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## EVALUATION OF HYDROXYL RADICAL SCAVENGING ACTIVITY OF ETHANOLIC EXTRACT OF *THYMUS SERPYLLUM*

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### Keywords:

Hydroxyl radical,  
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Antioxidant, Oxidative stress

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**ABSTRACT:** Ethanolic extract of the aerial parts of *Thymus serpyllum* was evaluated for antioxidant activity. As many diseases in today's world are due to free radicals, finding antioxidants of herbal origin can lead to development of safe herbal medicines for these diseases. Hydroxyl radical scavenging method was used to evaluate the antioxidant activity of *Thymus serpyllum* in the present study. The results obtained from the study proved the hydroxyl radical scavenging activity of *Thymus serpyllum*. Ethanolic extract (99.9%) of aerial parts of *Thymus serpyllum* was prepared, dried and sent to Deshpande Laboratories Private Limited, Madhya Pradesh Bhopal (An ISO 9001:2008 Certified Drug Testing Laboratory) for analyzing hydroxyl radical scavenging activity. The results obtained from the study proved the free radical scavenging of the extract. The antioxidant effect of *Thymus serpyllum* can be largely attributed to its chemical constitution. Since *Thymus serpyllum* showed significant antioxidant activity, it can be developed into a safe herbal formulation useful in stress and stress related disorders.

**INTRODUCTION:** In today's world many diseases are linked to oxidative stress. Oxidative stress is excess generation of and/or inadequate removal of highly reactive oxygen species (ROS) and reactive nitrogen species (RNS). ROS include superoxide radical, hydroxyl radical, hydrogen peroxide *etc.* RNS include peroxynitrite, nitrous oxide *etc.* <sup>1</sup> Oxidative stress is implicated in cancer, neurodegenerative diseases (Alzheimer's or Parkinson's disease), arteriosclerosis *etc.* <sup>2</sup> Antioxidants prevent oxidative damage by free radical and ROS, and may prevent the occurrence of various diseases. Current research is now directed towards finding naturally occurring antioxidants of plant origin.

Plant and plant products are being used as a source of medicine since long. The medicinal potentials of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no or very less side effects and economic viability.

*Thymus serpyllum* L. (Lamiaceae) is an aromatic plant. Flowers are pink in color and bloom in the month of April. It is found in regions of western Himalayas at an altitude of 1500 - 4500 meters. The chemical constituents of *Thymus serpyllum* are protein, fat, crude fibre, carbohydrate, minerals, Vitamin A, B and C. The main constituent of the volatile oil of *Thymus serpyllum* is thymol <sup>3</sup>. *Thymus serpyllum* also contains flavanoids and phenols <sup>4</sup>. Essential oil of *Thymus serpyllum* has stimulating, antispasmodic, antiseptic, expectorant, carminative and anthelmintic activities <sup>3</sup>. The present study is undertaken to investigate the antioxidant activity of the aerial parts of *Thymus serpyllum* using hydroxyl radical scavenging method.

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## MATERIAL AND METHODS:

**Plant Collection and Identification:** Aerial parts of *Thymus serpyllum* were obtained from the herbal garden of the Defence Institute of Bio-Energy Research, Pithoragarh, Uttarakhand, India. The plant sample was authenticated by Botanical Survey of India, Northern Regional Centre, Dehradun.

**Preparation of the Extract:** The aerial parts of *Thymus serpyllum* were dried, powdered and extracted with absolute ethanol (99.9%) using Soxhlet's assembly. The extract was then dried using rotatory vacuum flash evaporator<sup>5</sup>.

**Hydroxyl Radical Scavenging Activity:** The dried extract obtained was sent to Deshpande laboratories private limited, Bhopal, Madhya Pradesh, India (An ISO 9001:2008 Certified Drug Testing Laboratory) for analyzing hydroxyl radical scavenging activity and the following method was used by Deshpande laboratories according to in house Deshpande laboratories standard operating procedure (SOP): Hydroxyl radical scavenging activity was measured by the ability of the different fractions of extract to scavenge the hydroxyl radicals generated by the  $\text{Fe}^{3+}$ -ascorbate-EDTA- $\text{H}_2\text{O}_2$  system. The reaction mixture in a final volume of 1.0 ml contained 100  $\mu\text{l}$  of 2-deoxy 2-ribose (28 mM in 20 mM  $\text{KH}_2\text{PO}_4$  buffer, pH 7.4), 500  $\mu\text{l}$  of the extract at various concentrations (0.0001-50  $\mu\text{g}/\text{ml}$ ) in buffer, 200  $\mu\text{l}$  of 1.04 mM EDTA and 200  $\mu\text{M}$   $\text{FeCl}_3$  (1:1, v/v), 100  $\mu\text{l}$  of 1.0 mM hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and 100  $\mu\text{l}$  of 1.0 mM ascorbic acid. Test samples were kept at 37 °C for 1 h. The free radical damage imposed on the substrate, deoxyribose was measured using the thiobarbituric acid test. One ml of 1% thiobarbituric acid (TBA) and 1.0 ml 2.8% trichloroacetic acid (TCA) were added to the test samples and was incubated at 100 °C for 20 min. After cooling, the absorbance was measured at 532 nm against a blank containing deoxyribose and buffer<sup>6,7</sup>. Ascorbic acid was used as the standard antioxidant. The plate was read on BMG Fluostar (Germany) and the % inhibition data was analyzed on MARS software BMG (Germany).

