, 'tree of life', and is related to long life and vitality in Buddhist thoughts in China. The leaves of *Thuja orientalis* L [Syn. *Biota orientalis* (L.) Endl. (Cupressaceae)] have been used in Chinese medicine for treatment of gout, rheumatism,

*Correspondence: Iram Nizam, Department of Chemistry, Aligarh Muslim University, Aligarh - 202 002, India. Tel: +91-571-2703515; E-mail: iram2k5@yahoo.co.in

diarrhoea and chronic tracheitis (Jiangsu College of New Medicine, 1977). Some of the ethnobotanical uses and medicinal properties attributed to *Thuja occidentalis* (Grieve, 1994) are also attributed to *Thuja orientalis*. For example, *Thuja orientalis* is a nerve stimulant, expectorant, diuretic, astringent and counter irritant, and the plant extract can be used to treat warts and bronchitis with cardiac weakness (Mabey, 1988). The volatile oil of *Thuja orientalis* has shown antimicobial properties (Bagci *et al.*, 1996). Dietary *Thuja orientalis* seed oil suppresses anti-erythrocyte autoantibodies and prolongs survival of NZB mice (Lai *et al.*, 1994). Platelet activating factors, receptor binding antagonists have also been isolated from *Thuja orientalis*, and out of six compounds isolated, cedrol and pinusolide were found to be active (Yang *et al.*, 1995). The *Thuja orientalis* extract, quercetin and rutin, reduced serum urate levels of hyperuricemic mice caused by oxonate (Zhu *et al.*, 2004). The compounds isolated from the leaves inhibit expression of adhesion molecules induced by tumor necrosis factor-a on inflammatory cells (Lee *et al.*, 1999). The occurrence of terpenoids (Tomita *et al.*, 1969a,b; Tomita and Hirose, 1969), flavonoids (Natarajan *et al.*, 1970; Pelter *et al.*, 1970; Lee *et al.*, 1999) and coumarin (Lee *et al.*, 1999) in various parts of *Thuja orientalis* has been reported. Recent findings indicate that increased oxidative stress and/or defective antioxidant status contribute to the etiology of Rheumatoid arthritis (Karatas, 2003). The involvement of ROS in most of the age related disorders prompted us to investigate the antioxidant properties of *Thuja orientalis*, which has not been explored so far. The antioxidant activity was assessed by various models including red blood cell (RBC) membrane model.

MATERIALS AND METHODS

Instruments

Shimadzu UV-VIS Spectrophotometer (1240) was used for all spectrophotometric studies. Buchi rotavapor was used for vacuum drying and Remi R24 centrifuge was used for centrifugation. Remi Cyclomixer (CM-101 DX) was used for rapid mixing.

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Fluka Chemika (Switzerland). 2,2'-Azobis(2-amidinopropane) dihydrochloride (AAPH), calf thymus DNA, S_1 nuclease enzyme and gallic acid was from Sigma, St. Louis, MO. Folin-Ciocalteau reagent and Bovine serum albumin was obtained from Sisco Research Laboratories, Mumbai. All other chemicals were of analytical grade.

Plant material

The leaves of *Thuja orientalis* was collected in the month of March from the University Garden, Aligarh Muslim University, Aligarh (U.P.), India and identified by Dr. Athar Ali Khan, Department of Botany, A.M.U., Aligarh and the voucher specimen has been deposited in the herbarium of Department of Wildlife Sciences, A.M.U., Aligarh (Voucher No. 940).

Preparation of the extracts

Dried and powdered leaves of *Thuja orientalis* (50 g each) were extracted with ethanol and water, separately. The evaporation in vacuum yielded crude ethanol extract (8.2 g, 16.4%) and aqueous extract (4.5 g, 9%). The crude ethanol and aqueous extracts were dissolved in 99% ethanol and distilled water, respectively, for various studies.

Total phenolic content

We used the method of Saucier *et al.* (1999) with slight modification, and the results are expressed directly in absorbance units at 765 nm. In each analysis, 1.58 ml of water was pipetted into cuvettes, followed by addition of 20 µl of a standard solution, sample solution, or water, and the solutions were mixed well. Then 100 µl of Folin-Ciocalteau (FC) reagent was added to each

cuvette, and the solutions were mixed again. After 30 s and before 8 min, 300 μl of a 20% sodium carbonate solution was added. The solutions were left at room temperature for 2 h. Then the absorbance of the developed blue color was determined at 765 nm. The amount of light absorbed is proportional to the amount of oxidizable material present, that is, phenolic compounds. Gallic acid was used as a standard for the calibration curve. The total phenolic content is reported (Table 1) as gallic acid equivalents (mg) using the following linear equation based on the calibration curve:

$A = 0.0011x + 0.0025 \quad R^2 = 0.9995$

Where A is the absorbance and x is the gallic acid equivalents (μg).

