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Research article

ANTIOXIDANT POTENTIAL OF VARIOUS EXTRACTS OF STEM OF *THUJA ORIENTALIS: IN VITRO* STUDY

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ABSTRACT : The antioxidant activity of different extracts of *Thuja orientalis* was evaluated by employing three *in vitro* experiments namely (1, 1-Diphenyl-2-picrylhydrazyl) (DPPH), reducing power and chelating power assays. The stem powder of the plant was extracted with different solvents by maceration method in order of decreasing polarity and then partitioned (Flow Chart 1). The differential activities of the different extracts could be correlated with their respective phenolic and flavanoid contents and compared with standards L-ascorbic acid and butylated hydroxytoluene (BHT). All the extracts exhibit a remarkable concentration dependant scavenging activity. Moreover, among the different extract, methanol extract exhibited good scavenging response of 74.3%, 59.51% and 0.997% in DPPH, chelating power and reducing power assay in decreasing order of solvent polarity at maximum concentration, respectively. Studies are in progress to isolate and identify the active principle components responsible for this activity.

Keywords: Antioxidant; *Thuja orientalis*; polyphenol; Free radicals; DPPH

INTRODUCTION

Free radicals or ROS (reactive oxygen species) are highly reactive oxygen metabolites, which attract electrons from surrounding molecules (lipids, proteins, DNA) to induce accumulation of cellular damage and this result in skin aging (Ma, et. al., 2001; Cook & Samman, 1996), cardiovascular diseases, cancer, inflammatory diseases and a variety of other disorders (Finkel & Holbrook, 2000). Plants are potential sources of natural antioxidants. Plants produce various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive (Lu & Foo, 1995). ROS, which include free radicals such as superoxide anion radicals (O^{2-}), hydroxyl radicals (OH) and non free radical species such as H_2O_2 and singled oxygen (O_2), are various forms of activated oxygen.

In foods, ROS can cause lipid peroxidation, which leads to the deterioration of the food (Miller & Rice-Evans, 1997). The oxidative deterioration of the lipid-containing food is responsible for the rancid odours and flavours during processing and storage, consequently decreasing the nutritional quality and safety of foods; it is because of the formation of secondary, potentially toxic compounds. The addition of antioxidant is a method for increasing the shelf life of foods (Cook & Samman, 1996). Presently, most commonly added synthetic antioxidants such as L-ascorbic acid, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were restricted by legislative rules because of doubts over their toxic and carcinogenic effects. As a result, a considerable interest in the food industries has developed to find natural antioxidants to replace the synthetic ones. Bearing this in mind, the present work has been designed to investigate the antioxidative activity of different extracts of *Thuja orientalis* by employing DPPH, reducing power and chelating power *in vitro* assay and to evaluate total phenolic and flavanoid content of the plant, since single procedure cannot satisfy all possible findings, characterizing an antioxidant activity (Frankel & Meyer, 2000). *Thuja orientalis* is a common ornamental evergreen tree that is originally native to Northwest China belonging to family Cupressaceae. *Thuja orientalis* is hardy, large evergreen shrub or small to medium sized-tree rarely exceeding 20 m in nature. The tree is often stunted or prostrate. Traditionally it is used in the treatment of cough (Mabey, et. al., 1948). It is also used as a medicinal plant in various forms of traditional medicines like folk medicine, homeopathy, etc. for treatment of bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism (Shimada, 1956; Baran, 1991).

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MATERIALS AND METHODS

The organic solvents (methanol, acetone, ethyl acetate, chloroform and hexane) were purchased from Qualigens. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Aldrich, USA. All other chemicals used like tris HCl, ferrozine, ferric chloride, potassium ferricyanide, trichloroacetic acid, aluminum nitrate, potassium acetate, sodium carbonate, BHT (butylated hydroxytoluene), gallic acid, Folin's reagent, ascorbic acid, quercetin and other solvents were of analytical grade.

Preparation of extracts

The stem of *Thuja orientalis* was collected, washed and dried at room temperature. After grinding into the fine powder, it was extracted with different solvents in decreasing order of solvent polarity viz. water, methanol, acetone, ethyl acetate, chloroform and hexane (Flow chart 1). In this method of extraction, the stem powder was soaked for 24 h in each solvent and after recovering the supernatant, the respective solvents were added twice to the residue. The three supernatants obtained with each solvent were pooled and dried in rotary vacuum evaporator. Extraction in each solvent was done thrice and the whole procedure was repeated twice (Flow chart 1).

