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QUANTIFICATION OF TOTAL PHENOLICS AND THEIR ANTIOXIDANT ACTIVITY OF OCIMUM SPECIES: *OCIMUM BASILICUM* AND *OCIMUM SANCTUM*

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ABSTRACT: The genus Ocimum, a member of the Lamiaceae family. Ocimum species are mostly composed of phenolics, tannins, saponins, flavonoids, volatile oils, etc. We have investigated into the antioxidant activity and total phenolics of the two species of *Ocimum basilicum* and *Ocimum sanctum* since they all have therapeutic characteristics. The results were compared to those of other common antioxidants like ascorbic acid and gallic acid. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical, superoxide radical, hydroxyl radical, and lipid peroxidation can all be scavenged. These two plants' leaves were extracted using an ethanol solvent. By Soxhlation, the ethanolic extract was obtained. Several concentrations (ranging from 50g to 500g) were employed in this investigation. The maximum percentage activity of DPPH (76.67%), Superoxide radical (60.72%), Hydroxyl radical (57.97%), and Lipid peroxidation (66.61%) were found at the 500 g concentration. *Ocimum basilicum* demonstrated greater percent inhibitory activity than *Ocimum sanctum* among these radicals evaluated on the two species. *Ocimum basilicum* was recommended, however, both Ocimum species had the highest IC_{50} values.

INTRODUCTION: Medicinal plants are considered very rich sources of secondary metabolites and oils, which are of therapeutic importance. The use of medicinal plants in traditional medicine is widespread and still serves as lead for developing novel pharmaceutical agents. Many such medicinal plants have hepatoprotective, neuroprotective, anti-inflammatory, and antioxidant or radical scavenging properties [1]. The Ocimum species comprises 50 genera, and 150 above species belongs to the family Lamiaceae.

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Ocimum basilicum L., a sweet basil or basil, is a popular culinary herb that originated in India, Africa, and Southern Asia and is now cultivated worldwide [2]. Basil is used as a kitchen herb and an ornamental plant in traditional medicine [3, 4]. The leafy parts of basil had tonic, antiseptic, and insecticidal properties [5, 6]. It is also known that basil leaves are suitable for treating pain and cough [7]. Traditionally, basil has been extensively utilized in food as a flavoring agent and in perfumery and medicinal industries [8].

The leaves and flowering tops of the plants are perceived as carminative, galactogogue, stomachic, and antispasmodic in folk medicine [9] and also has been used as an antiseptic, preservative, sedative, digestive regulator, and diuretic and recommended for the treatment of headache, infections of the

upper respiratory tract, kidney malfunction to eliminate toxins [10]. However, the potential uses of *O. basilicum* essential oil, particularly as antimicrobial and antioxidant activity agents, have recently been investigated [11, 12, 13].

***Ocimum sanctum* L:** Commonly known as 'Tulasi'. Tulasi grows wild in the tropics and warm regions, distributed and cultivated throughout India. Its aromatic leaves are simple, opposite, elliptic, oblong, obtuse, and growing up to 5cms long [14, 15]. The leaves of *Ocimum sanctum* have 0.7% volatile oil comprising about 71% eugenol and 20% methyl eugenol. Fresh leaves and stems of *Ocimum sanctum* extract yielded some phenolic compounds (antioxidants) such as rosameric acid, apigenin, isothymusin, and eugenol [16]. Tulasi is a popular home remedy for many ailments such as wound, bronchitis, liver diseases, catarrhal fever, otalgia, lumbago, hiccough, ophthalmia, gastric disorders, genitourinary disorders, skin diseases, various forms of poisoning and psychosomatic stress disorders. It has also aromatic, stomachic, carminative, demulcent, diaphoretic, diuretic, expectorant, alexiteric, vermifuge and febrifuge properties. In Ayurveda, the *O. sanctum* leaves, flowers and occasionally the whole plant is used medicinally in the treatment of heart and blood diseases, leucoderma, strangury, asthma, bronchitis, lumbago and purulent discharge of the ear [17].