Reducing power

Total reducing power was determined as described by Zhu *et al.* (2002). *Thuja* extracts (50 - 500 μg) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide [$K_3Fe(CN)_6$]; the mixture was then incubated at 50°C for 30 min. Afterward, 2.5 ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3,000 rpm for 10 min. Finally, 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

DPPH radical scavenging activity

DPPH radical scavenging activity was evaluated by the method of Nagai *et al.* (2003). The assay mixture contained 0.3 ml of 1.0 mM DPPH radical solution, 2.4 ml of 99% ethanol, and 0.3 ml of extract solution of concentrations varying from 50 mg to 500 mg. The solutions were rapidly mixed and scavenging capacity was measured spectrophotometrically by monitoring the decrease in absorbance at 517 nm. Ascorbic acid (1 mM) was used as positive control while reaction mixture (DPPH radical solution) minus extract solution was taken as control. The percent (%) radical scavenging was calculated by the following equation.

Where A_c = Absorbance of control at 517 nm and A_s = Absorbance of sample at 517 nm.

Inhibition of Fe (II)-EDTA-H_2O_2 induced oxidative DNA damage

Solution of DNA was prepared by dissolving 2 mg of calf thymus DNA (Sigma Chemical Company, St. Loius, MO) in 1 ml of 10 mM tris-HCl pH 7.4, 500 μg DNA and varying concentration of extract (25 - 250 μg) solution, 0.08 mM EDTA, 0.08 mM $FeSO_4$, 0.03% H_2O_2 and 20 mM Na-ascorbate. All solutions were sterilized before use. After incubation at 37°C for 1 h S_1 nuclease digestion was performed as described by Rahman *et al.* (1989). The assay determines the acid soluble nucleotides released from DNA because of enzymatic digestion. Acid soluble nucleotides were determined colorimetrically using the diphenylamine method of Schneider (1957). To a 1.0 ml aliquot, 2.0 ml of diphenylamine reagent (freshly prepared by dissolving 1 g of recrystallized diphenylamine in 100 ml of glacial acetic acid and 2.75 ml of conc. H_2SO_4) was added. The tubes were heated in a boiling water bath for 20 min. The intensity of blue color was read at 600 nm.

RBC hemolysis

Blood was obtained from healthy human donor and collected into heparinized tubes through the Blood Bank, J. N. Medical College, Aligarh Muslim University, Aligarh. Erythrocytes were separated from plasma and the buffy coat, and washed three times with 5 volumes of phosphate buffered saline (PBS), pH 7.4. During every wash, erythrocytes were centrifuged at 4,000 rpm for 10 min to obtain a packed cell preparation (Miki *et al.*, 1987). After the last wash, the packed RBC was suspended in four volumes of PBS solution. RBC oxidative

hemolysis was induced by AAPH, a peroxyl radical initiator (Miki *et al.*, 1987). Addition of AAPH to the suspension of washed erythrocytes induces the oxidation of membrane lipids and proteins, resulting in hemolysis. Two ml of the erythrocyte suspension was mixed with 2 ml of PBS solution containing varying amounts of extracts of *Thuja orientalis*, 2 ml of 200 mM AAPH in PBS was then added to the mixture. The reaction mixture was shaken gently while being incubated at 37°C for 3 h. After incubation, the reaction mixture was removed, diluted with eight volumes of PBS and centrifuged at 4,000 rpm for 5 min. The absorbance (A) of the supernatant fraction was recorded at 540 nm. Percent inhibition was calculated by the following equation

$$\% \text{ Inhibition} = [A_{AAPH} - A_{EXTRACT}]/A_{AAPH}$$

Where A_{AAPH} is the absorbance of AAPH at 540 nm and $A_{EXTRACT}$ is the absorbance of alcohol or water extract at 540 nm.

