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In vitro Antioxidant and Free Radical Scavenging Activities of Methanolic Extract of *Ocimum Sanctum* Linn. Seed

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Abstract

In order to identify the potential sources of natural polyphenols and free radical scavenging activities, the edible seeds of methanolic extracts of *Ocimum sanctum* seed were studied for total phenolic and flavonoid contents, scavenging of free radicals of DPPH, Nitric oxide, super oxide, hydroxyl and hydrogen per oxide. Polyphenol and flavonoid content were observed sufficient amount in ocimum sanctum seed. The DPPH inhibitory and Nitric Oxide (NO) radical scavenging activities measured in-terms of their IC_{50} was 63.18µg/ml and 138.88µg/ml respectively. Other radicals like superoxide, hydroxyl and hydrogen peroxide scavenging activities were measured in-terms of IC_{50} value of 53.73µg/ml, 50.70µg/ml and 52.56µg/ml respectively.

Keywords: DPPH , Free Radical Scavenging, Hydroxyl and Hydrogen Peroxide, Nitric Oxide (NO) Superoxide, *Ocimum sanctum* Seed, Polyphenols

1. Introduction

Chemically Reactive Oxygen Species (ROS) produced through various physiological and biochemical metabolism are capable of damaging the tissues and reducing the function of a number of organs [1]. These radicals brings oxidative stress which lead to non-communicable diseases such as diabetes, atherosclerosis, neuro degeneration, aging and immunosuppression [2].

Antioxidants rich extract of several plants tends to reduce the stress caused due to free radicals. Accumulation of such Reactive Oxygen Species (ROS) attack the biological cells and reducing their potential. Use of medicinal plants with high level of antioxidant constituents have shown to have enormous therapeutic benefits [3].

India is an emporium of rich heritage of medicinal plants which contributes to prophylactic properties due to its antioxidant composition [4], [5].

Ocimum sanctum Linn. (*O. sanctum*), commonly known as Holy Basil or "Tulsi" belonging to the family Labiatae (Lamiaceae) has versatile therapeutic applications in Ayurveda, Siddha, Unani, Greek and Roman [6–8].

Besides adding flavour and distinctive aroma to food, it is a clinically proven cancer fighter, neuropathy healer, anti-inflammatory [9], antioxidant [10], immunomodulatory [11], antistress [12], radioprotective [13], neuroprotective [14] and anti-microbial properties [15–17]. β-carotene is a key component to prevent free radical damage [18]. Therefore, the main objective is to investigate the antioxidant potential of *O. sanctum seed* and its ability to scavenge the free radicals.

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2. Materials and Methods

2.1 Plant Material

Dried seeds of *O. sanctum* were collected locally. The plants were identified and authenticated (No:BSI/SRC/5/23/2013-14/Tech/1040) by Dr. M. Palanisamy, Scientist 'C'- Incharge, Botanical Survey of India (BSI), Tamilnadu Agricultural University, Coimbatore.

2.2 Preparation of the Plant Extract

The seeds of *O. sanctum* (Thulasi) were shade dried, powdered and extracted with solvent methanol in soxhlet apparatus and it was concentrated to dryness under pressure to obtain the dry extracts and stored at 4°C. 100 g of each powdered seeds were placed in conical flask and 100 ml of methanol was added and plugged with cotton. The powder material was extracted with methanol for 24 hours at room temperature with continuous stirring. After 24 hours the supernatant was collected by filtration and the solvent was evaporated to make the crude extract. The residues obtained were stored in airtight bottles in a refrigerator for further use.

2.3 Quantitative Phytochemical Analysis

Total phenolics and flavonoids are considered to be the most important phytochemicals that are responsible for the pharmacological activities. Total phenolic content and flavonoids were estimated [19] by using standard procedure.

2.4 In vitro Antioxidant Activity

The antioxidant activity of the extract was measured on the basis of the scavenging activity of the stable DPPH [20] free radical, nitric oxide radical scavenging method [21], super oxide radical scavenging method [22], hydroxyl radical scavenging method [23], and hydrogen peroxide radical scavenging method [24].

