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Pharmacognostic, phytochemical, and pharmacological investigation on bark of *Thuja Orientalis* Linn (Cupressaceae)

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Abstract

Antioxidants play an important role in protecting against damage by reactive oxygen species. The antioxidant activity of different extracts of *Thuja orientalis* was evaluated by employing two *in vitro* experiments namely Nitric oxide method and Hydrogen peroxide scavenging. The bark powder of the plant was extracted with different solvents by extraction method in order of decreasing polarity and then partitioned. The present study was designed to evaluate the plant potential as an antioxidant lead by using various *in vitro* models like Hydrogen peroxide scavenging method, and, Nitric oxide method. The plant exhibited significant antioxidant properties and could serve as a free radical inhibitor or scavenger.

Keywords: *Thuja orientalis* Linn, antioxidant, nitric oxide method, Hydrogen peroxide scavenging activity

Introduction

Oxygen is essential for the survival of all this earth. During the process of oxygen utilization in normal physiological and metabolic processes approximately 5% of get univalently reduced to oxygen derived free radical like Superoxide, Hydrogen peroxide, and Nitric oxide radicals. All these radicals known as reactive oxygen species (ROS) exert oxidative stress towards the cell of human body rendering each cell face about 10000 oxidative hits per seconds. When generation of ROS overtakes the cell, the free radical start to attaching to cell protein, lipids and carbohydrates and this leads to a number of physiological disorders. Many plants often contain substantial amounts of antioxidants including vitamin C and E, carotenoids, flavonoids and tannins etc. and thus can be utilized to scavenge the excess free radicals from human body [9].

Thuja orientalis is a common ornamental evergreen tree that is originally native to Northwest China belonging to family Cupressaceae. It is highly aromatic and resinous shrub that widely cultivated in gardens located in temperate and semi-temperate areas. *Thuja orientalis* is hardy, large evergreen shrub or small to medium sized-tree rarely exceeding 20 m in nature. It has a dense, pyramidal shape, but often exhibits a more open and spreading form. It prefers moist, well-drained soil and full sun. The bark is gray with brown highlights and has thin but deep furrows [5]. The bark has a rugged charm about it, especially on large mature specimens. Younger bark is a reddish-brown color and exfoliates in long, thin strips. *Thuja orientalis* leaves contain Rhodoxanthin, Amentoflavone, Hinokiflavone, Quercetin, Myricetin, Carotene, Xanthophylls and Ascorbic acid. Thujone is a ketone and a monoterpene that occurs naturally in two diastereomeric forms: α -Thujone and β -Thujone [18].

Traditionally it is used in the treatment of cough. 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. The plant has been exhibited extensively biological activities including anti-epileptic, anti-inflammatory, hair growth-promoting, antiviral, anti-allergic, antibacterial, antioxidant, and antifungal activities. The root bark is used in the treatment of burns and scalds. The stems are used in the treatment of coughs, colds, dysentery and parasitic skin-diseases. The leaves are antibacterial, antipyretic, antitussive, astringent, diuretic, refrigerant and stomachic [8].

Materials and Methods

Collection and identification of plant material: The fresh plants of *Thuja orientalis* Linn were collected in the months of October from Hadapsar, Pune, Maharashtra, India, and authenticated by P. Lakshminarasimhan, Botanical Survey of India, Pune.

Preparation of extracts

The bark of *Thuja orientalis* was collected, washed and dried at room temperature. Bark were grinded into the fine powder, extracted with different solvents in decreasing order of solvent polarity i.e n-Hexane, Chloroform, Alcohol each for 72 hrs. The extract was dried in a vacuum oven to obtained constant weight.

Phytochemical evaluation

The alcoholic extract was used to analyze qualitatively various phytoconstituents such as alkaloids, glycosides, steroids, phenolic compounds, tannins, flavonoids and carotenoids using standard procedures [1].

Table 1: Characterization of extracts by Chemical Tests.

SR. No.	Chemical Test	Bark Extract		
		n- exane	Chloroform	Alcohol
1	Test for sterol	Present	-	-
2	Test for Glycosides	-	-	Present
3	Test for Alkaloids	-	Present	-
4	Test for Flavonoids	-	-	Present
5	Test for Tannins	-	-	Present
6	Test for Carotenoids	Present	-	-

In Vitro Antioxidant Activity.

Nitric Oxide Radical Scavenging Assay

Chemicals: Sulfanilamide, N-1-naphylethylenediamine dichloride, Potassium dihydrogen phosphate, Ascorbic acid, sodium hydroxide, Methanol.

Equipment: UV- Spectrophotometer, Graduated pipette.

Preparation of Stock solution: 10 mg of Ethyl acetate soluble alcoholic extract (ETA) transfer in 10ml of volumetric flask dissolved in methanol up to the mark. From this solution prepared the different concentration of dilutions. (10-100µg/ml)

Preparation of ascorbic acid solution: 10mg of ascorbic acid dissolved in 100 ml phosphate buffer. From this solution prepared the different concentration of dilutions. (10-100µg/ml)

Preparation of Griess reagent: 0.665 ml of H₃PO₄, 0.25 gm of sulfanilamide, 0.25 gm of N-1-naphylethylenediamine dichloride mix in 25 ml of distilled water.