RESULTS

Amount of total phenolic compounds

As shown in Table 1, both water and alcohol extracts of *Thuja orientalis* have good amount of phenolic compounds. The water extract has greater amount of total phenolics as compared to alcohol extract.

Reducing power

The reducing powers determined by the present assay depend on the redox potentials of the compounds present in water and alcohol extracts of *Thuja orientalis*, characterized by the complexity of their constituents. As shown in Fig. 1, reducing power of water extract was higher than alcohol extract.

Table 1. Amount of total phenolic compounds in water and alcohol extracts of *Thuja orientalis*

Extracts (200 mg)	Absorbance (760 nm)	Gallic acid equivalents (mg)
Water extract	0.0724	63.545
Alcohol extract	0.0550	47.727

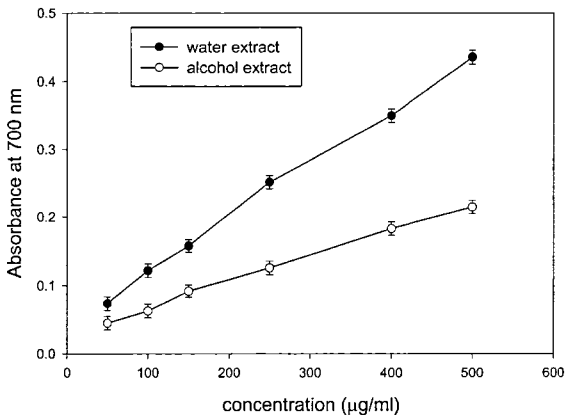

Fig. 1. Reducing power of water and alcohol extract of *Thuja orientalis*. All the points represent mean of triplicate samples and bars representing standard error of mean are shown.

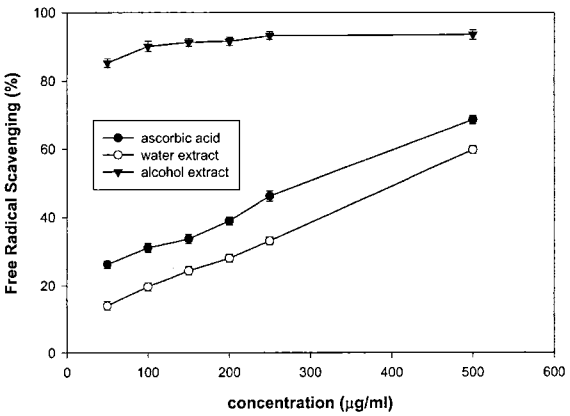

Fig. 2. Percent DPPH scavenging activities of water and alcohol extract of *Thuja orientalis*. Ascorbic acid was used as a positive control. All the points represent mean of triplicate samples and bars representing standard error of mean are shown.

DPPH radical scavenging activity

As shown in Fig. 2, DPPH decolorization was increased by the *Thuja orientalis* extracts in a concentration dependent manner. Both water and alcohol extracts are good scavengers of DPPH free

radical. Water extract of *Thuja orientalis* at a concentration of 500 mg was able to scavenge 68.59% of DPPH free radical while alcohol extract at the similar concentration was able to scavenge 59.73% of DPPH free radical. Thus, water extract is a better scavenger of DPPH free radical as compared to alcohol extract.

Inhibition of Fe (II)-EDTA-H_2O_2 induced oxidative DNA damage

We studied alcohol and water extracts of *Thuja orientalis* for their ability to modulate DNA damage produced by Fenton reaction. Control experiments (not shown) established that heat denatured DNA underwent 100% hydrolysis following treatment with S_1 nuclease, whereas native DNA resulted in < 10 % conversion. S_1 nuclease hydrolysis of DNA decreases in a dose dependent manner with the increasing concentration of water and alcohol extracts of *Thuja orientalis* (Fig. 3). At a concentration of 250 μg, water and alcohol extracts of *Thuja orientalis* inhibited the hydrolysis of DNA by 72.859% and 65.312%, respectively (Fig. 4). It shows that water extract is a stronger inhibitor of DNA cleavage, because it inhibits DNA degradation to a greater extent than the alcohol extract at all concentrations tested. Thus, *Thuja orientalis* extracts were able to prevent DNA hydrolysis and oxidative damage to DNA.

