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Evaluation of *In-Vitro* Antioxidant and Anti-inflammatory Activities of extract of Ocimum Sanctum linn

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ABSTRACT

The objective of the study was to investigate the anti-oxidant and anti-inflammatory effect of aqueous extract of Ocimum sanctum leaf. The antioxidant and anti-inflammatory activities were evaluated using *in vitro* Ferric reducing power, Free radical scavenging activity by DPPH method, hydroxyl radical scavenging activity, Nitric oxide radical scavenging activity, Hyaluronidase inhibition assay. The effect was compared with a known antioxidant agent (BHA/Ascorbic acid). It was found that the extract at different concentration exhibited significant dose dependent antioxidant and anti-inflammatory effect

Key words: Ocimum sanctum, Free radical, DPPH, Hyaluronidase inhibition

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INTRODUCTION

Plants are one of the most important sources of medicines. Today the large number of drugs in use are derived from plants, like morphine from Papaver somniferum, Ashwagandha from *Withania somniferum*, Ephedrine from *Ephedra vulgaris* , Atropine from *Atropa belladona* and Reserpine from *Rauwolfia serpentina* etc . The medicinal plants are reach in secondary metabolites (which are potential source of drugs). and essential oils of therapeutic importance [1]. In traditional systems of medicine the Indian medicinal plants have been use in successful management of various disease conditions like bronchial asthma , chronic fever , old cough , malaria , dysentery , convulsion , diabetics , diarrhea , arthritis Emetic syndrome and skin diseases ,insect bite etc. In Ayurveda Tulsi (Ocimum sanctum L.) has been well documented for its therapeutic potential for various diseases [2]

Reactive oxygen species (ROS) contribute various diseases in humans as well as in animal beings. Reactive oxygen species are formed intracellularly and are controlled by antioxidant defense. The generation of large amount of reactive oxygen species can overwhelm the intra cellular anti oxidant defense , causing activation of lipid peroxidation[3] , protein modification , DNA breaks .Reactive oxygen specie induced depletion of anti-oxidants is a key factor for the initiation of various diseases such as diabetes , atherosclerosis & the development of cardiovascular disease (CVD)[4] .

Inflammation is local response of living mammalian tissues to the injury. It is a body defence reaction in order to eliminate or limit the spread of injurious agents. There are various components to an inflammatory reaction that can contribute to the associated symptoms and tissue injury. Oedema formation, leukocyte infiltration and granuloma formation represent such component of inflammation[5]

MATERIALS AND METHODS

The powdered extract (aq.) of Ocimum sanctum (L.) was obtained as a gift sample from Amsar pvt. Ltd. M. P. All other chemicals used in this study were of Pharmaceutical grade.

Methods:

Antioxidant activity[6]:

The antioxidant activity of the extract was evaluated by using different *in vitro* methods such as, Ferric Reducing Antioxidant Power, Free radical scavenging activity by DPPH method, Hydroxyl Radical Scavenging Activity, Nitric oxide radical Scavenging Activity**.**

Ferric reducing antioxidant Power [6, 7]:

Various concentrations of extract in Dimethyl sulfoxide (10µg, 50µg, and test) were mixed with 2.5 ml of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50ºC for 20 minute. Next, 2.5 ml of 10% (w/v) trichloroacetic acid was added. 5 ml of above solution was mixed with 5 ml of distilled water and 1 ml of 0.1% of ferric chloride. The absorbance was measured spectrophotometrically at 700 nm. Butylated hydroxy anisole (BHA) was used as standard antioxidant.

Free radical scavenging activity by DPPH method [7]:

Different concentrations (10µg, 50µg, 100µg and 250µg) of extracts in Dimethyl sulfoxide and Butylated hydroxy anisole (BHA) were taken in different test tubes. The volume was adjusted to 500µl by adding Methanol. Five milliliters of a 0.1 mM methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH) was added to these tubes and shaken vigorously. A control without the test compound, but with an equivalent amount of methanol was maintained. The tubes were allowed to stand at RT for 20 min. The absorbance of the samples was measured at 517 nm. Radical scavenging activity was calculated using the following formula:

$$\% \text{ radical scavenging activity} = \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}} \times 100.$$

Hydroxyl Radical Scavenging Activity[8].