The commercial development of plants as sources of Antioxidants to enhanced health and food preservation is of current interest. Antioxidants can protect tissues from damage by stabilizing harmful free radicals. Free radicals containing oxygen, known as Reactive oxygen species (ROS) are most biologically significant free radicals. ROS can perform beneficial or deleterious function in the cells. Interaction of ROS with cell membrane and various cell components have deleterious effects on multicellular functions and viability [18]. Detrimental effects caused by ROS also occur due to imbalance between the formation and inactivation of these species. ROS-mediated oxidative damage to macromolecules namely lipids, proteins and DNA have been implicated in the pathogenicity of major diseases [19]. Free radical mediated oxidative stress is believed to be the primary cause of many age disorders such as cardiovascular diseases, brain dysfunction and cataracts [20]. In living organisms, various ROS can form in different ways. Normal aerobic respiration stimulates polymorphonuclear leukocytes and macrophages, and peroxisomes appear to be the main endogenous sources of most of the oxidants produced by cells. Exogenous sources of ROS include tobacco smoke, certain pollutants, organic solvents, and pesticides. ROS induces oxidative damage to biomolecules such as nucleic acids, lipids, proteins, and other macromolecules and has been regarded as one of the major endogenous causes leading to age [21, 22]. Fortunately, the body is not defenseless from these metabolic and environmental oxidation attacks. Antioxidant consumption is a defense mechanism that keeps oxidative stress manageable in the cellular accumulation of lipid peroxides and depletion of endogenerous antioxidants. Therefore, studies on antioxidants from medicinal plants help understand the potential therapeutic role in the antioxidant's disease and degradation of body cells, which are mainly responsible for the oxidative stress noticed by the increased production of superoxide radicals, hydrogen peroxide, and hydroxyl radicals.

MATERIALS & METHODS:

Plant Material: Ocimum seeds were collected from CSIR-CIMAP, Lucknow U. P. (India) i.e., *Ocimum sanctum* (OC 19) and *Ocimum basilicum* (OC 20) and then the plants were potted in the garden of Botany Department, Andhra University, Visakhapatnam.

Ethanolic Extract: The shade-dried leaf material was powdered. Then it was weighted of 200gm of powder has taken and transferred into a round bottom flask of the Soxhlet extractor. Then 2000 mL of ethanol was added to the plant material and soaked for 24 hrs at room temperature. The ethanolic extract of plant material was extracted by using a Soxhlet extractor. The extract was filtered through Whatman filter paper No 1. and the filtrate was collected; then the ethanol was removed by an evaporator at 50ºC and stored at 4ºC until use.

Determination of Total Phenolic Content: Total phenolic content was estimated as Gallic acid equivalents (GAE) [23]. Briefly, 100µl aliquot of the dissolved extract was transferred to a 10 mL volumetric flask containing 6 mL distilled water,

and subsequently added 500µl undiluted Folin-Ciocalteu reagent. After 1min, 1.5 mL Na_2CO_3 (20% w/v) was added, and the volume was made up to 10 mL with distilled water. After 30min incubation at 25ºC, the absorbance was measured at 760nm and compared to a Gallia acid calibration curve. Values are presented as the mean ± SE of each three replicates.

Dpph (1, 1-Diphenyl – 2 - Picrylhydrazyl) Scavenging Assay: In DPPH assay method is based on the reduction of alcoholic DPPH solution (Dark blue color) in the presence of hydrogen donating antioxidant converted to the non-radical form of yellow-colored diphenyl-picrylhydrazine [24]. 4mg of DPPH was dissolved in 100mL of ethanol and kept overnight in a dark place to generate DPPH radicals. An aliquot of 3mL of 0.004% DPPH solution in ethanol and 0.1mL of plant extract at various concentrations were mixed. The mixer was shaken vigorously and allowed to reach steady at room temperature for 30min. decolorization of DPPH was determined by measuring the absorbance at 517nm. A control was prepared using 0.1mL of the respective vehicle in the place of plant extract/Ascorbic acid.