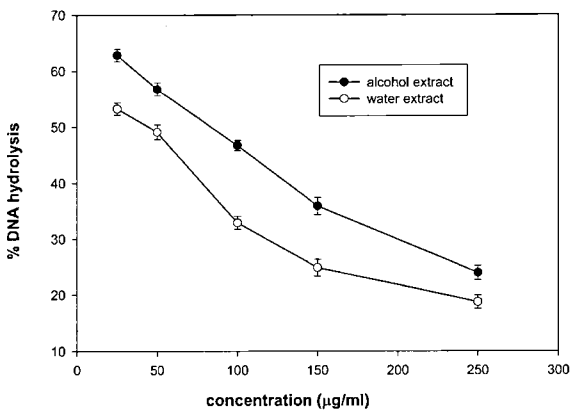

Fig. 3. Decrease in degradation of DNA in the presence of water and alcohol extracts of *Thuja orientalis*. All the points represent mean of triplicate samples and bars representing standard error of mean are shown.

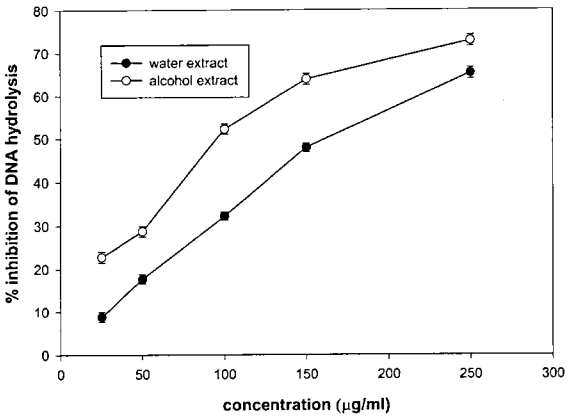

Fig. 4. Inhibition of DNA hydrolysis by water and alcohol extracts of *Thuja orientalis*. All the points represent mean of triplicate samples and bars representing standard error of mean are shown.

RBC hemolysis

Lipid oxidation of RBC membrane mediated by AAPH induces membrane damage and subsequent hemolysis (Miki *et al.*, 1987). Both water and alcohol extracts of *Thuja orientalis* showed inhibition of RBC hemolysis. As shown in Table 2, at a concentration of 12.5 μg/ml, water and alcohol extracts inhibited RBC hemolysis to the extent of

Table 2. Inhibitory effect (%) of water and alcohol extracts of *Thuja orientalis* on AAPH induced hemolysis of human RBC

Compound/extract	12.5 mg/ml	25 mg/ml	50 mg/ml
Ascorbic acid	80.44 ± 0.42	87.76 ± 0.75	92.38 ± 0.80
Water extract	69.30 ± 0.25	75.15 ± 0.84	88.14 ± 1.09
Alcohol extract	54.55 ± 0.66	68.34 ± 0.92	79.21 ± 1.31

Data are expressed as mean ± standard error of mean, n = 5.

69.30% and 54.55%, respectively. Both extracts demonstrated dose-dependent inhibition effects toward RBC hemolysis, with the protective effect reaching maximum at a concentration of 50 μg/ml.

DISCUSSION

In the present study, water and alcohol extracts of *Thuja orientalis* showed antioxidant activity, and protected DNA and human RBC from free radical induced damage. Since free radicals are involved in the etiology of several degenerative diseases and various inflammatory diseases (Ames *et al.*, 1993; Halliwell, 1994), the leaf extracts of *Thuja orientalis*, which are showing significant antioxidant activity, might be helpful in slowing the progress of various oxidative stress-related diseases.

Our findings further support the use of *Thuja orientalis* in Chinese medicine for the treatment of rheumatism, gout, diarrhoea and chronic tracheitis (Jiangsu College of New Medicine, 1977), because oxygen free radicals have also been implicated as mediators of tissue damage in patients with rheumatoid arthritis (Cimen *et al.*, 2000; Thabrew *et al.*, 2001; Taysi *et al.*, 2002; Karatas *et al.*, 2003).

Our study showed that the reducing capacity of water extract is higher than the alcohol extract. The greater reducing power of water extract might be due to presence of water-soluble polyphenols and flavanol glycosides, which are extracted in greater amount in the water extract.

