

ANTIOXIDANT ACTIVITIES OF *THUJA OCCIDENTALIS* LINN.S. K. DUBEY^{*1}, A. BATRA²

The present study was carried out to evaluate the antioxidant activities of an (EFTO) ethanol fraction of aerial part of *Thuja occidentalis* Linn. (Cupressaceae) in various systems. 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH) radical, superoxide anion radical, hydroxyl radical scavenging and lipid peroxidation were carried out to evaluate the antioxidant potential of the extract. The antioxidant activity of ethanol extract were increased in a concentration dependent manner. About 100, 150, 200, 250 & 300 µg EFTO (ethanol fraction of *Thuja occidentalis*) inhibited the FeSO₄ induced lipid peroxidation in a dose dependent manner and showed IC₅₀ value 195.60µg/ml. In DPPH radical scavenging assays the IC₅₀ values of the extract was 202.45µg/ml. EFTO also inhibited the hydroxyl radical generated by Fenton's reaction, with an IC₅₀ value of 158.59µg/ml. moreover, EFTO also scavenged the super oxide generated by PMS/NADH-NBT system and showed IC₅₀ 124.11µg/ml. The results obtained in this study indicate that EFTO can be a potential source of natural antioxidant.

Keywords : Antioxidant, *Thuja occidentalis*, lipid peroxidation, DPPH, superoxide, hydroxyl radical, Nitric oxide.

INTRODUCTION

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases.¹ Many plant extracts and phytochemicals have been shown to have antioxidant/free-radical scavenging properties² and it has been established as one of the mechanisms of their action. Some of the non-nutritive antioxidants of plants are phenolic compounds, flavonoids, coumarins, benzyl isothiocyanate³ etc.

Thuja occidentalis, commonly known as Arbor vitae or white cedar, is indigenous to North America and is grown in Europe as an ornamental tree. In folk medicine, *Thuja occidentalis* has been used to treat bronchial catarrh, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism.⁴ Extract of this plant has shown anti viral, anti diarrhoeal activity⁵⁻⁶. It has been reported to increase the proliferation of spleen cells as well as increase in TNF- α , IL-6 and IL-1 activity in serum and also have protective effect against radiation-induced toxicity.⁷ Today it is mainly used in homeopathy as mother tincture or dilution. The aim of the present investigation was to evaluate the possible anti ulcer activity of *Thuja occidentalis* aerial part.

MATERIALS AND METHODS

Plant material

Fresh aerial part (twigs) of *Thuja occidentalis* were collected from Jaipur, Rajasthan, India, in October-2007 and were

authenticated by experts of Deptt. Of Botany University of Rajasthan, Jaipur. The voucher specimen is preserved for further research in our laboratory.

Preparation of extract

Shade dried and powdered twigs (40-mesh size, 1kg) were soxhlet extracted with 90% EtOH (Dept. of Botany), the solvent was removed and the residue was triturated with hot (65°C) petroleum ether (60-80°C). Solvent was evaporated from the petroleum ether soluble portion and the residue dissolved in ethanol. On removal of the ethanol by evaporation, a semi solid reddish brown mass (12.76g) was obtained. Phytochemical investigations showed the presence of flavonoids (quercetin, kaempferol), tannic acids, polysaccharides and proteins.

Chemicals

Thiobarbituric acid was obtained from Loba Chemie, India. 1,1-Diphenyl-2-picryl hydrazyl (DPPH), NADH and Nitroblue tetrazolium (NBT) were obtained from Sigma Chemicals, St. Louis, USA. Deoxy ribose was obtained from Merck India. Dimethyl sulphoxide, ethylene diamine tetra acetic acid (EDTA), ferrous sulphate, trichloro acetic acid, Hydrogen peroxide (H₂O₂), Ascorbic acid, mannitol, potassium dihydrogen phosphate, potassium hydroxide, phenazine methosulfate were of analytical grade and obtained from Ranbaxy fine chemicals.

Determination of Anti oxidant activity

Assay of lipid per oxidation

The extent of lipid peroxidation in goat liver homogenate was measured *in vitro* in terms of formation of thiobarbituric acid reactive substances (TBARS) by using standard method⁸ with minor modifications⁹ with the help

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of spectrophotometer (Shimadzu model 1601).

