Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(8):283-286

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In vitro screening of antioxidant potential of *Thuja occidentalis*

Mir Z. Nazir, Sanjeev Chandel and Amit Sehgal*

Department of Zoology, School of Biotechnology and Biosciences, Lovely Professional University, Phagwara 144411, India

ABSTRACT

The present study was undertaken to investigate the phytochemical composition and antioxidant potential of methanolic extract (leaves) of Thuja occidentalis. The total phenolic and flavonoid content was determined using Folin-ciocalteau and Aluminum chloride method. The antioxidant activity was evaluated using DPPH, ABTS, NO and lipid peroxidation (LPO) assays. The results revealed that methanolic extract of T.occidentalis had an appreciable antioxidant activity. The IC50 value for DPPH, ABTS, NO and LPO are 0.47, 0.42, 0.82 and 0.42 mg/ml, respectively. The TPC and TFC was calculated to be 135.32 mg/g GAE and 3.46 mg/g QE. The antioxidant activity demonstrated by T. occidentalis may be due to its high phenolic content.

Key words: Antioxidant, *Thuja occidentalis*, phenolic, flavonoid.

INTRODUCTION

Medicinal plants play a crucial part in maintaining the health of people since ages. The Indian system of medicine, Ayurveda, mainly depends on the plant sources for the treatment of various diseases. Also because of easy availability, low cost, and high effectiveness the plant sources have not lost its importance of being medically beneficial [1]. Various plants also contain antioxidants, which help in shielding the cells against the harmful effects of reactive oxygen species. Antioxidants are present in our body in an appropriate quantity but to protect our body from these reactive oxygen species (ROS) there is need of antioxidants from natural sources [2]. The medicinal plants possess phenolic compounds that possess very strong antioxidant activity and in turn help the body against the damage by free radicals [3]. These phenolic compounds act as radical scavengers, reducing agents, nascent oxygen quenchers, hydrogen donors and metal chelators [4-5]. An antioxidant is defined as “any substance which when present at low concentrations significantly delays or prevents oxidation of an oxidizable substrate by scavenging free radicals” [6]. Free radicals and other reactive oxygen species are formed constantly in the human body. They can be useful for some physiological activities but in excess, they may prove very dangerous and harmful for human body. These free radicals generally damage the DNA, carbohydrates, lipids and proteins in body. Free radicals are also produced by environmental pollutants, cigarette smoke, automobile exhaust fumes, radiation, air pollutants and pesticides etc. [7]. There is a balance between the free radicals in body and the antioxidants to scavenge these radicals to protect against their harmful effect. However any increase in free radicals inside the body can produce harmful effects and stress in human body. Thus antioxidants are useful for maintaining the required quantity of free radicals inside the body and scavenge the extra free radicals so that no deleterious effects of these free radicals are found in human body.

Thuja occidentalis is a well known medicinal plant. It belongs to family Cupressacae. It is also known as American Arbor vitae or white cedar. It is indigenous to eastern North America and is grown in Europe as an ornamental tree [8]. *T.occidentalis* is effective for the treatment of bronchial infections, enuresis, cystitis, psoriasis, uterine carcinomas, amenorrhea and rheumatism [9]. The plant is also seen to be helpful for the treatment of the common cold [10]. *T.occidentalis* also demonstrated properties such as antifungal, insecticidal, larvicidal, antidiabetic, and antibacterial [11-13].

EXPERIMENTAL SECTION

Collection of Plant material
The plant material was collected from Herbal garden of Lovely professional university, Phagwara, Punjab.

Preparation of Extract
The dried and powdered leaves of *Thuja occidentalis* were subjected to soxhlet extraction to prepare various extracts. To 100 gm of powder 300 ml of solvent (methanol) was added in the flask. The solvent obtained after 4-5 hrs of extraction was dried in rotary evaporator. The extract obtained was stored in tight screw capped vial at 4˚C.

DPPH Scavenging Activity
The free radical scavenging activity of plant extracts were evaluated by using 1, 1-diphenyl-2-picyl-hydrazil (DPPH). A solution of DPPH was prepared (11 mg in 50 ml methanol). The OD of the DPPH solution prepared was set between 0.7 – 1 by diluting it with 50% methanol. Different concentrations of plant extract were added to every 2.5 ml of DPPH solution. After one min. the absorbance was measured at 520 nm using visible spectrophotometer. Methanol was taken as blank and 2.5 ml of DPPH solution was taken as control [14].