The capability of scavenging the DPPH radical was calculated using the following equation: percentage of inhibition by extracts of superoxide production was calculated using the formula;

% inhibition = Control OD – test OD / Control OD× 100

Determination of Superoxide Scavenging Activity by Riboflavin Photoreduction Method: Superoxide scavenging activity of the extracts was determined by the method of [25]. Which depends on light induced superoxide generation by riboflavin and the corresponding reduction of Nitroblue tetrazolium (NBT). The assay mixture contained the different concentrations of the extracts and EDTA (6µM containing 3µM NaCN), NBT (50µM), riboflavin (2µM) and phosphate buffer (58mM, pH 7.8) to give a total volume of 3mL the tubes were uniformly illuminated for 15 minutes and after that, the optical density (OD) was measured at 560nm. The percentage inhibition by the extracts of superoxide production was evaluated by comparing the absorbance values of control and experimental tubes.

The percentage of inhibition by the extracts of superoxide production was calculated using the formula;

% inhibition= Control OD – test OD / Control OD× 100

The OD obtained with each concentration of the extracts and ascorbic acids was plotted on a graph taking concentration on the X-axis and percentage inhibition on Y-axis; the graph was extrapolated to find the concentration needed for 50% inhibition.

Determination of Lipid Peroxidation Inhibiting Activity: The thiobarbituric acid method 26 determined the inhibition of lipid peroxidation. The reaction mixture (0.5 mL) containing rat liver homogenate (0.1mL, 25% w/v) in tris-HCl buffer (40mM, pH -7.0). KCl (30mM), Ascorbic acid (0.06mM) and ferrous ion (0.16mM) and various concentrations of the extracts were incubated for 1 hour at 37ºC. The reaction mixture (0.4mL) was treated with sodium dodecyl sulphate (SDS-0.2mL 8.1%), thiobarbiuric acid (TBA-1.5mL, 0.8%) and acitic acid (1.5mL, 20 pH-3.5). The total volume was then made up to 4mL by adding distilled water and kept in an oil bath at 100ºC for one hour. After the mixture had been cooled, 1mL of distilled water and 5mL of butanol-pyridine mixture (15: 1v/v) were added. Following vigorous shaking, the tubes were centrifuged, and the absorbance of the organic layer containing the chromophore was read at 532nm. The percentage inhibition of lipid peroxidation by the extract was determined by comparing the absorbance values of the control and the experimental tubes as calculated for superoxide radical assay.

Percentage of inhibition by the extracts of lipidi peroxidation production was calculated using the formula;

% inhibition = Control OD-Test OD / Control OD× 100

Determination of Hydroxl Scavenging by Deoxyribose Degradation Method: Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and the extracts for hydroxyl radicals generated from the Fe^{2+} / EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radicals attack deoxyribose, eventually resulting in thiobarbituric acid reacting substances (TBARS) formation [27].

The reaction mixture contains deoxyribose (2.8 mM), ferrous sulphate (10 mM), EDTA (10 mM), H_2O_2 (1.0 mM), phosphate buffer (0.1 M, pH 7.4) and various dilutions of the extracts. The reaction was incubated for 4 hours at 37ºC. Deoxyribos degradation was measured as TBA reactive substances by the method of Ohkawa *et al.,* [26] and the percentage of inhibition was calculated from the control where no test compound was added. The percentage inhibition of hydroxyl radicals by the extracts was determined by comparing the absorbance values using this formula;

% inhibition = Control OD-Test OD / Control OD × 100

RESULTS & DISCUSSION: Free radicals are defined as molecules having an unpaired electron in the outer orbit and they are produced in the body, primarily as a result of aerobic metabolism. They are generally unstable and very reactive. Examples of oxygen derived free radicals are superoxide, hydroxyl, peroxyl, alkoxyl and hydroperoxyl radicals and other reactive oxygen species are hydrogen peroxide and hypochlorous acid. The role of free radicals has been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, aging, arthritis, diabetes *etc*. [28] and the compounds that scavenge free radicals have great potential in ameliorating these disease process [29].