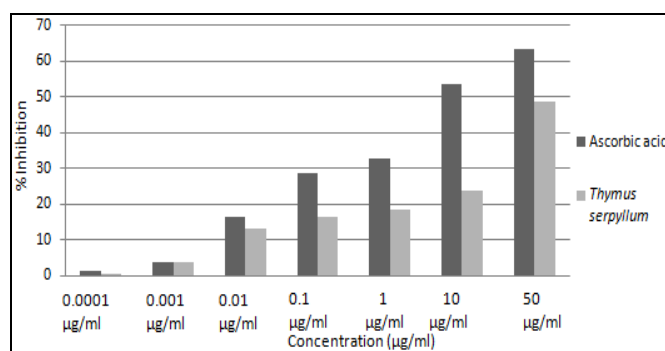
## RESULTS AND DISCUSSION:

**Results:** The table given below **Table 1** shows the percentage inhibition produced by ascorbic acid

and *Thymus serpyllum* at seven different concentrations. As the concentration goes on increasing, the percentage inhibition produced by *Thymus serpyllum* also goes on increasing. *Thymus serpyllum* has produced effect comparable to that of ascorbic acid (Standard) as shown by the table below.

**TABLE 1: PERCENTAGE INHIBITION PRODUCED BY ASCORBIC ACID AND *THYMUS SERPYLLUM* AT SEVEN DIFFERENT CONCENTRATIONS USING HYDROXYL RADICAL SCAVENGING METHOD**

Concentration ( $\mu\text{g}/\text{ml}$ )	% Inhibition (Ascorbic acid)	% Inhibition ( <i>Thymus serpyllum</i> )
0.0001	1.25	0.39
0.001	3.66	3.65
0.01	16.54	13.22
0.1	28.59	16.47
1	32.84	18.41
10	53.68	23.67
50	63.27	48.71



**FIG. 1: PERCENTAGE INHIBITION PRODUCED BY ASCORBIC ACID AND *THYMUS SERPYLLUM* AT SEVEN DIFFERENT CONCENTRATIONS USING HYDROXYL RADICAL SCAVENGING METHOD**

**DISCUSSION:** As discussed earlier, in oxidative stress there is overproduction of and/or inadequate removal of highly reactive oxygen species (ROS) and reactive nitrogen species (RNS). Reactive oxygen species generated in tissues and cells can damage DNA, proteins, carbohydrates and lipids. These potentially deleterious reactions are controlled in part by antioxidants that eliminate prooxidants and scavenge free radicals. Ethanolic extract of *Thymus serpyllum* has shown the ability to scavenge free radicals in the present study. Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage<sup>8</sup>. *Thymus serpyllum* has shown a concentration dependent effect in scavenging hydroxyl radicals. The minimum percentage inhibition shown by *Thymus Serpyllum* was 0.39 at

0.0001 µg/ml (lowest concentration) and maximum percentage inhibition shown was 48.71 at 50 µg/ml (highest concentration) **Table 1**. Thus as the concentration goes on increasing the percentage inhibition also goes on increasing, thus we can say *Thymus serpyllum* was effective in scavenging hydroxyl radical in a concentration dependent manner.

Ascorbic acid (Standard) showed better hydroxyl radical scavenging at all concentrations, still we can say that at most concentrations percentage inhibition shown by *Thymus serpyllum* was comparable to ascorbic acid **Fig. 1**. Thus we can say that *Thymus serpyllum* showed good antioxidant activity. Thus, hydroxyl radical scavenging activity of *Thymus serpyllum* might be due to one or more antioxidant phytochemicals present in it.

**CONCLUSION:** In today's modern life there are several factors which result in free radical generation. Generation of these free radicals can lead to a variety of diseases. Oxidative stress is a major problem which can cause the initiation of some fatal diseases. Variety of diseases nowadays is due to oxidative stress. Humans today consume fast foods and take a lot of stress. All these habits lead to oxidative stress that is oxidants are produced at a higher rate than they are scavenged by antioxidant defence mechanism of the body. Allopathic medicine has no answer to this problem so herbal drugs can play a significant role in reducing oxidative stress. *Thymus serpyllum* was collected from the Himalayan region of Pithoragarh, Uttarakhand, India. The unpolluted region of Pithoragarh provided a healthy growing

environment for *Thymus serpyllum* and thus ethanolic extract of aerial parts of *Thymus serpyllum* showed good antioxidant activity in the present study. Thus *Thymus serpyllum* can be developed into a herbal antioxidant formulation or a dietary herbal supplement for a variety of health disorders involving free radicals.

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**CONFLICT OF INTEREST:** There is no conflict of interest among the authors.

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