Goat liver was purchased from local slutter house. Its lobes were dried between blotting papers (to remove excess blood) and were cut into small pieces with a heavy-duty blade. They were then homogenized in glass-Teflon homogenizing tubes in cold phosphate buffer saline (pH 7.4). It was centrifuged at 2000 rpm for 10 min, and supernatant was diluted with phosphate buffer saline up to final concentration of protein 0.8-1.5 mg/0.1ml. protein concentration was measured by using standard method of Lowery *et.al.*¹⁰ to study the comparative response, the experiments was divided into seven groups. Liver homogenates (5%, 3ml) was aliquoted to seven different 35mm glass Petri dishes. The first two groups were treated as control and standard where buffer and Vit. E were added. In the third to seventh group, different concentrations of EFTO were added. Lipid peroxidation was initiated by adding 100 µl of 15mm ferrous sulphate solution to 3 ml of liver homogenate. After 30 min, 100µl of this reaction mixture was taken in a tube containing 1.5ml of 10% trichloro acetic acid. After 10 min, tubes were centrifuged and supernatant was separated and mixed with 1.5ml of 0.67% thio barbituric acid. The mixture was heated in a water bath at 85^o C for 30 min, and in boiling water bath to complete the reaction. The intensity of pink colored complex formed was measured at 535 nm .

The percentage of inhibition of lipid peroxidation was calculated by comparing the results of the test with those of controls as per the following formula i.e. Eq. (1);
Inhibition(%) = (Control Absorbance- Test Absorbance) X 100/Control absorbance.

DPPH radical scavenging activity

DPPH scavenging activity was measured by spectrophotometric method at 517 nm⁽¹¹⁾ To a methanolic solution of DPPH (100µM, 2.95 ml), 0.05 ml of EFTO and standard compound Vit. E were added in different concentrations. Equal amount of Methanol (0.05ml) was added to a control. After 30 min. absorbance was measured in triplicate. The percentage of scavenging was calculated by comparing the control and test samples with the Eq.-1

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity is a competition between deoxyribose and EFTO for hydroxyl radical generated by the Fe³⁺- ascorbate-EDTA-H₂O₂ system (Fenton reaction) according to the method of Kunchandy and Rao.¹² The reaction mixture containing final volume of 1.0 ml, 100µl 2-deoxy-ribose, 500µl of the various

concentrations of EFTO and standard compound (Mannitol 50mM) in KH₂PO₄-KOH buffer (20mM, pH 7.4), 200µl 1.04mm H₂O₂ and 100µl 1.0mM ascorbic acid was incubated at 37^oC for 1 hour. One milliliter 1% trichloro acetic acid was added to each test tube and incubated at 100^o C for 20 min. after cooling at room temperature; absorbance was measured at 532nm against a control preparation containing deoxyribose and buffer. Percent inhibition was determined by comparing the results of tests and control samples with the above mentioned Eq. -1

Super oxide anion scavenging activity

The superoxide scavenging activity of EFTO was determined by the method described by Nishimik *et.al.*¹³ with slight modification. About 1.0ml NBT solution containing 156µM NBT dissolved in 1.0 ml 100mM phosphate buffer , pH 7.4, 1.0 ml NADH solution containing 468µM NADH dissolved in 1.0 ml 100mM phosphate buffer, pH 7.4., and 0.1 ml of various concentrations of EFTO and standard compound Vit. E was mixed and the reaction was started by adding 100µl of phenazine methosulfate solution containing 60µM phenazine methosulfate in 100mM phosphate buffer, pH 7.4. The reaction mixture was incubated at 25^o C for 5 min, and absorbance at 560nm was measured against control sample. Percent inhibition was determined by comparing the results of the test and control samples with above mentioned Eq.-1.

Nitric oxide scavenging activity

Sodium nitropruside (10mm) in phosphate buffered saline was mixed with different concentrations of EFTO (100, 150, 200, 250 & 300µg) dissolved in methanol and incubated at room temperature for 150 min. The same reaction mixture without the sample but with equivalent amount of solvent served as control. After, the incubation period , 0.5ml of Griess reagent (1% sulphanilamide, 2% H₃PO₄ and 0.1% naphthylethylenedia mine dihydro chloride) was added. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine, was read at 546nm. Curcumin was used as positive control.¹⁴

RESULTS AND DISCUSION

Antioxidant activity

Assay of lipid peroxidation

The results presented in Table-1 showed that the ethanol extract of the *Thuja occidentalis* inhibited FeSo₄ induced

TABLE- 1 Effect of ethanol fraction of *Thuja occidentalis* (EFTO) on different antioxidant models.