The antioxidant activity of *Thuja orientalis* extracts was determined by DPPH radical scavenging ability. This method is based on the reduction of DPPH, a stable free radical. Because of the odd electron of DPPH, it gives a strong absorption maximum at 517 nm by visible spectroscopy (purple color). As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, that is, a free radical scavenging antioxidant, the absorption strength decreases, and the resulting decolorization is stiochiometric with respect to the number of electrons captured (Blois, 1958). This reaction has been widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate the antioxidative activity of foods and plant extracts (Ekanayake *et al.*, 2004; Zhou *et al.*, 2004; Panovska *et al.*, 2005; Rodriguez *et al.*, 2005). The water extract showed higher DPPH radical scavenging ability as compared to the alcohol extract. Better scavenging ability of water extract can be attributed to greater amount of phenolic compounds in water extract as shown in Table 1. It is further supported by the presence of flavonoid glycoside (Khabir *et al.*, 1985) and condensed tannin (Zhenwen *et al.*, 1983), which are highly soluble in water, and are easily extracted in water.

The oxidative stress due to oxygen and various radical species is associated with the induction of DNA single and double strand breaks, and is considered to be the first step in several human degenerative diseases, such as, cancer and aging (Lieber, 1998). In the Fe (II)-EDTA-H_2O_2 induced oxidative DNA damage assay, both water and alcohol extracts protected DNA from hydroxyl radical induced damage. At a concentration of only 250 μg/ml, water extract inhibited DNA hydrolysis by 72.859%. Alcohol extract also showed good protective ability in this assay, and at a concentration of 250 μg/ml, inhibited DNA hydrolysis by 65.312%. This is attributable to the presence of flavonoid glycosides (Khabir *et al.*, 1985), flavones (Yang *et al.*, 1995), biflavones (Natarajan *et al.*, 1970) and condensed tannin (Zhenwen *et al.*, 1983) with known chemopreventive properties (Hertog *et al.*, 1993; Chung *et al.*, 1998; Aherne and O'Brien, 2000). Therefore, *Thuja orientalis* extracts are potential chemopreventive agents.

It is well recognized that the oxidation of polyunsaturated fatty acids in biological membranes can lead to the formation and propagation of lipid radicals, uptake of oxygen, rearrangement of the double bonds in unsaturated lipids, and even destruction of membrane lipids. Many of these biochemical activities can lead to the production of breakdown products that are highly toxic to most

cell types. Excessive oxidative damage to cellular membranes contributes to the initiation and progression of numerous degenerative diseases, including certain types of cancer and cardiovascular diseases (Pryor, 2000; Young and Woodside, 2001). Red blood cells are vulnerable to lipid peroxidation due to their high content of polyunsaturated lipids, their rich oxygen supply, and the presence of transition metals. Lipid oxidation of human red blood cell membrane mediated by AAPH induces membrane damage and subsequent hemolysis (Miki *et al.*, 1987; Zhu *et al.*, 2002). *Thuja orientalis* extracts inhibited AAPH (an azo peroxyl radical initiator) mediated human red blood cell hemolysis. At a concentration of 12.5 μg/ml, water and alcohol extracts inhibited RBC hemolysis to the extent of 61.30% and 54.55%, respectively. At a concentration of 50 μg/ml, water extract inhibited RBC hemolysis by 88.14%, which is only 4% less than L-ascorbic acid at similar concentration. Alcohol extract at the similar concentration showed inhibition by 79.21%, which is 9% less than water extract. This may again be attributable to higher amount of polyphenolic compounds in water extract. Thus, there is direct relation between phenolic content and antioxidant activity. Since *Thuja orientalis* extracts showed protection against lipid peroxidation of RBC membrane; it can also be used in the prevention of cardiovascular diseases.

The data reported in the present study demonstrates that water and alcohol extracts of *Thuja orientalis* have free radical scavenging activity and reducing power, and can provide protection against DNA oxidation and RBC hemolysis. Therefore, the herbal formulations based on *Thuja orientalis* extracts can be used for the prevention and treatment of oxidative stress related disorders, such as, cancer, vascular diseases, gout and rheumatism.

ACKNOWLEDGEMENTS

Research facilities provided by the Department of Chemistry, Aligarh Muslim University, Aligarh is gratefully acknowledged. Authors are grateful to Prof. NU Khan and Prof. SM Hadi for useful discussions, and to Dr. Athar Ali Khan for the identification of the plant.

REFERENCES

Aherne SA, O'Brien NM. (2000) Mechanism of protection by the flavonoids, quercetin and rutin, against *tert*-butylhydroperoxide and menadione-induced DNA single strand breaks in Caco-2 cells. *Free Radic. Biol. Med.* **29**, 507-514.

Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. (2004) Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem.* **84**, 551-562.

Ames BN, Shigenaga MK, Hagen TM. (1993) Oxidants, Antioxidants, and the Degenerative Diseases of Aging. *Proc. Natl. Acad. Sci. U.S.A.* **90**, 7915-7922.

Aruoma OI. (2003) Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutat. Res.* **523-524**, 9-20.

Bagci E, Digrak M. (1996) The antimicrobial activities of some forest trees essential oils. *Tr. J. Biol.* **20**, 191-198.

Blois, MS. (1958) Antioxidant determinations by the use of a stable free radical. *Nature* **26**, 1199-1200.

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

Chung KT, Wong TY, Wei CI. (1998) Tannins and human health: a review. *Crit. Rev. Food Sci. Nutr.* **38**, 421-464.

Cimen MY, Cimen OB, Kacmaz, Ozturk HS, Yorgancioglu R, Durak I. (2000) Oxidant/antioxidant status of the erythrocytes from patients with rheumatoid arthritis. *Clin. Rheumatol.* **19**, 275-277.

Ekanayake P, Lee YD, Lee J. (2004) Antioxidant Activity of Flesh and Skin of Eptatretus Burgeri (Hag Fish) and Enedrias Nebulosus (White Spotted Eel). *Food Sci. Technol. Int.* **10**, 171-177.

Freeman B A (1984) Biological sites and mechanism of free radical production, In: *Free radicals in molecular biology, aging, and disease, edited by* Armstrong D, Sohal R, Culter RG, Slater T (eds), pp. 43-52, Raven Press, New York.

Grieve MA. (1994) *Modern Herbal*, pp. 176-177, Tiger Books International, London.

Hertog MGL, Hollman PCH, van de Putte B. (1993) Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. *J. Agric. Food Chem.* **41**, 1242-1246.

Hirano R, Sasamoto W, Matsumoto A, Itakura H, Igarashi O, Kondo K. (2001) Antioxidant ability of various flavonoids against DPPH radicals and LDL oxidation. *J. Nutr. Sci. Vitaminol.* **47**, 357-362.

Karatas F, Ozates I, Kanatan H, Halifeoglu I, Karatepe M, Colak R. (2003) Antioxidant status and lipid peroxidation in patients with rheumatoid arthritis. *Indian J. Med. Res.* **118**, 178-181.

Khabir M., Khatoon F, Ansari WH. (1985) Phenolic constituents of *Thuja orientalis*. *Curr. Sci.* **54**, 1180-1181.

Lai LTY, Naiki M, Yoshida SH, German BJ, Gershwin ME. (1994) Dietary *Platycladus orientalis* seed oil suppresses anti-erythrocyte autoantibodies and prolongs survival of NZB mice. *Clin. Immunol. Immunopathol.* **71**, 293-302.

Lee H-K, Ahn K-S, Park SH, Lee IS, Kim JH. (1999) Compounds from *Biota orientalis* leaves inhibit expression of adhesion molecules induced by TNF-a on inflammatory cells In: *Recent Adv. Nat. Prod. Res Proc. Int. Symp., 3 rd., Seoul National University* edited by Shin KH, Kang SS, Kin YS, pp. 54-62, Seoul, South Korea.

Lieber MR. (1998) Pathological and Physiological Double-Strand Breaks: Roles in Cancer, Aging, and the Immune System. *Am. J. Pathol.* **153**, 1323-1332.

Mabey R. (1988) *The New Age Herbalist*, p. 56, Macmillan Publishing Company, New York.

Miki M, Tamai H, Mino M, Yamamoto Y, Niki E. (1987) Free radical chain oxidation of erythrocytes by molecular oxygen and its inhibition by alpha-tocopherol. *Arch. Biochem. Biophys.* **258**, 373-380.

Nagai T, Inoue R, Inoue H, Suzuki N. (2003) Preparation and antioxidant properties of water extract of propolis. *Food Chem.* **80**, 29-33

Natarajan S, Murti VVS, Seshadri TR. (1970) Biflavonoids IV. Biflavones of some Cupressaceae plants. *Phytochemistry* **9**, 575-579.

Panovska TK, Kulenova S, Stefova M. (2005) *In vitro* antioxidant activity of some *Teucrium* species *(Lamiaceae). Acta Pharm.* **55**, 207-214.