Various concentrations (10µg, 50µg, and 100µg) of extracts in water were taken in different test tubes and made up to 250µl with 0.1M phosphate buffer. One milliliter of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA (0.018%), and 1 ml of Dimethyl sulphoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml of 0.22% ascorbic acid. These reaction mixtures were incubated at room temperature for 15 min. The reaction was terminated by the addition of 1 ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (150 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against reagent blank. Ascorbic acid was used as reference standard. The percentage hydroxyl radical scavenging activity was calculated by the following formula:

$$\% \text{ hydroxyl radical scavenging activity} = 1 - \frac{(\text{difference in absorbance of sample})}{(\text{difference in absorbance of blank})} \times 100$$

d) Nitric oxide radical Scavenging Activity: [9]

Various concentrations (10µg, 50µg, and 100µg) of extracts in water and Butylated hydroxy anisole (BHA) were taken in different test tubes and made up to 3ml with 0.1M phosphate buffer (pH 7.2). Sodium Nitroprusside (5mM) prepared in buffered saline (pH7.2) was added (1 ml) to each tube. The reaction mixture was incubated for 30 min at RT. A control without the test compound, but with an equivalent amount of methanol was maintained. After 30 min, 1.5 ml of above solution was mixed with 1.5 ml of Griess reagent (1% Sulphanilamide, 2% phosphoric acid and 0.1% N-1- Naphthyl ethylene diamine dihydro chloride).The absorbance of the samples was measured at 546 nm. Nitric oxide radical scavenging activity was calculated using the following formula:

$$\% \text{ NO radical scavenging activity} = \frac{(\text{control OD} - \text{sample OD})}{\text{control OD}} \times 100.$$

e) Hyaluronidase Inhibition Activity[10]:

The assay was performed according to Ling et al (2003) and Sigma Protocol. The assay medium consisting of 3-5U hyaluronidase (from Sigma –Aldrich, Bangalore) in 100µl 20mM sodium phosphate buffer pH 7.0 with 77mM sodium chloride, 0.01% BSA was pre incubated with different concentrations of the test compound (in Dimethyl sulfoxide; DMSO) for 15 min at 37 °C. The assay was commenced by adding 100µl hyaluronic acid (from Sigma –Aldrich, Bangalore; 0.03% in 300mM sodium phosphate, pH 5.35) to the incubation mixture and incubated for a further 45 min at 37 °C. The undigested hyaluronic acid was precipitated with 1ml acid albumin solution made up of 0.1% bovine serum albumin in 24mM sodium acetate and 79mM acetic acid, (pH 3.75). After standing at room temperature for 10 min, the absorbance of the reaction mixture was measured at 600 nm. The absorbance in the absence of enzyme was used as the reference value for maximum inhibition. The inhibitory activity of test compound was calculated as the percentage ratio of the absorbance in the presence of test compound *vs.* absorbance in the absence of enzyme. The enzyme activity was checked by control experiment run simultaneously, in which the enzyme was pre incubated with 5µl DMSO instead, and followed by the assay procedures described above. Compounds were tested in a range of 10µg - 100µg in the reaction mixture. Indomethacin was used as reference standard.

RESULTS AND DISCUSSION:

The extract of Ocimum sanctum (L.) was evaluated for antioxidant activity by using *in vitro* ferric reducing power method. In this method various concentrations of extract in Dimethyl sulfoxide were used as a test drug where as Butylated hydroxyl anisole (BHA) was used as

standard antioxidant. The test compound at the conc. of 250 µg exhibited significant effect (Table: 1, Figure: 1). the anti oxidant activity of the extract was evaluated by using free radical scavenging activity. The extract exhibited a dose dependent free radical scavenging activity, the result is depicted in table: 2 and figure: 2. the result of hydroxyl radical scavenging activity revealed that the extract at the different concentrations produced dose dependent effect (Table: 3, figure: 3) .The potency of the extract was also evaluated for its Nitric Oxide radical scavenging activity. The extract at various concentrations showed dose dependent effect as depicted in table: 4 and figure: 4. The Hyaluronidase inhibition activity was carried out to evaluate the anti inflammatory potential of the extract. The potency of the extract was compared with the standard Indomethacin. The extract at different concentrations i.e. 10, 50,100µg showed dose dependent effect where as the standard indomethacin at 50 µg and 100 µg exhibited much higher activity Table:5 , figure :5 .

