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Superoxide and Hydrogen Peroxide Scavenging Action of Ocimum Sanctum Extracts and their Fractions

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Abstract – *Ocimum sanctum*, the Indian holy basil has significant abilities to scavenge highly reactive free radicals. Shade dried leaf powder of plant has extracted with water and alcohol, fractionated with different solvents. Both extracts and their fractions were found to be good scavengers of Superoxide and hydrogen peroxide. Free radical scavenging action of these compared with ascorbic acid, a known antioxidant.
Key words – *Ocimum sanctum*, antioxidant

Introduction

Ocimum sanctum – Holy basil (family *Labiatae*) commonly known as sacred Tulsi is a fragrant bushy plant found in semi tropical and tropical parts of India Different parts of the plant are traditionally used in Ayurveda and Siddha system of medicines for treating infections, skin diseases, hepatic disorders, cold, cough, malarial fever and as an antidote for snake bite (Satyavathi *et al.*, 1987).

Aqueous extract of *Ocimum* showed strong protective effect against radiation injury (Umadevi, Ganasoundari, 1995). It protected mouse liver against radiation induced lipid peroxidation and increased the levels of cellular antioxidants (Umadevi, Ganasoundari, 1999). Flavonoids isolated from aqueous extract namely orientin and vicenin found to be very effective in protecting against radiation induced lipid peroxidation in mouse liver and also showed significant inhibition of Fenton reaction induced hydroxyl radical scavenging activity *in vitro*. Hydroxyl radical scavenging activity was better than DMSO (Dimethyl sulfoxide) and synthetic radioprotector WR-2721 (Ganasoundari *et al.*, 1997; 1998). Flavonoids isolated from *Ocimum sanctum* scavenged free radicals *in vitro* and showed antilipoperoxidant activity *in vivo* at a very low concentration (Umadevi *et al.*, 2000). Therefore in the present investigation it has been undertaken to see *in vitro* antioxidant efficacy of *Ocimum sanctum* extracts and their fractions. Free radical scavenging action was studied with respect to Superoxide and Hydrogen peroxide and was compared with known antioxidant ascorbic acid. Scavenging action was expressed as inhibitory concentration of test compound required for bringing 50% inhibition of free radicals i.e. IC_{50} values.

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Experimental

Leaves of *Ocimum sanctum* were collected, dried in shade and powdered. The powder was used for extraction.

Aqueous extract – 100gms of leaf powder was refluxed with 750 ml of double distilled water (DDW) for 1 hr at 75-80°C, cooled and filtered. This was repeated in 3 trials, extracts were pooled and evaporated using Lyophiliser (Ganasoundari *et al.*, 1998).

Alcoholic extract – 75 gms leaf powder was extracted with 700 ml of 95% ethanol in a Soxhlet apparatus at 60-75°C as explained by Suffness and Douros (Suffness, Douros, 1979). Extract was concentrated.

Fractionation of Extract

Both aqueous and alcoholic extracts were re-extracted with organic solvents ranging from polar to non-polar in succession. They were successively re-extracted with petroleum ether (60-80°C) 200 ml×3, diethyl ether (200 ml×3) and ethyl acetate (200 ml×3) and in methanol (200 ml×3) in succession. Petroleum ether, diethyl ether, ethyl acetate fractions were washed separately in a separating funnel with small quantity of DDW dried over anhydrous

sodium sulphate concentrated by distillation and were evaporated to dryness. Un-dissolved material left after, methanol extraction, was dissolved in DDW and concentrated in Lyophiliser. Final yield of solvent free fractions of aqueous extract and alcoholic extracts were used in study.

Scavenging activity against Superoxide anion radical – Superoxide scavenging activity of the compounds were determined by monitoring the competition of those with NBT for Superoxide anion generated by PMS-NADH system (Liu *et al.*, 1997). Superoxide radicals were generated in 1 ml 20 mM Tris-HCl buffer pH 8.0 contained 0.05 mM nitroblue tetrazolium (NBT), 0.01 mM phenazine methosulphate (PMS) and test compound were preincubated for 2 min. The reaction was initiated by the addition of 0.078 mM NADH. Blue chromogen formed due to NBT reduction was read at 560 nm. Results were expressed in percentage of inhibition. Superoxide scavenging action of *Ocimum* extracts and their fractions were compared with known antioxidant Ascorbic acid.

Hydrogen peroxide scavenging action – This was measured by peroxidase based assay system (Paya *et al.*, 1992), where Horseradish peroxidase (HRP) uses H_2O_2 to oxidise guaiacol, which produces brown colour measured at 436 nm. If an antioxidant is incubated with H_2O_2, the rates of H_2O_2 disappearance can be calculated.

Different concentrations of test compounds were incubated with 2 mM H_2O_2 for 30 min at 25°C and remaining H_2O_2 was measured by peroxidase system. 2.0 ml of reaction mixture contained 150 mM KH_2PO_4-NaOH buffer pH 7.4, 0.1 µl guaiacol and 0.1 units of HRP. Percentage of inhibition of H_2O_2 by test compounds was calculated and was compared with that of ascorbic acid.

Results and Discussion

Figure 1 shows the Superoxide scavenging action of *Ocimum sanctum* extracts and their fractions, which indicates a dose dependent inhibition of radicals. These are compared with known antioxidant and free radical scavenger ascorbic acid. (Table No. 1) Crude aqueous extract is found to be better scavenger (IC 50 at 65 µg) than the alcoholic extract. Further water fraction of aqueous extract is found to contain the active inhibitor of Superoxide (IC 50 at 62 µg). Ethyl acetate and methanol fractions of both aqueous and alcoholic extract showed same level of scavenging action (IC 50 at 90 µg). IC 50 values of all these compounds are greater than that of ascorbic acid where IC 50 achieved at 5 µg concentration.