Preparation of Sodium nitroprusside solution (10mM): 0.065 gm in 25 ml of phosphate buffer.

Preparation of phosphate buffer (7.4): 50 mml of K₂PO₄ (0.2M) and 38.1 ml of NaOH (0.2M) solution.

- 1) K₂PO₄ (0.2M): 2.718 g in 100ml of distilled water.
- 2) NaOH (0.2M): 0.8 g NaOH in 100 ml of distilled water.

Method

Incubate stock solution with different concentration of sample at 25 °C for 150 minute. Control experiment without the test sample but equivalent amount of buffer was conducted in identical manner. After incubation take 0.5 ml of solution add 0.5 ml of Griess reagent (1% Sulfanilamide, 0.1% N-1-naphylethylenediamine dichloride, 2% phosphoric acid) was added. The absorbance was taken at 546 nm. Ascorbic acid used as standard. The percentage inhibition activity was calculated by following formula [2]:

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

Where,

A₀ = is the absorbance of the control

A₁ = is the absorbance of extract/standard taken as Ascorbic acid.

Table 2: Antioxidant activity by Nitric oxide scavenging activity.

No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	ETA extract
1	25	23.5	22.56
2	50	29.84	24.36
3	75	46.25	40.85
4	100	60.57	53.32
5	125	68.96	62.35
6	IC ₅₀	24.10	20.40

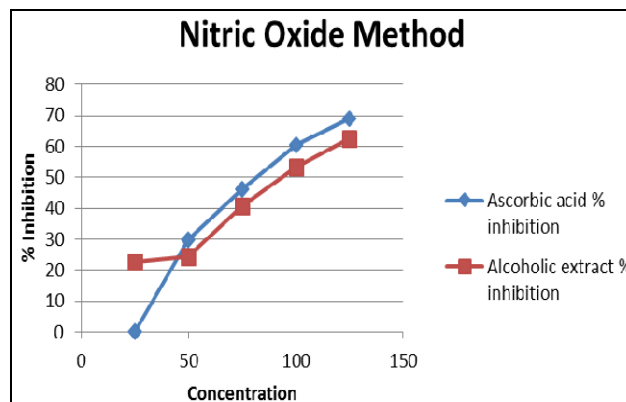


Fig 1: Nitric oxide scavenging activity

B) Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity was measured with titrimetric method of estimation. 1ml of 0.01mM of H₂O₂, 2 drops of 3% Ammonium molybdate indicator, 10ml Sulphuric acid and 7 ml of 2M KI. The mixed solution was titrated with 5Mm Sodium thiosulphate until yellow colour disappeared. Ascorbic acid was used as positive control and percentage hydrogen scavenging was determined [1].

$$\% \text{ Inhibition} = (\text{Blank} - \text{Test}) / \text{Blank} \times 100$$

Table 3: Antioxidant activity by Hydrogen Peroxide Scavenging activity.

No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	ETA extract
1	25	23.5	21.73
2	50	29.84	26.84
3	75	46.25	43.54
4	100	60.57	59.32
5	125	68.96	65.51
6	IC ₅₀	25.58	23.79

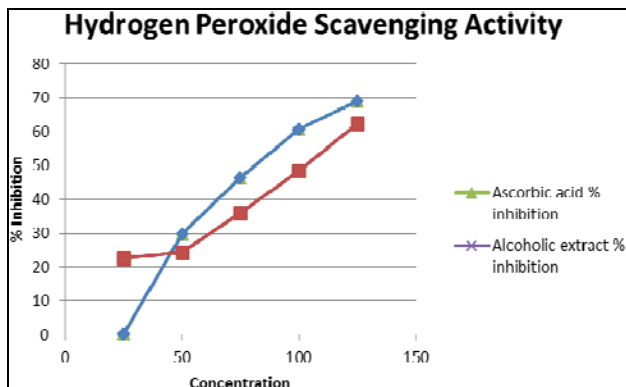


Fig 2: Hydrogen peroxide scavenging activity.

Results and Discussion

Ethyl acetate soluble alcoholic extract of bark of *Thuja orientalis* was screened for Anti-oxidant Activity. The fraction isolated from ethyl acetate soluble alcoholic extract of bark is 8(17), 13- Labdine- 16, 15 olide-18-ate.

Anti-oxidant activity, of extract was compared with standard drug i.e. Ascorbic acid. IC₅₀ value of ETA extract and Ascorbic acid by Nitric oxide radical scavenging assay method was found to be 20.40 µg/ml and 24.10 µg/ml respectively. IC₅₀ value of ETA extract and Ascorbic acid by Hydrogen peroxide scavenging activity method was found to be 23.79 µg/ml and 25.58 µg/ml respectively. This study shows that ETA extract had comparable Nitric oxide radical scavenging activity to standard Ascorbic acid.

Conclusion

From all the experiments done on bark of *Thuja orientalis*. It is concluded that the *Thuja orientalis* plant shows presence of sterols, carotenoids, alkaloids, flavonoids, tannins.

The phytochemical studies showed the presence of most of the biologically active compounds in the plant. 8(17), 13- Labdine- 16, 15 olide-18-ate was found as compound in ethyl acetate soluble alcoholic extract.

Ethyl acetate soluble alcoholic extract of *Thuja orientalis* bark was found to be significant in Antioxidant activity.

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