2.5 Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration (mg/ml) of the plant extracts required to scavenge 50% of the radicals was calculated by using the percentage scavenging activities at seven different concentrations of the extract. Percentage inhibition was calculated using the formula

$$\text{Percentage inhibition} = \frac{\text{Absorbance of the control - Absorbance of the sample}}{\text{Absorbance of the control}} \text{x100}$$

2.6 Statistical Analysis

The antioxidant assays and identified values are expressed as means of triplicate analysis of the samples (n=3) ± SD.

3. Results and Discussion

3.1 Quantification of Phytochemicals

The quantitative analysis of phytochemicals in the methanolic extracts are given in Table 1.

Table1. Quantitative analysis of phytochemicals in methanolic extracts of seed of *O. sanctum*

Phytochemicals	Concentration (µg/ml)	Absorbance
Phenolic concentration of standard Gallic acid(µg/ml)	2.5	0.021
	5	0.041
	10	0.062
	15	0.082
	20	0.103
	25	0.123
Sample *O.sanctum* seed	**13**	**0.076**
Flavonoid concentration of standard Rutin (µg/ml)	10	0.009
	20	0.018
	40	0.028
	60	0.037
	89	0.047
	100	0.057
Sample *O.sanctum* seed	**70**	**0.042**

Phenolic compounds in plant materials retard the oxidative degradation of lipids and improves the nutritional value of food [25]. The methanolic extract of *O.sanctum* seed exhibited the highest total phenolic content (13µg/ml) hence confers improved scavenging ability.

Flavonoids had wide range of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities to scavenge singlet oxygen and free radicals implicated in several diseases [26], [27]. The concentration of standard flavonoids expressed as rutin equivalents, varied from 10 µg/mL to 100 µg/mL and the absorbance was noted from 0.009 to 0.057. The amount of total flavonoid compounds present in the methanolic extracts of the *O. sanctum* seed was estimated as 70 µg/mL by visible-spectroscopy and its absorbance being 0.042. The current study is in par with the findings of the earlier literature of plant products that suggests that occurrence of phenolic acids and flavonoids are contributors of the antioxidant property.

3.2 Free Radical Scavenging Activity

3.2.1 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

DPPH is widely used as an index to estimate the antioxidant potential of medicinal plants. Dose response curve of scavenging activity of methanolic extracts of *O. sanctum* seed for radicals of DPPH was perceived and shown in Figure 1. Antioxidant activity in the form of IC_{50} values of methanolic extracts of *O. sanctum* seed was calculated and shown in Table 2.

Table 2. DPPH Radical Scavenging Activity

Concentration (µg/ml)	Inhibition (I %)	
	Standard – Ascorbic acid*	*O. sanctum* seed*
50	54±2.6	50±0.8
100	58±0.8	52±0.8
150	62±1.7	57±2.7
200	65±2.4	60±1.2
250	70±2.1	66±1.5
300	75±0.5	70±0.8
350	78±1.7	73±0.4
IC_{50} value(µg/ml)	03.56	63.18

*Mean ± S.D of three replicates

The highest percentage of scavenging activity on DPPH was seen in methanolic extracts of *O. sanctum* seed at 350µg/ml concentration. The IC_{50} value of the methanolic extracts of seed of *O. sanctum* was 63.18µg / ml (lower IC_{50} value indicates higher antioxidant activity). The test sample had higher IC_{50} value compared to the standard. It is evident that the percentage inhibition of sample was greater than the standard ascorbic acid.

DPPH radical scavenging is considered to be good in vitro model widely used to assess antioxidant efficacy of single compound as well as for different plant extracts within a very short period of time.