ABTS (2, 2 – azino- bis – 3- ethyl benzthiazoline – 6 – sulphonic acid) Scavenging Assay
The antioxidant effect of the plant extracts was studied using ABTS radical cation decolourisation assay. ABTS and sodium persulphate solution was prepared in distilled water (36 mg ABTS in 100 ml distilled water & 57 mg sodium persulphate in 100 ml distilled water).10ml ABTS solution and 10ml sodium persulphate solution prepared were mixed to generate ABTS radical cations. This mixture was kept in dark for 18 hours at room temperature. After incubation the ABTS solution was diluted with 50% methanol for an initial reading of 0.7–0.9 at 745nm [15].

Lipid Peroxidation Assay
Fresh chicken liver was collected from slaughter house and was kept in cold or chilled PBS and maintained at 4^0C till use. Thin slices were cut using a sterile scalpel. 1g of liver was taken in 2ml of PBS. This mixture was homogenized using homogenizer and centrifuged. The supernatant was taken for use. Various concentrations of plant extracts were added to every 0.5ml of liver homogenate. Appropriate controls were set. This mixture of 0.5ml of liver homogenate, different concentrations of plant extract and 100µl of FeSO4 were incubated at 37^0c for 30 minutes. After incubation 2ml of TBA, TCA and HCl (TBA-0.5g, TCA-20ml, HCl-0.5ml) mixture were added to this mixture and was kept in water bath for 20 minutes at 100^0c. The light pink color was developed. This color was estimated at 532 nm against in spectrophotometer [16].

Nitric Oxide Scavenging Activity
Sodium nitroprusside solution (0.262g/100ml dH2O) was prepared in PBS at pH 7.4. Different concentrations of plant extracts were mixed with 2ml of sodium nitroprusside and 1 ml of PBS. An appropriate control (reagents without plant extract) was set. Samples were incubated at 37^0C for 2 ó hours. After incubation 0.5ml of Griess reagent was added and absorbance was measured at 546nm [17].

Estimation of Total Phenolic Content
Total phenolic content of plant extracts were determined by the Folin-ciocalteau method [18]. The extract samples were mixed with 0.5ml of 0.2 N Folin-ciocalteaureagents. After 5 minutes 2.0 ml of 20% sodium carbonate were added. The absorbance of reaction was measured at 765 nm after 30 minutes of incubation at 37 ºC. The standard curve was prepared by 200,400,600,800 µg/ml solution of gallic acid in methanol. The total phenolic content was measured in reference to gallic acid curve.

Estimation of Total Flavonoid Content
Total flavonoid content was measured by Colorimetric Aluminum chloride method[18]. 0.5ml of plant extract of each plant extracts were mixed with 1.5 ml of methanol, 0.1ml of 10% aluminum chloride, 0.1ml of 1 M potassium acetate, and 2.8 ml of distilled water. The solution was incubated at 37ºC for 30 minutes. The absorbance of mixture was measured at 415 nm. Total flavonoid contents were calculated from the curve obtained from quercetin which was taken as standard. The standard curve was prepared by 10 to 40 µg/ml concentration of quercetin.

Statistical analysis
The data were expressed as mean±sd for triplicate readings. The statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Tukey`s honestly significant test using SPSS software (version 16). The results were considered statistically significant if the p-values were 0.05 or less.

RESULTS AND DISCUSSION

In vitro antioxidant capability of the methanolic extract (leaves) of *Thuja occidentalis* was measured in the present study by DPPH, ABTS, NO and Lipid peroxidation assays. The total phenolic and flavonoid content was also evaluated.

The percentage of scavenging effect on DPPH free radical increased significantly with the increase in the concentration of methanolic extract of *T.occidentalis* from 0.125 mg/ml to 0.5 mg/ml (Figure 1a). The %inhibition deviated from 13.15% at 0.125 mg/ml to 50.88% at 0.5 mg/ml of methanolic extract ($p \leq .05$) and the IC_{50} value was calculated to be 0.49 mg/ml. A previous study revealed the %scavenging activity of 73.35% at 0.3 mg/ml and the IC_{50} value was found to be 202.46µg/ml [13]. Our study showed that ABTS scavenging activity of methanolic extract from 19.81% at 0.125 mg/ml to 54.98% at 0.5 mg/ml ($p \leq .05$) (Figure 1b) and the IC_{50} value was evaluated to be 0.45 mg/ml.

The percentage of scavenging effect on nitric oxide radical increased with the increase in the concentration of methanolic extract. The %inhibition was varying from 17.28% at 0.25 mg/ml to 59.75% at 1 mg/ml ($p \leq .05$) of methanolic extract of *T.*occidentalis (Figure 1c). The IC50 value of the extract was calculated to be 0.73 mg/ml. The previous report demonstrated the %scavenging activity of 71.55% at 0.3 mg/ml. The IC_{50} values were found to be 155.84µg/ml [13]. Lipid peroxidation involves the stealing of electrons by free radicals from membrane lipids thus resulting in the damage of cells. Antioxidants scavenge these free radicals and thus prevent cell damage. The %inhibition was varying from 10.98% at 0.125 mg/ml to 57.85% at 0.5 mg/ml ($p \leq .05$) (Figure 1d) and the IC50 value was calculated to be 0.42 mg/ml of methanolic extract.