Table 1: Ferric Reducing Antioxidant Power

(µg of sample)	Sample	BHA
10	0.075	0.116
50	0.09	0.333
100	0.151	0.558
250	0.249	0.91

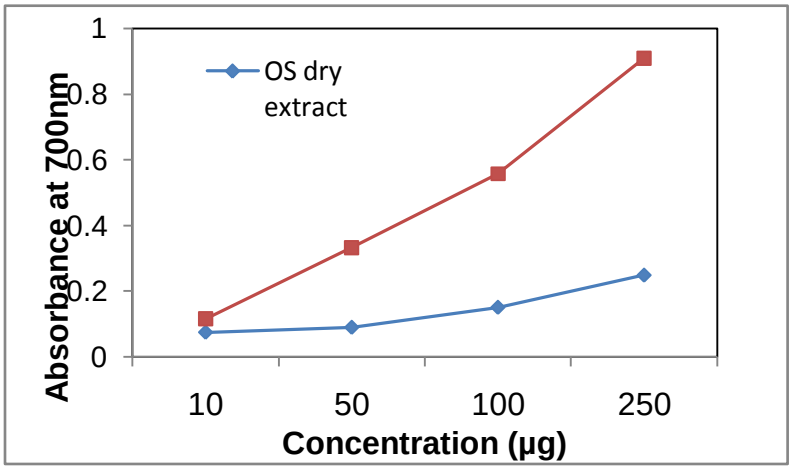

Figure 1: Ferric Reducing Antioxidant Power

Table 2: Percentage free radical scavenging activity

µg of sample	OS dry extract	BHA
10	15.54	22.89
50	19.39	69.10
100	41.25	83.82
250	65.01	*

***** Beyond measurable range: Much higher activity

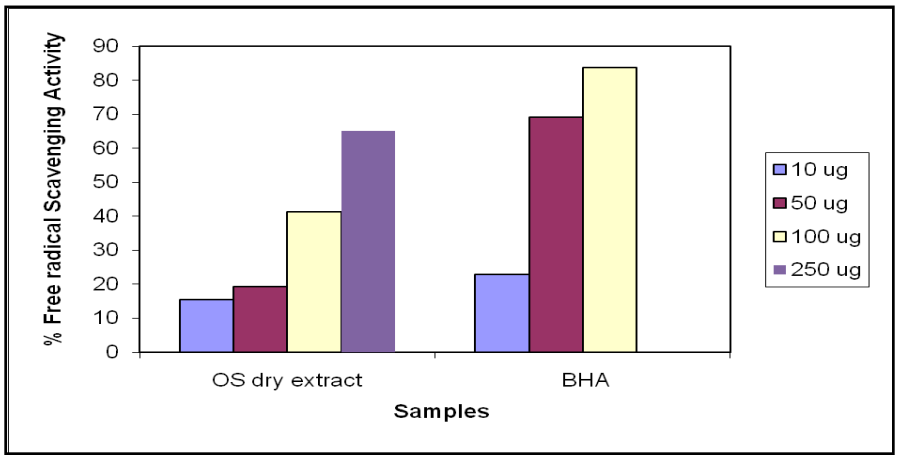

Figure 2: Percentage free radical scavenging activity

Table 3: Percentage hydroxyl radical scavenging activity

µg of sample	OS dry extract	Ascorbic Acid
10	3.23	18.38
50	10.51	24.65
100	21.41	35.35