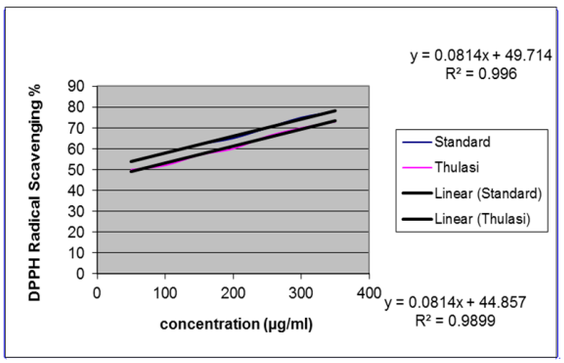

Figure 1. DPPH Radical Scavenging Activity of *Ocimum sanctum* (Thulasi) seed and Ascorbic Acid.

The percentage of DPPH scavenging effect increases with the concentration of samples as well as in standard from 50µg to 350µg/ml. As compared to 50µg concentration, 350µg concentration of *O. sanctum* seed (31.5%) and standard ascorbic acid (30.7%) increased DPPH radical scavenging activity. Among the sample and standard, the methanolic extract of *O. sanctum* seed appeared to have the highest potential for DPPH radical scavenging activity as indicated by its lowest IC_{50} value. The reducing capacity of the extract is associated with the probable presence of reductones. Further it was noted that these compounds can delay or meliorate chronic degenerative diseases [28–30].

3.2.2 Nitric Oxide Radical Scavenging Activity

Nitric oxide reveals abundant physiological properties and implicated in several pathological situations [31]. The seed of *O. sanctum* was subjected to nitric oxide radical scavenging activity and the results are shown in Table 3 and Figure 2. Inhibition of nitric oxide radical is also a measure of anti-oxidant activity. The antioxidant fractions compete with oxygen to react with nitric oxide and hence reduction in nitrite ions [32]. The results showed that methanolic extracts of *O. sanctum* seed exhibited the nitric oxide scavenging of 83±0.5 per cent and standard ascorbic acid exhibited 92±0.5 per cent scavenging effect with the concentration of 350µg/ml. The IC_{50} value of the methanolic extracts of *O. sanctum* seed and standard ascorbic acid in this assay was 94.38µg and 138.88µg/ml respectively.

Table 3. Nitric Oxide Scavenging Activity

Concentration (µg/ml)	Inhibition (I %)	
	Standard – Ascorbic acid*	*O. sanctum* seed*
50	41±0.8	36±0.5
100	52±0.5	45±0.5
150	61±0.9	53±0.5
200	68±0.5	58±0.5
250	74±0.8	64±0.8
300	85±0.8	77±0.9
350	92±0.5	83±0.5
IC_{50} value(µg/ml)	94.38	138.88

*Mean ± S.D of three replicates

The extract of *O. sanctum* seed effectively reduced the generation of nitric oxide from decomposition of sodium nitro prusside *invitro* and IC_{50} value showed nitric oxide scavenging activity at the concentration of 138.88µg/ml

for sample, while the standard vitamin C showed 94.38µg/ml (Table 3). The methanol extract recorded maximum percentage of NO activity of 56% at the concentration 350 µg/ml.

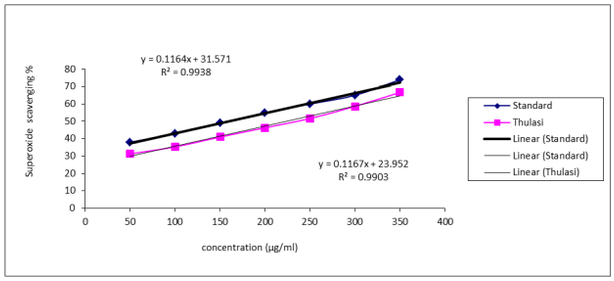

Figure 2. Nitric Oxide Scavenging Activity of *Ocimum sanctum* (Thulasi) seed and Ascorbic Acid.

3.2.3 Super Oxide Radical Scavenging Activity

Superoxide radical is a key biological cause of reactive oxygen species [33]. The seed of *O. sanctum* was subjected to super oxide radical scavenging assay and the results are shown in Table 4 and Figure 3.