Total Phenolic Content: Phenolic compounds are a broad group of secondary bioactive substances that are produced by plants as a result of plant adaptation to biotic and abiotic stress. Both edible and inedible plants and plant parts typically include bioactive substances including phenolics, tannins, flavonoids, *etc* [30, 31].

They have a variety of biological properties that have been noted, including antioxidant activity. The redox characteristics of phenolic compounds, which can be useful in absorbing and neutralizing free radicals, quenching singlet, and triplet oxygen, or dissolving peroxides, are primarily responsible for their antioxidant action [32]. Furthermore, phenolic extracts of plant materials have been demonstrated in several model systems to neutralize free radicals [33.] However, the antioxidant properties of plant extracts are predominantly attributable to phenolic compounds. The total phenolic content of the *Ocimum basilicum* and *Ocimum sanctum* leaf extracts in this study was expressed as Gallic acid equivalents (GAE), and the values were 48.19 ± 0.91 and 28.22 ± 0.38 g/100g, respectively. This study showed that Ocimum basilicum is richer in polyphenols and has more antioxidant-activity than *Ocimum sanctum* [34].

DPPH Radical Method: Due to its simplicity and convenience, the DPPH radical has been frequently employed to evaluate the action of radical scavengers. The antioxidant successfully turned the stable radical DPPH into the yellow-colored diphenyl-picrylhydrazine in the DPPH test. The technique relies on reducing an alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant because the reaction produces the non-radical form of DPPH-H [35]. A decrease in absorbance at 517 nm was used to assess the DPPH radicals' capacity for reduction. By donating hydrogen, antioxidants could scavenge more DPPH radicals, reducing their absorption. The scavenging effect of Ocimum extracts on DPPH radical is shown in **Fig. 1** and **Table 1**. The extent of DPPH radical scavenging at different concentrations (50µl-500µl) of *O. basilicum* and *O.sanctum* extracts compared with Ascorbic acid as the standard. Among all the concentrations, at 500µl concentration the percentage of activity in *O. basilicum* (76.67%) and *O. sanctum* (70.60%). The antioxidant activity of the *Ocimum basilicum* and *Ocimum sanctum* have been studied [36, 37] and the results were similar to our observations.

TABLE 1: *IN-VITRO* CONCENTRATION DEPENDENT INHIBITION OF DPPH RADICAL BY ALCOHOLIC EXTRACT OF *OCIMUM SANCTUM, OCIMUM BASILICUM* AND ASCORBIC ACID

Concentrations (µg/Ml)	% Inhibition of *O. basilicum*	% Inhibition of *O. sanctum*	% Inhibition of ascorbic acid
50	14.11±0.22	10.32±0.98	21.46±2.60
100	20.42±0.92	18.02±1.02	30.32±3.26
200	31.32±1.50	26.11±2.63	51.22±2.90
300	51.55±2.92	48.23±1.32	58.66±2.22
400	58.66±3.99	51.55±3.16	66.61±4.40
500	76.67±4.82	70.60±4.60	79.22±3.69

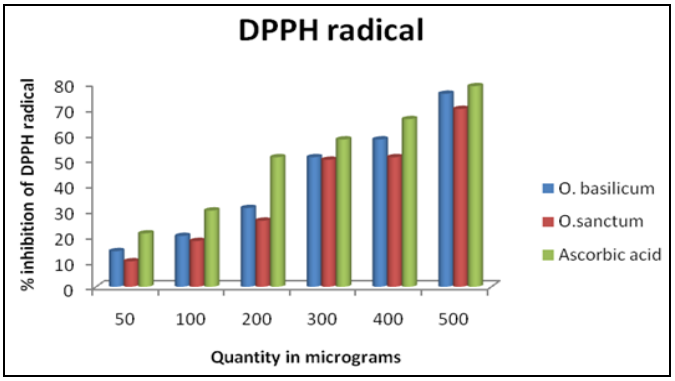

FIG. 1: DPPH (1,1-DIPHENYL-2-PICRYLHYDRAZYL) RADICAL BY ALCOHOLIC EXTRACT OF *OCIMUM SANCTUM, OCIMUM BASILICUM* AND ASCORBIC ACID

