PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIOXIDANT ACTIVITY OF DIFFERENT SPECIES OF *OCIMUM* PLANT

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***Abstract*: Tulsi is described in Ayurveda having many medicinal properties and a wide therapeutic range. It is used specially in the management of kasa (cough), Shwasa (Ashtma), Jwara (fever) and pratishaya (common cold). In India, the herb tulsi also called holy basil (sometimes spelled "Tulasi") has been widely known for its health promoting and medicinal value for thousands of years. Commonly called sacred or holy basil, it is a principal herb of Ayurveda, the ancient traditional holistic health system of India. Holy basil is also known as "The Incomparable One", "The Mother Medicine of Nature", and "The Queen of Herbs". Tulsi is identified by botanists primarily as *Ocimum sanctum* (Rama and Krishna Tulsi varieties) or more recently Ocimum tenuiflorum, and *Ocimum gratissimum* (Vana Tulsi variety). Belonging to the Lamiaceae/Labiatae mint family, these and other closely related species and varieties (e.g., *Ocimum canum*) are cousins of the familiar sweet basil cooking herb *Ocimum basilicum*. In parts of India, all of the basils are honoured as tulsi. An antioxidant is a substance capable of preventing or slowing the oxidation of other molecules. Generally, an antioxidant can protect against metal toxicity by trapping free radicals thus terminating the chain reaction, by chelating metal ion and preventing the reaction with reactive oxygen species or by chelating metal and maintaining it in a redox state leading to its incompetency to reduce molecular oxygen.**

***Keywords*: *Ocimum canum*, Pratishaya, The Queen of Herbs, Antioxidant and Tulasi.**

INTRODUCTION

Ancient Indian, Chinese and European discovered origin of medicinal herbs. They have been using them for curative purposes successfully. The records are available in ancient texts. In India itself, there are more than 20000 medicinal plants grown all over the wild forests. Of these, some 60 genus are used immensely in medicinal preparation. Despite their demands today, they are not grown in controlled manner. Rather tribes use them as their livelihood in some belts where they are grown in the wild. Unlike India, in china, the spurts in demand for traditional medicine have made government to allow growth of these plants for further research and development. About 100 units have nearly 600 plant type, grown for their medicinal value. Herbal medicines are used in Ayurveda, Naturopathy and Homeopathy, tradition and Native American medicine [1].

The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs. In almost all the traditional medicine, the medicinal plant plays a major role and constitutes the backbone of traditional medicine [2].

A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal Plants”. Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants, which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils, contain minerals and vitamins, possess medicinal properties [3].

Tulsi is described in Ayurveda having many medicinal properties and a wide therapeutic range. It is used specially in the management of kasa (cough), Shwasa (Ashtma), Jwara (fever) and pratishaya (common cold). In India, the herb tulsi also called holy basil (sometimes spelled "Tulasi") has been widely known for its health promoting and medicinal value for thousands of years. Commonly called sacred or holy basil, it is a principal herb of Ayurveda, the ancient traditional holistic health system of India. Holy basil is also known as "The Incomparable One", "The Mother Medicine of Nature", and "The Queen of Herbs" [4].

SCIENTIFIC CLASSIFICATION

Kingdom : Plantae
Division : Magnoliophyta

Class : Magnoliopsida
Order : Lamiales
Family : Lamiaceae
Genus : *Ocimum*
Species : (*Sanctum, basilicum, kilimandscharicum, americanum, gratissimum)* [5]