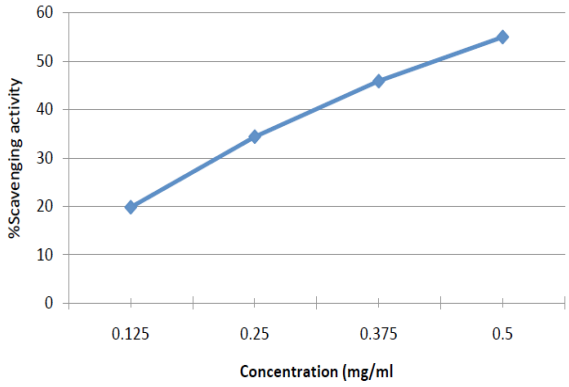

Fig. 1a

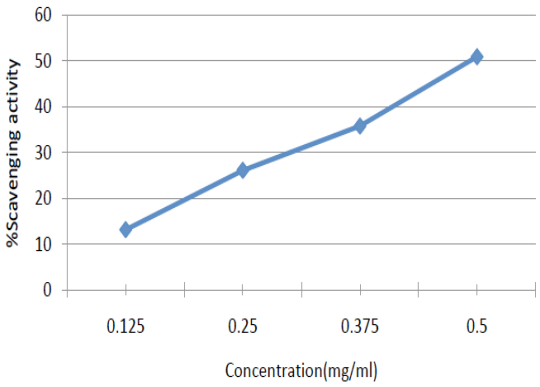

Fig.1b

Figure 1: a) Radical scavenging activity (%) of methanolic extract of *Thuja occidentalis* (mean±sd, n=3) against (a) DPPH and (b) ABTS

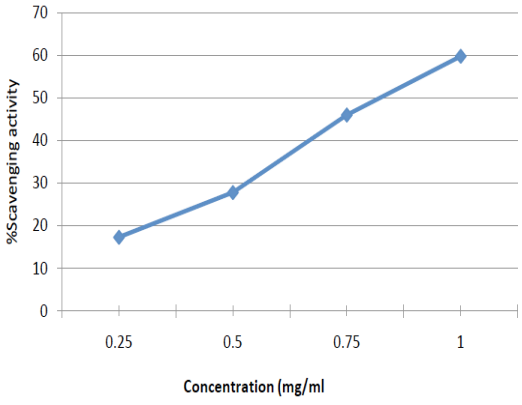

Fig. 2a

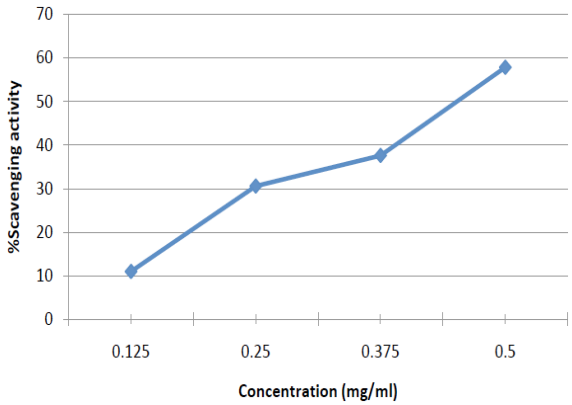

Fig. 2b

Figure 2: a) Nitric oxide radical scavenging and (b) Anti-lipid peroxidation activity of methanolic extract of *Thuja occidentalis* (mean±sd, n=3)