Determination of total phenolics

Total phenolics content was determined according to the Folin-Ciocalteu method, using gallic acid as standards. Extract powders (1 mg) were dissolved in 1 ml 50% methanol solution. Extract solution (0.5 ml) was mixed with 0.5 ml of 50% Folin- Ciocalteu reagent. After 2-5 min, 1.0 ml of 20% Na_2CO_3 was added to the mixture and incubated for 10 min at room temperature. The mixture was centrifuged at 150 g for 8 min and the absorbance of the supernatant was measured at 730 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram sample.

Determination of total flavanoids

Sample (1 mg) was added in 1ml of 80% ethanol. An aliquot of 0.5 ml was added to test tubes containing 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M potassium acetate and 4.3 ml of 80% ethanol. The absorbance of the supernatant was measured at 415 nm after 40 min at room temperature. Total flavonoid content was calculated using quercetin as standard.

Antioxidant testing assays

The antioxidant activity of the extracts was determined by using standard methods. The DPPH assay (Hsu, 2006) was used to evaluate the ability of antioxidants to scavenge free radicals which are considered a major factor in biological damages caused by oxidative stress. One mg extract powder was dissolved in 1 ml of 50% ethanol solution to obtain 1000 µg/ml sample solution. This solution was serially diluted into 100, 250, 400, 550, 700, 850 µg/ml with 50% ethanol. In each reaction, the solutions were mixed with 1 ml of 0.1 mM 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 0.45 ml of 50 mM tris-HCl buffer (pH 7.4) and 0.05 ml samples at room temperature for 30 min. Fifty percent ethanol solution was used as negative control. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. DPPH is a purple-colored stable free radical; when reduced it becomes the yellow-colored diphenylpicrylhydrazine. L-ascorbic acid was used as positive control. The antioxidant activity of test samples was evaluated by calculating the percent inhibition of superoxide anion radical by applying the following formula:

$$\% \text{ inhibition} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance of the extract. The antioxidant activity of each sample was expressed in terms of IC_{50} (micro molar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve.

The chelating effect on ferrous ions was determined according to the method of Dinis, et. al., (1994). Measurement of the rate of color reduction allows estimation of the chelating activity of the coexisting chelator (Yamaguchi, et. al., 2000). Ferrozine can quantitatively form complexes with Fe2+. Each extract (150, 300, 450, 600, 750 and 1000 µg ml^{-1}) in methanol (2 ml) was mixed with 0.1 ml of 2 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine solutions. After reaction for 10 min, the absorbance was measured at 562 nm. A lower absorbance indicates a stronger chelating ability. BHT (butylated hydroxytoluene) was used as the control. The percentage of ferrous ion chelating effect of test samples was evaluated by calculating the percent inhibition of superoxide anion radical by applying the following formula: % inhibition= $[(A_0 - A_1)/A_0] \times 100$