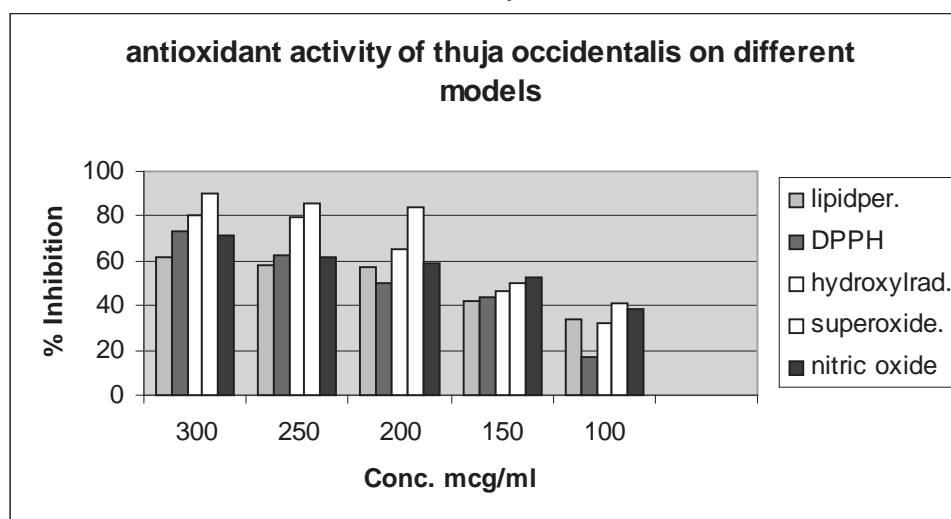
Concentration (µg/ml)	% Inhibition				
	Lipid peroxidation	DPPH	Hydroxyl radical	Superoxide radical	Nitric oxide scavenging
300	61.52±0.13*	73.35±1.04*	80.38±2.28*	89.79±0.29*	71.55±0.95*
250	58.17±0.33*	62.66±1.34*	79.54±1.24*	86.05±.24*	61.95±0.85*
200	57.39±0.65*	50.40±1.73*	65.37±2.27*	83.52±0.14*	58.54±0.23*
150	42.14±0.37	43.51±2.41	46.28±1.89	50.06±0.62*	52.98±0.66*
100	33.95±1.80	16.82±2.75	32.14±2.24	41.38±0.56	38.34±1.45
Vitamin E(5mM)	68.32±0.42*	67.29±0.71*	--NT--	70.39±0.13*	--NT--
Mannitol(50mM)	--NT--	--NT--	89.64±1.10*	--NT--	--NT--
Curcumin	--NT--	--NT--	--NT--	--NT--	85.38±0.22*
IC ₅₀ (µg/ml)	195.60	202.46	158.59	124.11	155.84

Values are mean ±SEM of three replicates. (NT- Not Tested) *P<0.05 vs control; student's t-test.

lipid peroxidation in a dose dependent manner. The extract at 300µg/ml exhibited maximum inhibition (61.516 ±0.131 %) of lipid peroxidation nearly equal to the inhibition produced by Vit. C. The IC₅₀ value was found to be 195.60µg/ml. The inhibition could be caused by the absence of ferryl-perferryl complex or by changing the ratio of Fe³⁺/Fe²⁺ or by reducing the rate of conversion of ferrous to ferric or by changing the iron itself or combination thereof.¹⁵

been widely used to test the ability of compound/plant extracts to act as free radical scavengers. Reduction of the DPPH radicals can be observed by the decrease in absorbance at 517nm. The DPPH scavenging activity of the extract was found to be 73.346±1.040 % at 300µg/ml compared with standard drug Vit. C at 5mM 78.296 ±0.708. The IC₅₀ value was found to be 202.457µg/ml, listed in Table -1.

FIGURE 1. Effect of Ethanol Fraction of *Thuja occidentalis* Linn. on different models



DPPH scavenging activity

DPPH is a stable free radical that can accept an electron or hydrogen radical to become a diamagnetic molecule. Due to its odd electron, the methanolic solution of DPPH shows a strong absorption at 517nm. DPPH radical reacts with suitable reducing agent, then electrons become paired off, and the solution loses color stoichiometrically with the number of electrons taken up.¹⁶ Such reactivity has

Hydroxyl radical scavenging activity

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage.¹⁷ Ferric-EDTA was incubated with H₂O₂ and ascorbic acid at pH 7.4. Hydroxyl radicals were formed in free solution and were detected by their ability to degrade 2-deoxy-2-ribose into fragments that formed a pink chromogen upon heating with TBA at low pH.¹⁸ When

the test compound were added to the reaction mixture they removed hydroxyl radicals from the sugar and prevented their degradation. The ethanol extract of *Thuja occidentalis* significantly inhibited (80.38±2.28%) degradation of deoxy-ribose mediated by hydroxyl radicals at the dose of 300µg/ml (Table-1), compared to that of known scavenger Mannitol (50mM). the concentration of ethanol extract of *Thuja occidentalis* needed for 50% inhibition was 158.59µg/ml.