Figure 2 shows the H_2O_2 scavenging action of *Ocimum sanctum* extracts and their fractions compared with that of

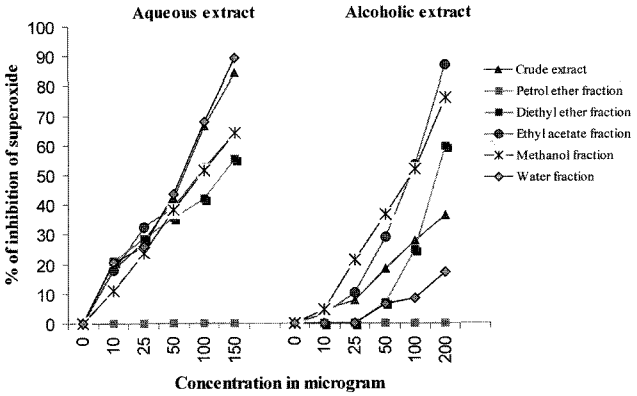

Fig. 1. Showing Superoxide radical scavenging action of aqueous and alcoholic extracts of *Ocimum sanctum* and their fractions.

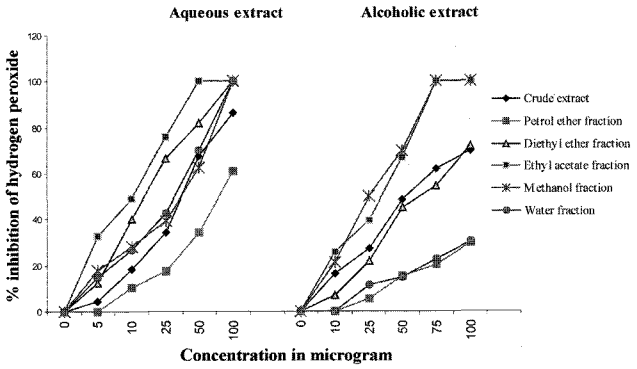

Fig. 2. Showing Hydrogen peroxide scavenging action of aqueous and alcoholic extracts of *Ocimum sanctum* and their fractions.

ascorbic acid, which are found to linear along with concentration. Crude aqueous extract is better scavenger (IC 50 at 37 µg) than alcoholic extract. Among fractions

Table 1. Comparison of Superoxide and Hydrogen peroxide scavenging action of *Ocimum sanctum* extracts and their fractions with Ascorbic acid

Extract	Concentration needed for 50% inhibition (IC 50 Values)	
	Super oxide	Hydrogen peroxide
Crude aqueous extract	65 µg	37 µg
Fractions:		
a) Petrol ether	No inhibition	75 µg
b) Diethyl ether	150 µg	12.5 µg
c) Ethyl acetate	90 µg	10 µg
d) Methanol	90 µg	35 µg
e) Water	62 µg	35 µg
Crude Alcoholic extract	450 µg	55 µg
Fractions:		
a) Petrol ether	No inhibition	175 µg
b) Diethyl ether	250 µg	60 µg
c) Ethyl acetate	90 µg	28 µg
d) Methanol	90 µg	25 µg
e) Water	700 µg	350 µg
Ascorbic acid	5 µg	8 µg

Diethyl ether and ethyl acetate fractions of aqueous extract showed IC 50 at 12.5 µg and 10 µg respectively, which is almost equal to known antioxidant ascorbic acid (IC 50 at 8 µg). (Table 1)

Present study reveals that *Ocimum sanctum* extracts and their fractions are potent scavengers of deleterious free radicals such as $O^{\bullet -}$ and H_2O_2. These extracts were found to be very effective at very low concentration. Aqueous extract showed better scavenging activity with respect to superoxide and hydrogen peroxide. Among fractions Diethyl ether and Ethyl acetate fractions were found to be good scavengers at very low concentrations. ROS have been implicated in more than 100 diseases from Malaria to Haemorrhagic shock to AIDS (Alho *et al.*, 1999). Oxidative stress causes various forms of tissues damage and inflammation and plays a main role in the development of several degenerative changes in cells and tissues, which lead to several degenerative disorders. Bodily defenses are not completely efficient to prevent on going oxidative damage to DNA, lipids and proteins. Dietary antioxidants, Vitamins, Flavonoids, plant phenolics, herbal formulations and Ayurvedic preparations are very essential in protecting against oxidative stress (Weiss *et al.*, 2000). Flavonoids and poly phenolics are the main components of *Ocimum sanctum* (Skaltsa *et al.*, 1999). In a study it was concluded that plant Flavonoids which show antioxidant activity *in vitro* also function as antioxidants *in vivo* (Shimoi *et al.*, 1996). From present study it is evident that *Ocimum sanctum* extracts and fractions are good scavenger of reactive free radicals. Scavenging actions of some of the fractions are on par with Ascorbic acid. Many antioxidants similar to ascorbate and phenolic compounds possess prooxidant properties (Narla *et al.*,1995). But *Ocimum sanctum* extracts and their fractions are free from such prooxidant properties. In recent years there is a growing interest in antioxidant supplements for the prevention of many diseases. In this context *Ocimum sanctum* can be exploited for its impressive free radical scavenging activities.

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