Absorbance of the solution increased with increased concentration of the sample. The results showed that inhibition percentage of methanolic extract of *O. sanctum* seed exhibited 31± 0.9 to 67±0.5 percent and standard ascorbic acid exhibited 38±0.9 to 74±0.8 with a concentration of 50µg/ml to 350µg/ml.

Table 4. Super oxide Scavenging Activity

Concentration (µg/ml)	Inhibition (I %)	
	Standard*	*O.sanctum* seed*
50	38±0.9	31±0.9
100	43±0.5	35±0.9
150	49±0.5	41±1.4
200	55±0.5	46±0.5
250	60±0.5	52±0.5
300	65±0.5	59±0.5
350	74±0.8	67±0.5
IC_{50} value(µg/*ml*)	158.32	223.20

*Mean ± S.D of three replicates

The superoxide radical scavenging effect of seed of *O. sanctum* was compared with the same doses of ascorbic acid ranging from 50 – 350µg/ml.

The superoxide radical (SO) scavenging effect obtained for the *O. sanctum* seed extract results showed dose dependent free radical scavenging activity and the percentage inhibition is shown in (Figure 3). The scavenging activity of methanolic extract of *O. Sanctum* seed (IC_{50} 223 µg/ml) was compared with the vitamin C (158 µg/ml) as standard. The extract of *O. Sanctum* seed in a concentration of 100 µg/ml showed 35% inhibition while, in 350 µg/ml concentration the percentage of inhibition was noted as 67%. This evidently shows that the inhibition activity was dose dependent.

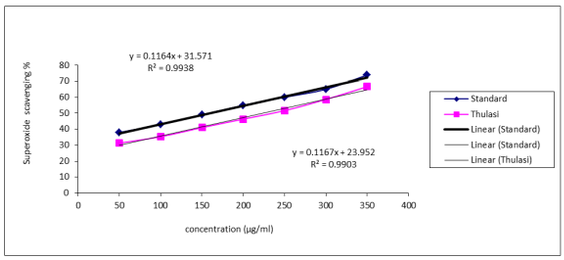

Figure 3. Super oxide Scavenging Activity of *Ocimum sanctum* (Thulasi) seed and Ascorbic Acid.

3.2.4 Hydroxyl Radical Scavenging Activity

Hydroxyl radicals causes peroxidation and enormous biological damage. Hydroxyl radical scavenging activity of methanolic extract of *O. sanctum* seed is shown in Table 5 and Figure 4. The methanolic extract of *O. sanctum* seed (350µg/ml) exhibited 71±0.5% hydroxyl radical scavenging activity and standard ascorbic acid exhibited 76±2.1% hydroxyl radical scavenging activity. The value of IC_{50} of the extract of seed of *O. sanctum* and standard ascorbic acid in this assay was 178.88µg/ml and 92.71µg/ml respectively.

Hydroxyl radical scavenging activity was quantified by measuring the inhibition of the degradation of 2-deoxyribose by the free radicals generated by the Fenton reaction [34]. In the current research, the IC_{50} value of hydroxyl radical scavenging activity for the *O. sanctum* seed extract was 178.88µg/ml and for ascorbic acid it was 92.0±71 µg/ml . The strong antioxidant response of *O. sanctum* seed in comparison with ascorbic acid might be helpful in characterizing the significant sources of natural antioxidant reaction.

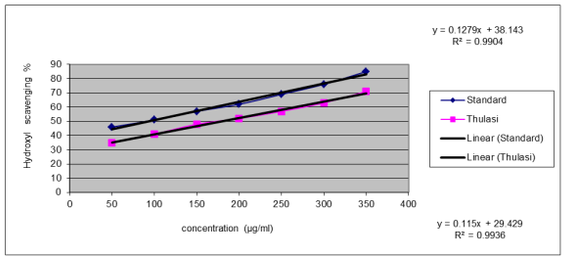

Figure 4. Hydroxyl Radical Scavenging Activity of *Ocimum sanctum* (Thulasi) seed and Ascorbic Acid