Superoxide scavenging activity

Superoxide radical O₂⁻ is a highly toxic species and is generated by numerous biological and photochemical reactions. Both aerobic and anaerobic organisms possess superoxide dismutase enzymes that catalyze the breakdown of superoxide radical.¹⁹ Reduced phenazine methosulfate assay was used to measure the superoxide dismutase activity of EFTO. The results presented in Table -1 showed that the scavenging activity of the extract was 89.79±0.296% at 300µg/ml and IC₅₀ was found to be 124.11µg/ml.

Nitric oxide scavenging activity

EFTO (ethanolic fraction of *Thuja occidentalis*) showed moderate NO scavenging activity, with IC₅₀ of 155.85µg/ml and has its activity was comparable with that of curcumin (Table-1).

This fraction of ethanol extract of *Thuja occidentalis* Linn. has showed very good results in all the models of antioxidant activity tested (Fig.1). Therefore, it can be recommended for the *in vivo* pharmacological activities based on antioxidant activity like, hepatoprotective activity, antidiabetic activity, wound healing activity, anti ulcer activity, anti-inflammatory activity and many some others. The role of free radical oxidative stress in various disease conditions has been well established.¹⁹

REFERENCES

- Gutteridge JMC. Free radicals in Disease Processes: A Compilation of cause and consequence. Free radic. Res. Comm 1995;19: 141.
- Tiwari A. Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidant therapy . Curr. Sci. 2001; 81;1179.
- Joyce DA. Oxygen radicals in disease. Adv. Drug Rec. Bull. 1987; 127:476.
- Chang LC, Song LL, Park EJ, Luyengi L, Lee K J& Norman R. Bioactive Constituents of *Thuja occidentalis*. Journal of Natural Product 2000; 63:1235.
- Nam SH & Kang MY . Antioxidant activity of Medicinal Plants. Pharmaceutical Biotechnology 2005; 42: 409.
- Deb L, Dubey SK, Jain AK, Jain A, Pandian GS & Rout SP. Antidiarrhoeal activity of *Thuja occidentalis* Linn. ethanol extract on experimental animal. Indian Drugs 2007; 44:319.
- Belal N, Bodinet C, Tegtmeier M & Lindequist U. *Thuja occidentalis*(Arbor vitae): A Review of its Pharmaceutical, Pharmacological and Clinical Properties. Advance Access Publication 2005. 69-78.
- Ohkawa H, Oshishi N & Yagi K. Assay for lipid peroxidation in animal tissues by thiobarbituric acid. Anal Biochem 1979; 95: 351.
- Pandey S, Sharma M, Chaturvedi P & Tripathi YB. Protective effect of *R. cordifolia* on lipid peroxide formation in isolated in liver homogenate. Experimental journal of Biology 1995; 193:265.
- Lowery OH, Rosenbrough NJ, Farr AL & Randall RJ. Protein estimation with Folin phenol reagen. Biol. Chem 1951; 193:265.
- Sreejayan N & Rao MNA. Free radical scavenging by curcuminoids. J. Pharm. Pharmacology 1990;58:237.
- Kunchandy E & Rao MNA. Oxygen radical scavenging activity of curcuma. Int. J. Pharmacog 1990; 58: 237.
- Nishimiki M, Rao NA, Appaji N & Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophy Res Comm 1972; 46: 849.
- Sreejayan N & Rao MNA., Journ. Of Pharmacy and Pharmacology.1997; 49, 105.
- Braughler JM, Duncan CA & Chase IR. The involvement of iron in lipid peroxidation. Importance of ferrous to ferric ration in initiation. J. Biol. Chem 1986; 261: 10282.
- Blois MS. Antioxidant determinations by the use of stable free radical. Nature 1958;29: 1199.
- Aurand LW, Boonnmen NH & Gidding GG. Super oxide and singlet oxygen in milk lipid peroxidation. J Diar Sci 1977;60: 363.
- Aruoma OI, Laughton MJ and Halliwell B. Carnosine, Homocarnosine and Anserine: could they act as antioxidants *in vivo*. Biochem J 1989; 264:863.
- Govindarajan R, Vijaykumar M, Rawat AKS and Mehrotra S. Free radical scavenging potential of *Picrobiza kurrooa* Royle ex Benth. Ind. J. Exp. Bio. 2003; 41: 875.