Superoxide Radical: Superoxide anion plays an important role in forming more reactive oxygen species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which include oxidative damage in lipids, proteins, and DNA [38]. Therefore, studying the scavenging activity of plant extract on superoxide radicals is one of the most important ways of clarifying the mechanism of antioxidant activity. In the present study, ethanolic extracts of *O. sanctum* and *O. basilicum* L. was found to posse's superoxide radicals generated by photoreduction of riboflavin.

The concentration-dependent percent inhibition of superoxide radical activity by ethanolic extract of *O. sanctum* and *O. basilicum* and Ascorbic acid were given in the **Table 2** and shown in the **Fig. 2**. The concentration of 500µl was the most potent to *O. sanctum* (58.23%) and *O. basilicum* (60.72%).

TABLE 2: *IN-VITRO* CONCENTRATION-DEPENDENT INHIBITION OF SUPEROXIDE RADICAL BY ALCOHOLIC EXTRACT OF *OCIMUM SANCTUM, OCIMUM BASILICUM* AND ASCORBIC ACID

Concentrations (µg/ml)	% inhibition of *O. basilicum*	% inhibition of *O. sanctum*	% inhibition of ascorbic acid
50	17.17±0.64	7.25 ± 0.54	19.06 ± 1.60
100	21.10 ±0.82	18.10 ±0.62	28.32 ± 2.26
200	37.21±1.63	27.26 ± 2.63	52.22 ± 3.90
300	46.23 ± 2.32	39.20 ± 2.92	56.66±3.22
400	55.45 ± 1.16	48.15 ± 3.16	76.61±5.40
500	60.72 ± 3.60	58.52 ± 4.60	80.12± 4.69

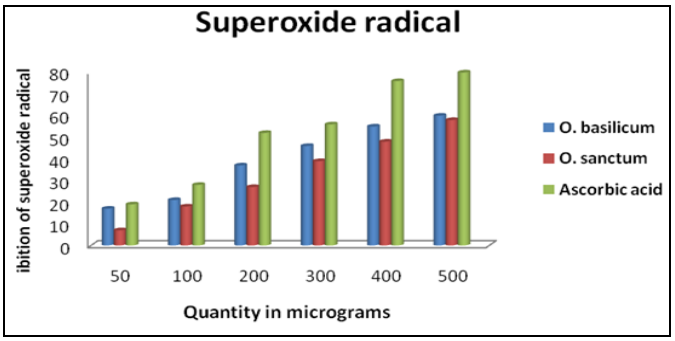

FIG. 2: SUPEROXIDE RADICAL BY ALCOHOLIC EXTRACT OF *OCIMUM SANCTUM, OCIMUM BASILICUM* AND ASCORBIC ACID

Hydroxyl Radical: Among the ROS, the hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism. Due to the high reactivity, the radicals have a very short biological half-life. The generated hydroxyl radicals initiate the lipid peroxidation process and/or propagate the chain process via the decomposition of lipid hydroperoxides [39]. A single hydroxyl radical can form many lipid hydroperoxides molecules in the cell membrane, which may severely disrupts its function and leads to cell death. In the present study, the ethanolic extract of *Ocimum sanctum* and *Ocimum basilicum* was found to possess concentration dependent scavenging activity on hydroxyl radicals generated by Deoxyribose degradation method. The concentration dependent percent inhibition of hydroxyl radical activity by ethanolic extracts of *O. sanctum* and *O. basilicum* and ascorbic acid were given in the **Table 3** and shown in the **Fig. 3**. At a concentration of 500µl the scavenging activity of *O. sanctum* (55.51%) and *O. basilicum* (57.97%).