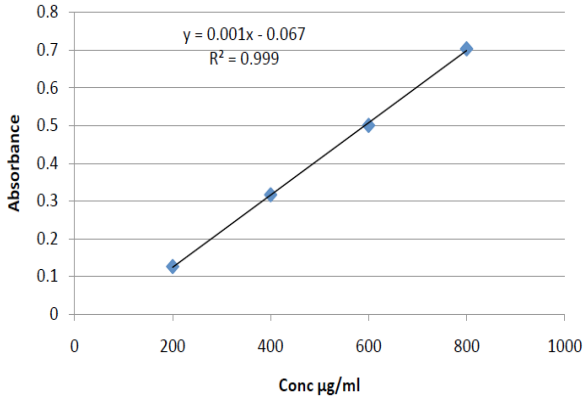

Fig. 3a

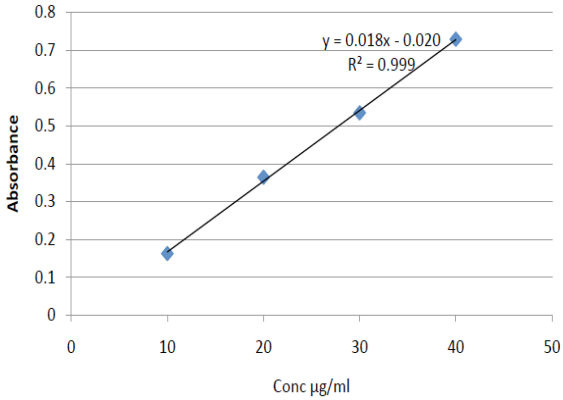

Fig. 3b

Figure 3: Standard curve of (a) gallic acid (b) quercetin

In a recent study, the %scavenging activity of *T. occidentalis* was found to be 61.52% at 0.3 mg/ml. The IC_{50} value was 195.60µg/ml [13].

TPC was calculated using the standard curve of gallic acid. TPC in methanolic extracts of *T.occidentalis* was measured to be 135.32 mg GAE/g (Figure 2a). Previous finding reported that the total phenolic content in *T.occidentalis* was found to be 78.08 mg GAE/g [19]. The total flavonoid content was also calculated in reference to quercetin. The TFC in the methanolic extracts of *T.occidentalis* calculated was 3.46 mg QE/g (Figure 2b). The Total flavonoid content in previous study came out to be 7.5 mg QE/g [19].

The methanolic extract of *T.occidentalis* was found to be a potential source of antioxidants. This may be ascribed to its high phenolic content. Further, in vivo studies are required to prove the efficacy of this plant as a potential health promoting agent.

Acknowledgments
We acknowledge the financial support by Lovely Professional University to do the necessary research work and to use departmental facilities.

REFERENCES

[1] G Agbor; J Oben; J Ngogang; C Xinxing; J Vinson. *Journal of Agricultural and Food Chemistry*, **2005**, 53 (17), 6819-24.
[2] M S Brewer; *Comprehensive Reviews in Food Science and Food Safety*, **2011**, 10(4), 221-47.
[3] M Kahkonen; A Hopia; H Vuorela; J Rauha; K Pihlaja; T Kujala; M Heinonen; *Journal of Agricultural and Food Chemistry*., **1999**, 47(10), 3954-62.
[4] C Proestos; I Boziaris; G Nychas, M Komaitis; *Food Chemistry*, **2006**, 95 (4), 664-71.
[5] S Kapoor; *International Journal of Advanced Research*. **2011**, 4(2), 10-11.
[6] B Halliwell; M C Gutteridge; *The International Journal of Biochemistry & Cell Biology*, **1999**, 31(12), 1454.
[7] H Sies; *The American Journal of Medicine*, **1991**, 91(3), 31-8.
[8] L Chang; L Song; E Park; L Luyengi; K Lee; N Farnsworth; J Pezzuto; *Journal of Natural Products*. **2000**, 63(9), 1235-38.
[9] D Baran; *EVISTA Medico -Surgical Society of Physicians and Naturalists*, **1991**, 95 (3-4), 347-49.
[10] C Bodinet; R Mentel; U Wegner; U Lindequist; E Teuscher; J Freudenstein; *Planta medica*., **2002**, 68(10), 896-900.
[11] M Ahmad; F Saeed; N Jahan; *Journal of Pharmacognosy and Phytochemistry*., **2013**, 2 (3), 153-58.
[12] P Sharma; L Mohan; N Srivastava, N; *Journal of Environmental Biology*, **2005**, 26 (4), 657-60.
[13] S Dubey; A Batra; *Asian Journal of Pharmaceutical and Clinical Research*, **2009**, 2(1), 73-76.
[14] L Mensor; F Menezes; G Leit; A Reis; T Santos, C Coube, C; *Phytotherapy research*., **2001**, 15 (2), 127-30.
[15] A Shirwaikar; K Rajendran; I Punitha; *Biological & Pharmaceutical Bulletin*. **2006**, 29(9), 1906-10.
[16] H Ohkawa; N Ohishi; K Yagi; *Analytical Biochemistry*, **1979**, 95(2), 351-58.
[17] M Ebrahimzadeh; S Nabavi; F Pourmorad; *African journal of Biotechnology*, **2013**, 9 (32), 5212-217.
[18] A Ebrahimzadeh; K Ghasemi; Y Ghasemi; *Pak Journal of Pharmaceutical sciences*, **2009**, 22 (3), 277-81.
[19] K Yogesh; J Ali; *Journal of Food Science and Technology*. **2012**, 51(8), 1547-553.