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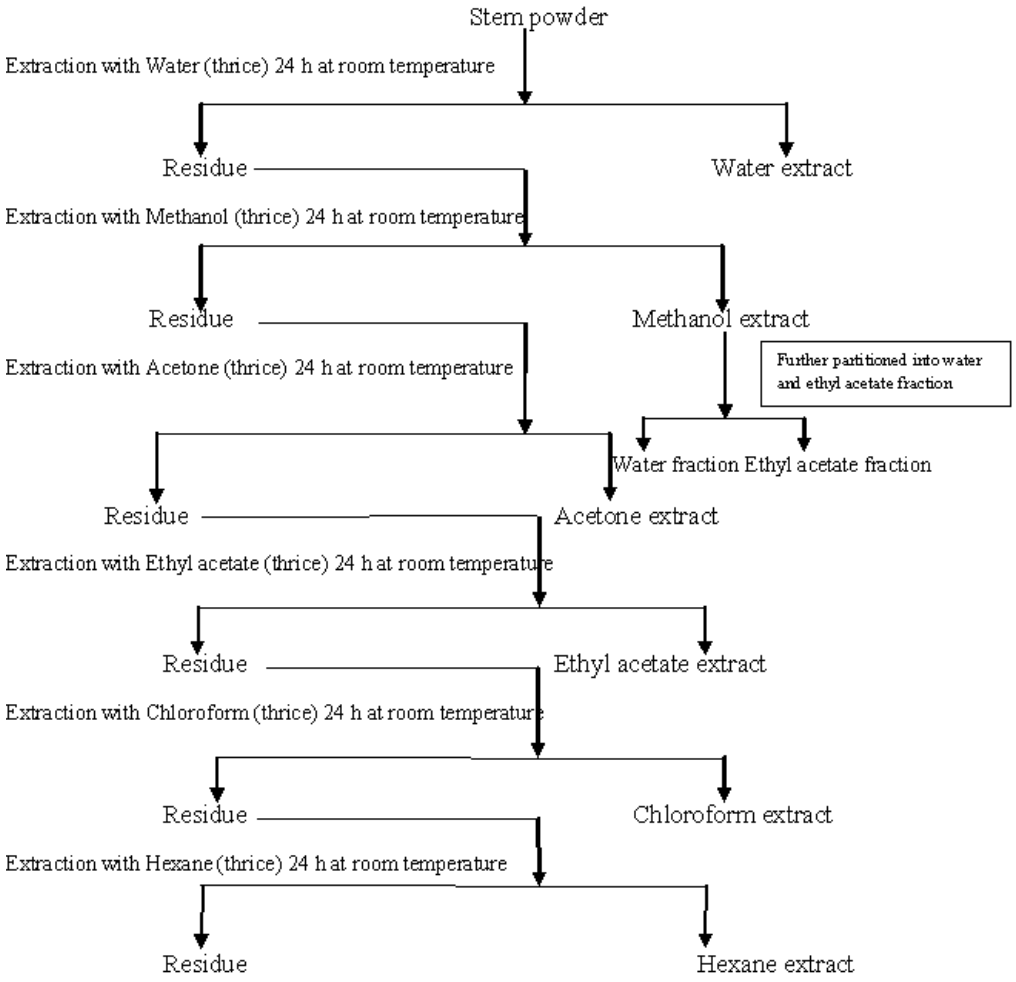
Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance of the extract. The percentage of ferrous ion chelating effect of test samples were determined and compared with that of BHT (butylated hydroxytoluene), which was used as the standard (positive control).

The reducing power of the extracts was measured using the method described by Oyaizu (1986). Different concentrations of each extract i.e. 50, 100, 150, 200, 250 and 300 µg ml^{-1} in methanol, (2.5 ml) of each were mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50^0 C for 20 min. Aliquots (2.5) ml of 10% trichloroacetic acid (w/v) was added to the mixture which was then centrifuged for 10 min at 1036 g. The upper layer (5 ml) of the solution was mixed with 5 ml of deionized water and 1 ml of 0.1% ferric chloride. The mixture was shaken and left to stand for 10 min in the dark, and the absorbance was read at 700 nm in spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power. BHT (butylated hydroxytoluene) was used as the control.

Statistical analysis

All experiments were repeated three times. Results are reported as means ± SE (not shown in graphs). IC_{50} values have been calculated. T-test was applied to see the significance of the data.



Flow chart 1. Extraction of stem powder of *Thuja orientalis* by decreasing order of solvent polarity.

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RESULTS AND DISCUSSION

A single testing method can't provide a comprehensive picture of antioxidant profile of studied samples because of the complexity of the oxidation-antioxidation processes. Therefore, multi-method approach has been used in the assessment of antioxidant activity.

DPPH radical scavenging assay

DPPH is a stable radical that has been widely utilized to appraise the antioxidant activity of various natural products (Hu and Kitts, 2000). The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm, and also for visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized which can be quantitatively measured from the changes in absorbance. DPPH radical scavenging activity of different extracts of stem of *Thuja orientalis* and ascorbic acid are presented in Fig. 1. In this assay, it was observed that different extracts of *T. orientalis* scavenged DPPH free radical in a dose dependant manner because, as the concentration of extracts increased the DPPH scavenging activity also increased (Fig.1). The order of effectiveness of the extracts was methanol extract (74.31%) >acetone extract (61.16%) >ethyl acetate extract (54.43%) respectively at maximum concentration. The amount of methanol extract of stem of *T. orientalis* needed for 50% inhibition of DPPH radical was 329.68 µg/ml which is higher than IC_{50} value of ascorbic acid (362.50 µg/ml), as shown in Table 1.While IC_{50} value of acetone and ethyl acetate extracts of stem was observed to be 512.50 and 756.00 µg/ml, respectively. It means that methanol extracts of stem is more effective than the ascorbic acid as an antioxidant agent.