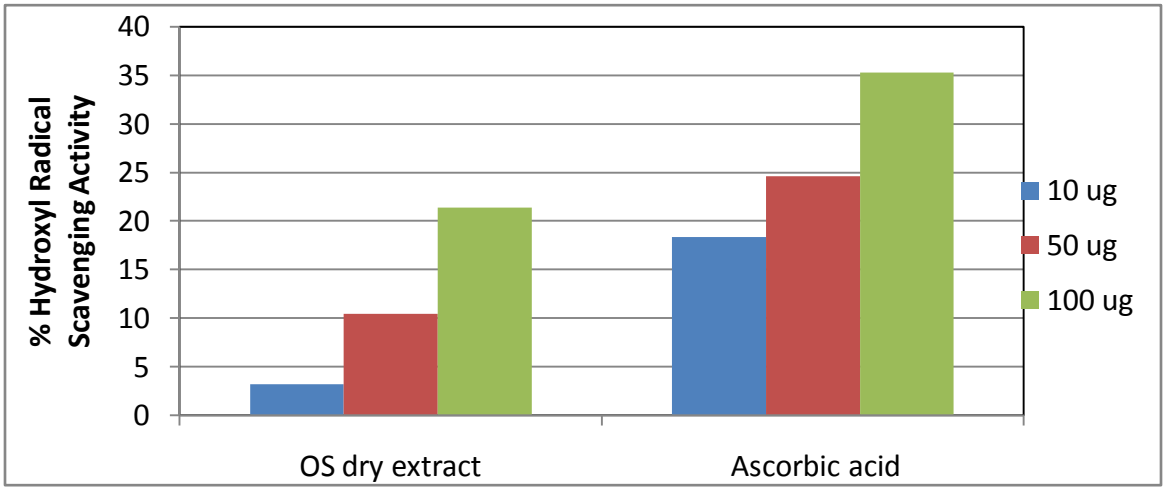

Figure 3: Percentage hydroxyl radical scavenging activity

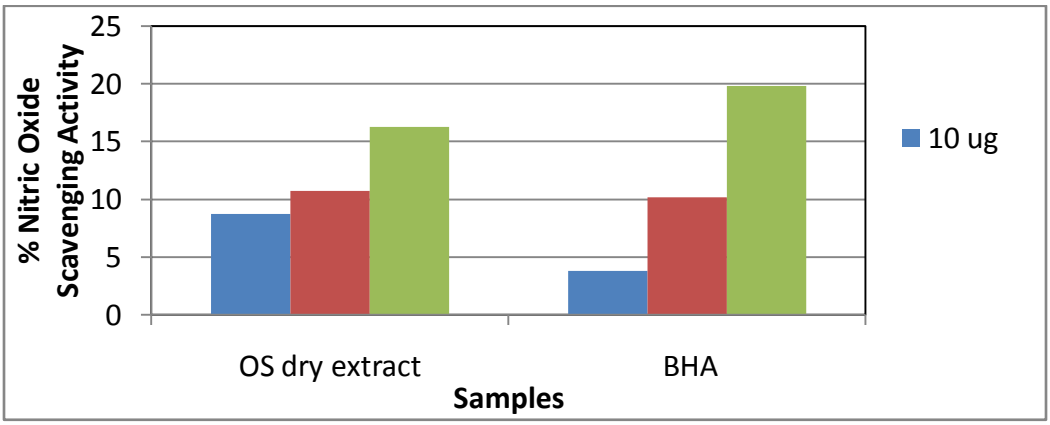

Figure 4: Percentage Nitric oxide scavenging activity

Table 4: Percentage Nitric oxide scavenging activity

µg of sample	sample	BHA
10	8.72	3.8
50	10.73	10.2
100	16.24	19.8

Table 5: Percentage inhibition of Hyaluronidase enzyme by the samples

Sample	Test con. (in µg)	O.D. at 600nm	% inhibition
		0.269	100
OS dry extract	10	0.023	8.55
	50	0.144	53.53
	100	0.162	60.22
Indomethacin	10	0.208	77.32
	50	0.556	*
	100	0.585	*