Pelter A, Warren R, Hameed N, Khan, NU, Ilyas M., Rahman W. (1970) Biflavonyl pigments from *Thuja orientalis* (Cupressaceae). *Phytochemistry* **9**, 1897-1898.

Pryor WA. (2000) Oxidation and atherosclerosis. *Free Radic. Biol. Med.* **28**, 1681-1682.

Rahman A, Hadi SM, Parish JH, Ainley K. (1989) Strand scission in DNA induced by quercetin and Cu (II): role of Cu (I) and oxygen free radicals. *Carcinogenesis* **10**, 1833-1839.

Ramarathnam N, Ochi H, Takeuchi M. (1997) Antioxidant defense system in vegetable extracts. In: *Natural Antioxidants: Chemistry, Health Effects, and Applications*, edited by Shahidi F, pp. 76-87, AOCS Press: Champaign, IL.

Rodríguez R, Jaramillo S, Rodriguez G, Espejo JA, Guillén R, Fernández-Bolaños J, Heredia A, Jiménez A. (2005) Antioxidant Activity of Ethanolic Extracts from Several Asparagus Cultivars. *J. Agric. Food Chem.* **53**, 5212-5217.

Saucier CT, Waterhouse AL. (1999) Synergetic Activity of Catechin and other Antioxidants. *J. Agric. Food Chem.* **47**, 4491-4494.

Schneider WC. (1957) Determination of nucleic acid in tissues by pentose analysis. *Methods Enzymol.* **3**, 680-684.

Singh A. (1989) Physicochemical and physiological aspects. In: *CRC handbook of free radicals and antioxidants in biomedicine* edited by Miquel J, Quintanilha AT, Weber H, Vol. I, pp. 17-25, CRC press Inc., Boca Raton, Florida.

Squadrito GL, Pryor WA. (1998) Oxidative chemistry of nitric oxide: the roles of superoxide, peroxynitrite, and carbon dioxide. *Free Radic. Biol. Med.* **25**, 392-403.

Taysi S, Polat F, Gul M, Sari RA, Bakan E. (2002) Lipid peroxidation, some extracellular antioxidants, and antioxidant enzymes in serum of patients with rheumatoid arthritis. *Rheumatol. Int.* **21**, 200-204.

Thabrew MI, Senaratna L, Samarawickrema N, Munasinghe C. (2001) Antioxidant potential of two polyherbal preparations used in Ayurveda for the treatment of rheumatoid arthritis. *J. Ethnopharmacol.* **76**, 285-291.

Tomita B, Hirose Y. (1969) Terpenoids. XXIII. Chemotaxonomy of the Cupressaceae (2) Sesquiterpenes in the Wood of *Biota orientalis* ENDL. *Mokuzai Gakkaishi* **15**, 339-340.

Tomita B, Hirose Y, Nakatsuka T. (1969a) Terpenoids. XIX. New Constituents of *Biota orientalis*. *Mokuzai Gakkaishi* **15**, 46.

Tomita B, Hirose Y, Nakatsuka T. (1969b) Terpenoids. XX. New Constituents of *Biota orientalis*. *Mokuzai Gakkaishi* **15**, 47.

Yang HO, Suh D-Y, Han BH. (1995) Isolation and characterization of platelet - activating factor receptor binding antagonists from *Biota orientalis*. *Planta Med.* **61**, 37-40.

Young IS, Woodside JV. (2001) Antioxidants in health and disease. *J. Clin. Pathol.* **54**, 176-186.

Zhenwen X, Shilan B, Juanjuan Z, Qiang W. (1983) Study on hemostatic constituents in *Biota orientalis* (L.) Endl. leaves. *Zhongyao Tongbao* **8**, 30-32.

Zhou K, Su L, Yu L. (2004) Phytochemicals and antioxidants in Wheat Bran. *J. Agric. Food Chem.* **52**, 6108-6114.

Zhu JX, Wang Y, Kong LD, Yang C, Zhang X. (2004) Effects of *Biota orientalis* extract and its flavonoid constituents, quercetin and rutin on serum uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver. *J. Ethnopharmacol.* **93**, 133-140.

Zhu QY, Hackman RM, Ensunsa JL, Holt RR, Keen CL. (2002) Antioxidative Activities of Oolong Tea. *J. Agric. Food Chem.* **50**, 6929-6934.