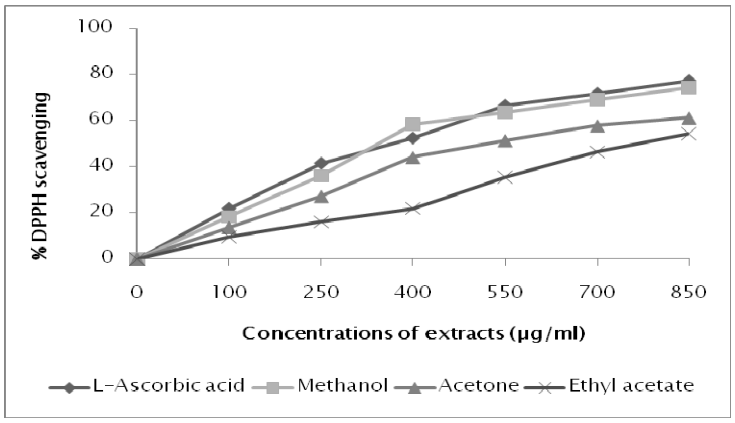

Figure 1: Scavenging of the DPPH radical by different extracts of stem of *Thuja orientalis* by DPPH assay.

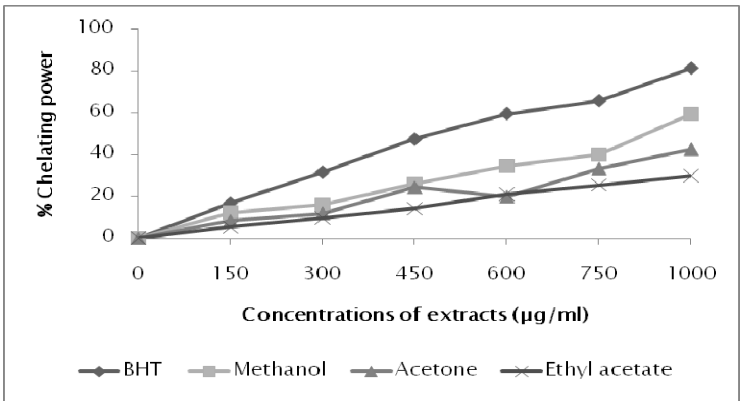

Figure 2: Chelating power potential of different extracts of stem of *Thuja orientalis* by chelating power assay.

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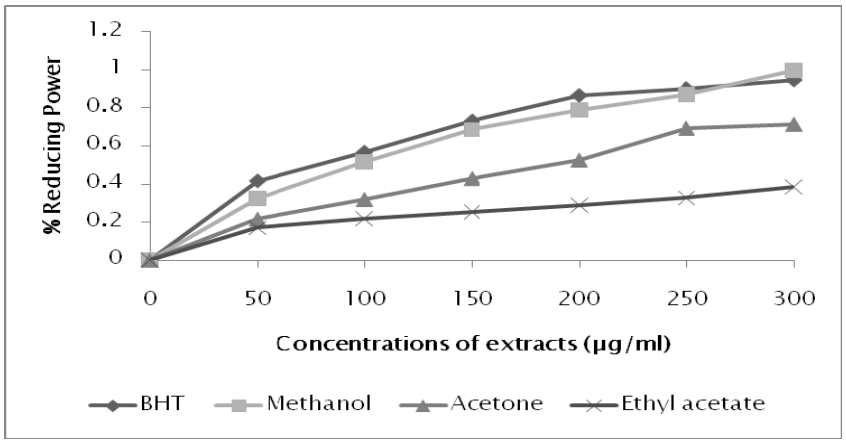

Figure 3: Reducing power potential of different extracts of stem of *Thuja orientalis* by reducing power assay.