***** Beyond measurable range: Much higher activity

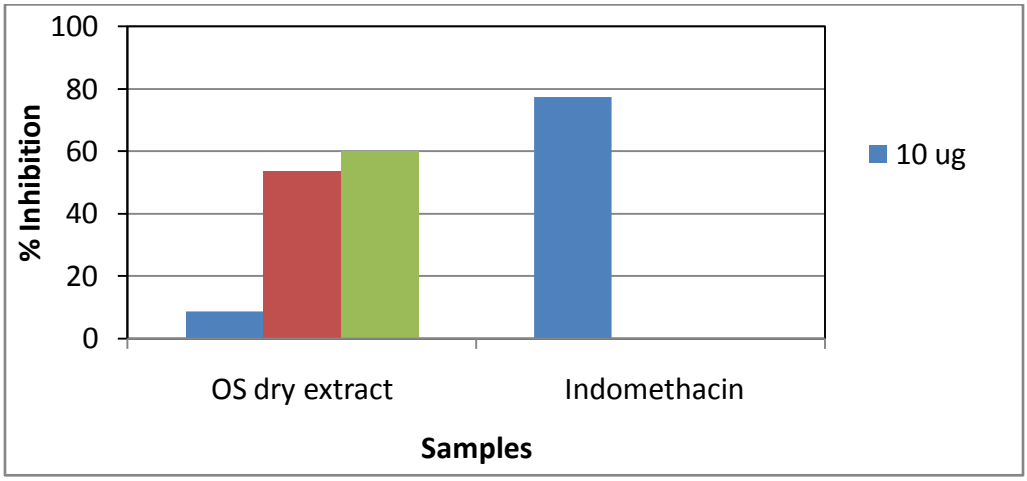

Figure 5: Percentage inhibition of Hyaluronidase enzyme by the samples

Free radical reactions have been implicated in the pathology of many human diseases like atherosclerosis, ischemic heart disease, diabetes and neurodegenerative disease etc, and disease conditions like aging process, inflammation, immune suppression etc. A number of plant and plant isolates have been reported to protect free radical induced damaged in various experimental models [11]. Antioxidants may offer resistance against the oxidative stress by scavenging free radicals, inhibiting lipid peroxidation and thus prevent disease. In the present study our efforts have been devoted to evaluate the antioxidant activity of *Ocimum sanctum* (L) by using various parameters.

The total antioxidant capacities of the extract of *Ocimum sanctum* (L.) was determined by FRAP method, a simple, speedy, inexpensive, and reproducible method, which can be applied to the assay of antioxidants in plasma or botanicals. In this method various concentrations of extract in Dimethyl sulfoxide were used as a test drug where as Butylated hydroxyl anisole (BHA) was

used as standard antioxidant. The antioxidant activity of *O sanctum* extract, at all the concentrations exhibited significant effect. DPPH is usually used as a reagent to evaluate free radical scavenging activity of antioxidant. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The extract and fractions are able to reduce the stable free radical to the yellow coloured diphenyl picryl hydrazine. The potential decrease in the concentration of DPPH radical due to the scavenging ability of *Ocimum sanctum* (L) at different concentration 10, 50,100, 250 µg was found to be 15.54%, 19.39%, 41.25% and 65.01% respectively. The extract of *O. sanctum* was examined for its ability to act as OH^- radicals scavenging agent. The extract at the different concentrations i.e. 10, 50 &100 µg produced dose dependent hydroxyl radical scavenging activity i.e. 3.23 %, 10.51%, 21.41 % respectively. Nitric Oxide (NO) is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases. Nitric oxide is a very unstable species under aerobic condition .it is react with O_2 to produce stable product nitrate and nitrite to intermediates NO_2, N_2O_4, and N_3O_4. It is estimated by using Griess reagent. In presence of test compound which is a scavenger the amount of nitrous acid will decrease. The potency of the extract was evaluated for its Nitric Oxide radical scavenging activity. The extract at various concentrations showed dose dependent effect as depicted in table: 4 and figure: 4. The Hyaluronidase inhibition activity was carried out to evaluate the anti inflammatory potential of the extract. The potency of the extract was compared with the standard Indomethacin. The extract at different concentrations i.e. 10, 50,100µg showed dose dependent effect i.e. 8.55, 53.53, 60.22% inhibition where as the standard Indomethacin at10µg concentration exhibited 77.32% inhibition but at 50 µg and 100 µg exhibited much higher activity i.e. beyond the measurable range.

CONCLUSION:

There are many herbal plants in the world but the *Ocimum sanctum* (Tulsi) is considered to be the queen of herbs due to its greater medicinal values. It is well documented in the Hindu mythology about the Tulsi. Considering the health beneficial effects of Tulsi our ancestors in India insisted to plant a Tulsi sapling in everyone's house. Keeping the various medical benefits in view, our efforts has been devoted to evaluate the *in vitro* pharmacological activity of Ocimum *sanctum* (L). Probably, such natural components might prove to be potentially beneficial but comparatively less toxic. Eventually, plants belonging to *Ocimum* genus could contribute a lot towards economy and healthy problem. From the above study it has been

concluded that, the aqueous extract of Ocimum sanctum have a good dose dependent *in vitro* anti oxidant and anti inflammatory activity. Although a variety of work has been done to explore the various potential of the different parts of this plant on experimental animals but very few work has reported for its in vitro activity. Thus in this present study the aqueous extract of Ocimum sanctum provided the effectiveness of the extract in a dose dependent manner for inhibition of different enzymes responsible for oxidation and inflammation. The important and significant preliminary finding can be taken as the basis upon which further in vivo studies can be carried out to delineate the detailed profile of the anti oxidant and anti-inflammatory property of *Ocimum sanctum* .

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