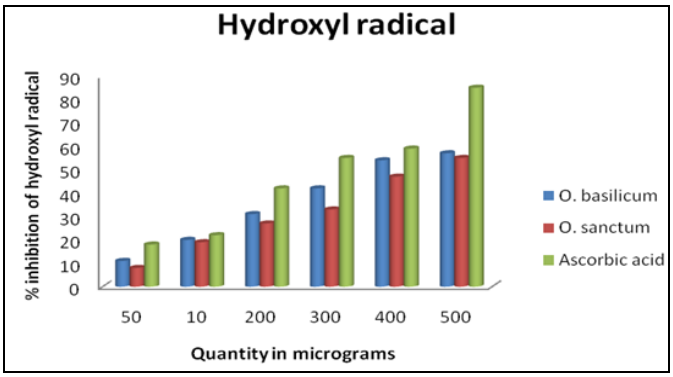

FIG. 3: HYDROXYL RADICAL BY ALCOHOLIC EXTRACT OF *OCIMUM SANCTUM, OCIMUM BASILICUM* AND ASCORBIC ACID

TABLE 3: *IN-VITRO* CONCENTRATION-DEPENDENT INHIBITION OF HYDROXYL RADICAL BY ALCOHOLIC EXTRACT OF *OCIMUM SANCTUM, OCIMUM BASILICUM* AND ASCORBIC ACID

Concentrations (µg/ml)	% inhibition of O. *basilicum*	% inhibition of *O. sanctum*	% inhibition of ascorbic acid
50	11.77±0.94	8.25 ± 0.84	18.06 ± 1.60
100	20.10 ±0.92	19.20 ±0.72	22.32 ± 2.26
200	31.11±2.53	27.26 ± 1.63	42.22 ± 4.90
300	42.13 ± 3.32	33.20 ± 2.92	55.66±2.22
400	54.15 ± 2.16	47.15 ± 3.16	59.11±4.40
500	57.97 ± 4.60	55.52 ± 3.60	85.12± 4.19

Lipid Peroxidation Radical: Lipid peroxidation, which involves a series of free radical-mediated chain reaction processes, is also associated with several types of biological damage.

Therefore, much attention has been focused on using natural antioxidants to initial lipid peroxidation and protect from damage due to free radicals. In the present study, ethanolic extracts of *O. sanctum* and *O. basilicum* were found to inhibit the lipid peroxides generation induced by Fe^{2+}/ascorbate on rat liver homogenate in a concentration-dependent manner.

The concentration-dependent lipid peroxidation inhibition activity by alcoholic extracts of *O. sanctum* and *O. basilicum* and ascorbic acid were given in **Table 4** and shown in **Fig. 4.**

TABLE 4: *IN-VITRO* CONCENTRATION DEPENDENT INHIBITION OF LIPID PEROXIDATION BY ALCOHOLIC EXTRACT OF *OCIMUM SANCTUM, OCIMUM BASILICUM* AND ASCORBIC ACID

Concentrations (µg/ml)	% inhibition of *O. basilicum*	% inhibition of *O. sanctum*	% inhibition of ascorbic acid
50	15.65 ± 1.14	10.25 ± 0.44	20.06 ± 2.60
100	22.10 ± 0.82	15.20 ± 0.52	37.01 ± 1.26
200	35.21 ± 1.53	29.26 ± 2.53	51.02 ± 2.90
300	45.23 ± 2.32	36.20 ± 1.92	59.56 ± 2.22
400	56.15 ± 5.16	48.15 ± 5.16	69.61 ± 4.40
500	66.66 ± 4.64	59.52 ± 4.60	74.12 ± 5.69