Table 1: IC_{50} values of different extracts/fractions of stem of *Thuja orientalis* in different antioxidant systems

S.No	Assay	Extract/fraction/Standard	IC_{50} value (µg/ml)
1	DPPH-radical scavenging assay	L-ascorbic acid	362.50
		Methanol	329.68
		Acetone	512.50
		Ethyl acetate	756.25
2	Chelating effect on ferrous ions assay	BHT	475
		Methanol	875
		Acetone	NA
		Ethyl acetate	NA

Table 2: Total flavonoids contents in different extract/fractions of stem of *Thuja orientalis*

S.No	Extract/fractions	Phenol content *	Flavonoids content **
1	Methanol	106	11.00
2	Acetone	79	9.33
3	Ethyl acetate	74	7.33

*mg gallic acid equivalent/g of extract powder, **mg quercetin equivalent/g of extract powder

Table 3: Co-relation coefficient (r) between total phenol content and antioxidant activities of different extracts of stem of *Thuja orientalis*

S.No	Antioxidant assays	Methanol	Acetone	Ethyl acetate
1	DPPH	0.914^{5}	0.938^{1}	0.988^{1}
2	Chelating effect on ferrous ions	0.992^{1}	0.898^{5}	0.994^{1}
3	Reducing power	0.971^{1}	0.977^{1}	0.987^{1}

Superscript values denotes % level of significance

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Table 4: T-test statistics of total phenol content and antioxidant activities of different extracts of stem of *Thuja orientalis*

S.No	Antioxidant assays	Methanol	Acetone	Ethyl acetate
1	DPPH	6.035^{2}	5.617^{2}	4.266^{8}
2	Chelating effect on ferrous ions	4.454^{7}	4.428^{7}	4.549^{6}
3	Reducing power	6.961^{1}	5.903^{2}	8.702#

Superscript values denotes % level of significance, # not significant

The antioxidant constituents are the vital substances that posses the ability to protect the body from damage which is caused by free radical (like DPPH) that induced oxidative stress (Souri, et al., 2004). These antioxidant molecules quench DPPH free radicals either by donating electrons or by providing hydrogen atoms (Naik, et. al., 2003) via a free radical attack on the DPPH molecule and result into discolouration with decrease in absorbance at 517 nm. On the other hand, antioxidant are also believed to inhibit the free radical chain of oxidation and donate hydrogen from the phenolics hydroxyl group, forming a stable end product, which does not initiate or propagate further oxidation of lipid (Sherwin, 1978). The data obtained show that among the four extracts of stem analysed for DPPH scavenging activity, methanol extract shows higher free radical inhibition activity which is comparable with that of standard (i.e. L-ascorbic acid).

Chelating effect on ferrous ions

Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation gets disrupted, resulting in decrease of red colored complex. Measurement of colour reduction is the measurement of metal chelating activity. Fig. 2 shows the effect of different extracts in the chelating power assay. It was observed that methanol extract (59.51%) showed more metal chelating activity than the acetone extract did (42.70%) or the ethyl acetate extract (29.87%), respectively obtained at maximum concentration. The IC_{50} value for the methanol extract was observed to be 875µg/ml (Table 1). In this assay various extracts of stem of *T. orientalis* along with standard compound interfere with the formation of ferrous and ferrozine complex suggesting that they have chelating activity and are able to capture the ferrous ions before the formation of ferrozine.

The possible explanation of chelating power of the extracts is the ability to the extracts to reduce iron and then to form Fe^{2+}- extracts complex that are inert. This study is in conformity with the observation made in literature that non-flavanoid polyphenolics can reduce iron and the form Fe^{2+}- polyphenol complexes that are inert (Laughton, et. al., 1987).

The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, et. al., 1994). For the measurements of the reductive ability, it has been found that the Fe^{3+}– Fe^{2+} transformation occurred in the presence of extract samples which had been postulated previously by Oyaizu (1986).