Table 5. Hydroxyl Radical Scavenging Activity

Concentration (µg/ml)	Inhibition (I %)	
	Standard*	Thulasi*
50	46±0.5	35±2.1
100	51±0.8	41±2.2
150	57±0.9	48±1.4
200	62±0.5	52±2.5
250	69±1.2	57±1.2
300	76±2.1	63±2.1
350	85±0.9	71±0.5
IC_{50} value(µg/ml)	92.71	178.88

*Mean ± S.D of three replicates

3.2.5 Hydrogen Peroxide Radical Scavenging Activity

Methanolic extract of *O. sanctum* seed were screened for their possible antioxidant activity by H_2O_2 scavenging assay and the results were shown in Table 5 and Figure 4.

In H_2O_2 assay, percentage inhibition in *O. sanctum* seed extract was in range of 37±0.8% to 78±0.5% while in standard ascorbic acid it was 42±0.8% to 85±0.8%. The IC_{50} value of *O. sanctum* seed extract was 139.34µg/ml and while it was 94.97 µg/ml in case of standard ascorbic acid. The higher antioxidant activity is reflected with lower IC_{50} value.

Table 6. Hydrogen Peroxide Scavenging Activity

Concentration (µg/ml)	Inhibition (I%)	
	Standard*	Thulasi*
50	42±0.8	37±0.8
100	51±0.5	44±1.8
150	59±0.0	53±1.0
200	66±0.3	66±0.6
250	72±0.4	64±0.4
300	80±0.4	71±0.7
350	85±0.5	78±0.9
IC_{50} value(µg/ml)	94.97	139.34

*Mean ± S.D of three replicates

The hydrogen peroxide radical scavenging activity of methanol extract of *O. sanctum* seed was compared with standard ascorbic acid with different concentration ranging from 50-350µg/ml. Inhibition percentage of methanol extract of *O. sanctum* seed exhibited 37±0.8 to 78±0.9 per cent and the standard ascorbic acid percentage of inhibition was 42±0.8 to 85±0.5 , it is represented in Figure 5. The IC_{50} value of *O. sanctum* seed extract was found to be 139.34µg/ml.

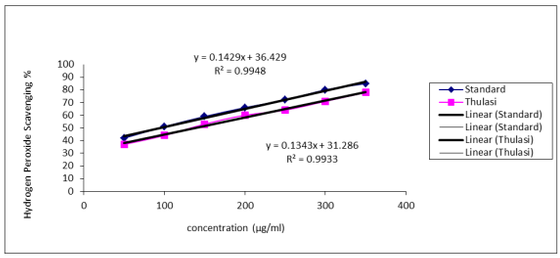

Figure 5. Hydrogen Peroxide Scavenging Activity of *Ocimum sanctum* (Thulasi) seed and Ascorbic Acid.

Hydrogen peroxide is a weak oxidizing agent that can cross biological membranes can initiate cytotoxicity than chemical reactivity. Hence removing H_2O_2 is significant for the protecting living systems [35].

In the present study, hydroxyl radical scavenging activity of methanolic extracts of seed of O.*sanctum* and standard ascorbic acid showed a dose dependent hydroxyl radical scavenging activity. Out of the seven different concentrations tested for hydroxyl radical scavenging activity, higher concentrations (250 to 350 µg/ml) have demonstrated good hydroxyl radical scavenging activity. The concentrations 150 and 200µg/ml showed slightly better inhibition than 50 and 100µg concentration. *O.sanctum seed* has recorded 52.56% and standard ascorbic acid shows 50.58% increased hydroxyl radical scavenging activity at 350 µg/ml concentration along with increased inhibition capacity. IC_{50} values of the extracts were more than the standard ascorbic acid.

4. Conclusion

From the study it is evident that the methanolic extracts of seeds of *O. sanctum* have the considerable amounts of polyphenols and flavonoids. The added health benefits are due to the presence of antioxidants that helps to prevent several degenerative diseases.

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