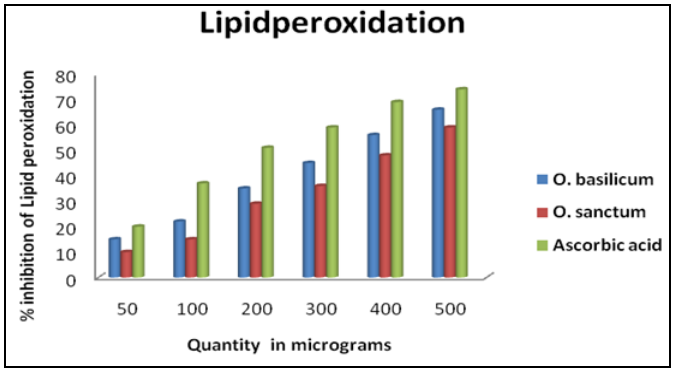

FIG. 4: LIPID PEROXIDATION BY ALCOHOLIC EXTRACT OF *OCIMUM SANCTUM, OCIMUM BASILICUM* AND ASCORBIC ACID

The highest parentage activity showed both plants at 500µl in *O. sanctum* (61.77%) and *O. basilicum* (66.61%). The radical system was previously conducted in several medicinal plants [40, 41] and cereal crop [42]. Antioxidant activity of these two plant extracts were analyzed and compared with natural antioxidants like ascorbic acid.

The free radical scavenging effects were found to increase with increasing concentrations of ethanolic extracts (µg/mL). In all free radicals tested on these two plants maximum IC_{50} values have been observed in *Ocimum basilicum* than *Ocimum sanctum* **Fig. 5**. This study demonstrates that Ocimum species have significant antioxidant levels and antioxidant potentials.

These observations further enhance medicinal plants' therapeutic and pharmaceutical applications, giving scope for further investigation.

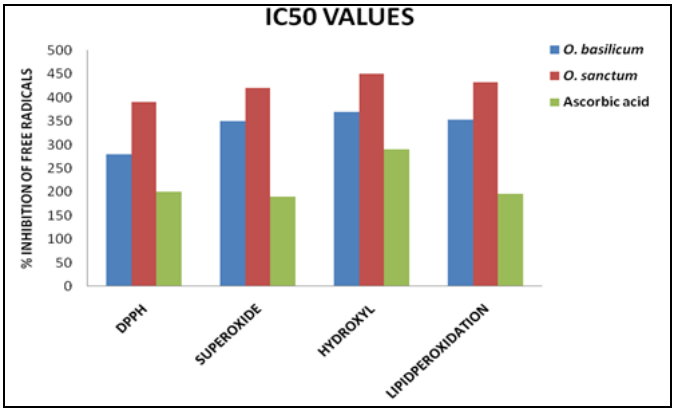

FIG. 5: IC_{50} VALUES OF FREE RADICALS BY ALCOHOLIC EXTRACT OF *OCIMUM SANCTUM, OCIMUM BASILICUM* AND ASCORBIC ACID.

TABLE 5: IC_{50} VALUES OF *OCIMUM BASILICUM* AND *OCIMUM SANCTUM*

Free radicals	*O. basilicum* (µg/mg)	*O. sanctum* (µg/mg)	Ascorbic acid (µg/mg)
DPPH	280	390	200
Superoxide	350	420	190
Hydroxyl	370	450	290
Lipidperoxidation	354	433	196

CONCLUSION: The design could be reasonable to assume that this extract can prevent all types of radicals and further suggest that the extract is a potential therapeutic agent for the control of oxidative damage caused by reactive oxygen species, given the antioxidant potential of the investigated Ethanolic Extract of *Ocimum basilicum* and *Ocimum sanctum* for the potent of DPPH radical, Superoxide radical, Hydroxyl radical, and Lipid peroxidation scavenging activity. The results indicate that the aroma of the leaf has antioxidant properties, which suggests that the plant has certain chemicals that may act as antioxidants. Reactive oxygen species are believed to play a role in the pathogenesis of inflammatory diseases, chronic infections, and food deterioration; therefore, the observed inhibitory potential could contribute to an understanding of the way *O. basilicum* and *O. sanctum* are effective in treating various disease conditions. The findings also point to the possibility of using leaf extract from both plants in the food sector as a natural antioxidant and food taste. It's possible that the antioxidants in this plant extract function by partnering with oxygen or by preventing oxygen from interacting with dietary components. However, it is essential to investigate each plant's potential *in-vivo* and determine its chemical compounds, as these processes could eventually lead to their application in pharmaceutical formulations or the food industry as an antioxidant flavor.

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