Reducing power assay

The reducing capacity of a compound may serve as a significant indicator of its being a potential antioxidant. In this assay, the yellow colour of the test solution changes to various shades of green and blue, depending on the reducing power of each compound. The relative reducing power of different extracts of *T. orientalis* express marked variations. The methanol extract possesses maximum reduction potential (0.997%) at concentration of 300 µg/ml, which is significantly greater than reduction potential of BHT (0.947 %) used as standard reducing agent (Fig. 3) followed by acetone extract (0.712%), ethyl acetate (0.385%).Reducing power potential of different extracts of stem increase with the increase in concentration of extracts.

The reducing properties are generally associated with the presence of reductones (Duh, et. al., 1999) which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom (Gordon, 1990). Reductones are also reported to react with certain precursors of peroxide, thus preventing peroxide formation.

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Determination of total phenolics

A critical analysis of the results obtained in different assay shows that the methanol and acetone extracts were comparatively more effective than the ethyl acetate extract.

In order to identify the antioxidant principle in the extracts the total phenolic content (standard curve equation: $y = 0.004x + 0.118$, $R^2 = 0.976$), mg/g as gallic acid equivalent (GAE). The total phenolic content was found to be highest in methanol extract i.e. 106 mg/g as gallic acid equivalent followed by acetone and ethyl acetate (79, 76 mg/g as gallic acid equivalent respectively).

Determination of total flavanoids

The content of flavonoids was expressed in terms of quercetin equivalent (the standard curve equation: $y = 0.0033x + 0.4723$, $R^2 = 0.9907$), mg Qu/g extract. Summary of identified quantities of flavonoids in the tested extracts is shown in Table 2. The flavanoid content was found to be highest in methanol extract i.e. 11.00 mg. The amount of phenolic as well as flavonoid compounds was observed to be greater in methanol followed by acetone and ethyl acetate. The study revealed that flavonoids also the contributors to the antioxidant activity exhibited by the plants.

Relationship between Antioxidant activity and Total phenolic content

Total phenolics content was significantly correlated with the antioxidant activities in all the three extracts of stem of *Thuja orientalis* in all the three tested methods (Table 3). Methanol extract was found to be more significant followed by acetone and ethyl acetate extracts (Table 4).

All plants produce an amazing diversity of secondary metabolites. One of the most important groups of these metabolites is phenolic compounds. The conception of antioxidant action of phenolic compounds is not novel (Bors, et. al., 1990). There have been many reports of induced accumulation of phenolic compounds and peroxidise activity in plants treated with high concentrations of metals. Antioxidant action of phenolic compounds is due to their high tendency to chelate metals. Phenolics possess hydroxyl and carboxyl groups, able to bind particularly iron and copper (Jung, et. al., 2003). The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kahkonen, et. al., 1999; Valenzuela, et. al., 1996). In addition, they have a metal chelation potential (Rice-Evans, et. al., 1995).Further studies are in progress to identify and elucidate the chemical nature of the bioactive principles (active antioxidative agents) by using different chemical techniques.

The free radical scavenging activity in different assays can be linked to the presence of phenolic and flavonoid compounds in the extract because these compounds exhibit the important mechanism of antioxidative activities (Yildirim, et. al., 2000). Other antioxidants may also be present in these extracts, phenolic and flavonoid compounds could make significant contribution to their bioactivity.

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REFERENCES

D. Baran (1991). *Arbor vitae*, a guarantee of health. Revista Medico-Chirurgicala a Societatii de Medici si Naturalisti din Lasi., 95(3-4): 347–349.

W. Bors, E.R.W. Hell, E.L.C. Mich and M. Saran (1990). Flavonoids as antioxidants: determination of radical-scavenging efficiency. Meth. Enzymol, 186: 343.

N.S. Cook, S. Samman (1996). Flavonoids-chemistry, metabolism,cardioprotective effects, and dietary sources. Journal of Nutritional Biochemistry, 7: 66-76.

T.C.P. Dinis, V.M.C. Madeira and L.M. Almeida (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-amino salicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophysics, 315: 161-169.

P.D. Duh, Y.Y. Tu and G.C.Yen (1999). Antioxidant activity of the aqueous extract of harn jyur (Chrysanthemum morifolium Ramat). Lebensmittel-Wissenschaft Technologie, 32: 69-277.

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T. Finkel and N.J. Holbrook (2000). Oxidant, oxidative stress and the biology of aging. Nature, 408: 239-247.

E.N. Frankel and A.S. Meyer (2000). The problems of using one dimensional method to evaluate multifunctional foods and biological antioxidants. Journal of Science Food and Agriculture, 80: 1925-1941.

M.H. Gordon (1990).The mechanism of antioxidant action in vitro. In: Hudson BJF (ed) Food antioxidants London: Elsevier Applied Science, pp, 1-18.

C.Y. Hsu (2006). Antioxidant activity of extract form Polygonum aviculare L. Biological Resources, 39: 281-288.

C. Hu and D.D. Kitts (2000). Studies on the antioxidant activity of Echinacea root extract. Journal of Agriculture Food Chemistry, 48: 1466-1472.

C.H. Jung, V. Maeder, F. Funk, B. Frey, E.R.H. Stich and E. Fross Erd (2003). Release of phenols from Lupinus albus L. roots exposed to Cu and their possible role in Cu detoxification. Plant & Soil, 252: 301.

M.P. Kahkonen, A.I. Hopia, H.J. Vuorela, J.P. Rauuha, K. Pihlaja, T.S. Kujala and M. Heinonen (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agriculture Food Chemistry, 47: 3954-3962.

M.J. Laughton, B. Halliwell, P.J. Evans and J.R.S. Holult (1987).Antioxidant and pro-oxidant actions of the plant phenolics quercetin, gossypol and myricetin. Biochem Pharmacol, 36: 717-720.

F. Lu and L.Y. Foo, 1995. Toxicological aspects of food antioxidants In Madhavi DL, Deshpande SS, Salunkhe SS(ed) Food antioxidants. New York: Marcel Dekker.

W. Ma, M. Wlaschek, I. Tantcheva-Poor, L.A. Schneider, L. Naderi, Z. Razi-Wolf, J. Schuller and K.S. Kochanek (2001). Chronological ageing and photoageing of the fibroblasts and the dermal connective tissue. Clinical. Expt Dermatol, 26: 592-599.

R.M. Mabey, P. Mcintyre, G. Micheal, R. Duff and J. Stevens (1948). The New Age Herbalist, New York: Collier Books Macmillian Publishing Company.55-56.

N.J. Miller and C.A. Rice-Evans (1997). The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidants to the activity of orange and apple fruit juices and black currant drink. Food Chemistry, 60: 331-337.

G.H. Naik, K.I. Pryadarshini, J.G. Satav, M.M. Banavalikar, P.P. Spohoni and M.K. Biyani (2003).Comprehensive antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry, 63: 97-104.

T. Osawa, I. Uritani, V.V. Garcia and E.M. Mendoza (1994).Novel natural antioxidants for utilization in food and biological systems. Post harvest biochemistry of plant food-materials in the tropics. Japan Scientific Societies Press, Japan.241-251.

M. (Oyaizu 1986). Studies on products of browning reaction prepared from glucoseamine. Japanese Journal of Nutrition, 44: 307-314.

C.A. Rice-Evans, N.J. Miller, P.G. Bolwell, P.M. Bramley and J.B. Pridham (1995).The relative antioxidant activities of plant-derived polyphenolic flavonoids. Free Radical Research, 23: 375-383.

E.R. Sherwin (1978).Oxidation and antioxidants in fat and oil processing. Journal of American Oil Food Chemistry, 40: 945-948.

K. Shimada (1956).Contribution to anatomy of the central nervous system of the Japanese upon the vermal arbour vitae. Okajimas Folia Anat Jpn, 28: 207–227.

C.A. Valenzuela, N.J. Miller and G. Paganga (1996). Cholesterol oxidation: Health hazard and the role of antioxidants in prevention. Biological Research, 36: 291-302.

F. Yamaguchi, T. Ariga, Y. Yoshimira and Nakazawa (2000). Antioxidant and anti-glycation of carcinol from Garcinia indica fruit rind. Journal of Agriculture Food Chemistry, 48: 180-185.

A. Yildirim, A. Mavi, M. Oktay, A. Kara, F. Algur and V. Bilaloglu (2000). Comparison of antioxidant and antimicrobial activities of Tilia (Tilia argentea Desf Ex DC), Sage (Salvia triloba L.), and Black Tea (Camellia sinesis) extracts. Journal of Agriculture Food Chemistry, 48: 5030-5034.