Figure 1 *Ocimum basilicum* Linn, Ocimum gratissimum Linn, Ocimum canum Sims

BOTANICAL DESCRIPTION

Basil is an annual herb to 2-3 ft (0.6-0.9 m) tall with green stems (usually woody at the base) that are square in cross section. Basil has opposite leaves, 2-4 in (5.1-10.2cm) long, and tiny purple or white flowers arranged in flattened whorls that encircle the stems, one whorl above another. Plants are leafy and branch freely with a pair of opposing branches in a flat plane, then another pair above in a plane perpendicular to the last, and so on. It is known as lime, hairy or hoary basil, It is An annual aromatic much-branched herb, 15 - 60 cm high. Leaves 2.5-3.8 cm long, elliptic-lanceolate, acute at both ends [6]. Flowers small, white, in rather close whorls, in spiciform racemes, up to 20 cm long. Nutlets 1.25 mm long, ellipsoid, black. ***Ocimum gratissimum*** is a shrub up to 1.9m in height with stems that are branched [7]. The leaves measure up to 10 x 5 cm, and are ovate to ovate-lanceolate, sub-acuminate to acuminate at apex, cuneate and decurrent at base with a coarsely crenate, serrate margin, pubscent and dotted on both the sides. The leaves show the presence of covering and glandular trichomes. Stomata are rare or absent on the upper surface while they are present on the lower surface. Ordinary trichomes are few, while the long ones up to 6-celled are present on the margins mostly; the short ones which are 2 celled, are mostly found on the lamina. Petioles are up to 6 cm long and racemes up to 18 cm long. The peduncles are densely pubescent. Calyx is upto 5mm long, campanulate and 5-7 mm long, greenish-white to greenish-yellow in colour. Nutlets are mucilaginous when they are wet [8].

GEOGRAPHICAL DISTRIBUTION

Sweet basil is native to tropical Asia. It is cultivated commercially in southern Europe, Egypt, Morocco, Indonesia, and California. Basil is very sensitive to cold, with best growth in hot, dry conditions. It behaves as an annual if there is any chance of a frost. In Northern Europe, Canada, the northern states of the U.S., and the South Island of New Zealand it will grow best if sown under glass in a peat pot, then planted out in late spring/early summer (when there is little chance of a frost). Additionally, it may be sown in soil once chance of frost is past. It fares best in a well-drained sunny spot. Although basil grows best outdoors, it can be grown indoors in a pot and, like most herbs, will do best on an equator-facing windowsill. It should be kept away from extremely cold drafts, and grows best in strong sunlight, therefore a greenhouse or row cover is ideal if available. It can, however, be grown even in a basement, under fluorescent lights [9]. The plant grows widely in tropical Africa. It is also grown in South-East Asia, largely in India and Hawaii. The native areas of the plant are mostly found to be regions that are 1500 m above sea level. The plant also grows well in lake shores, coastal bush lands and in sub-montane regions [10].

PHYTOCHEMISTRY

Ocimum basilicum L. contains linalol (54.95%), methylchavikol (11.98%), methylcinnamat (7.24%), and linolen (0.14%).Essential oil is also found in sweet basil, along with rosmarinic acid, citral, eugenol, and geraniol. Aerial parts yield an essential oil, which contains camphor and linalool (major constituents), citronellal, methylcinnamate, citronellic acid, eugenol, citronellol, geraniol, citral and methylheptenone. On fractionation the oil yields dipentene, terpinolene, crithmene, limonene, pinene, sabinene, camphene, caryophyllene, traces of phenol and acetic acid. Polysaccharide present in the plant contains xylose, arabinose, rhamnose, galactose, galacturonic acid and glucuronic acid detected from the plant. Photochemical evaluation of this plant has shown that it is rich in alkaloid, tannis, phytates, flavonoids and oligosaccharides. It has tolerable cyanogenic content [11]. The volatile aromatic oil from the leaves consists mainly of thymol (32-65%) and eugenol; it also contains xanthones, terpenes and lactones. Characterization of its ethanolic extracts revealed the presence of non–cyclic sesquiterpenes, phenols. It is characterized by presence of high amount of camphor in essential oil. It is pale yellow in color and its content varies in different samples fro m 61 to 80.5% [12]. Leaves contain the maximum amount of camphor and oil followed by flowers; stems contain only minute quantities. It contains d-camphor, d-α-pinene, d-limonene, terpinolene and unidentified sesquiterpenes and sesquiterpenes of alcohols [13].

MATERIALS AND METHODS

Preparation of Extracts through Soxhlet extraction

1. Mature leaves of *Ocimum species* were, collected from healthy plant from garden of government Senior secondary school, Pindwara.
2. The leaves were shaded, dried at room temperature and were grounded to very fine powder followed by sieving in 75 micron size sieve and was stored at room temperature.
3. In soxhlet apparatus, little amount of glass wool was placed in thimble where the siphon arm was open, and then 120gm of powdered leaves of *Ocimum* species were filled into the thimble of the Soxhlet assembly.
4. The glycerin wasplaced at the mouth of extraction chamber and at the mouth of condenser to avoid any leakage.
5. The extraction chamber was than filled with petroleum ether through the open end.
6. After the completion of extraction with petroleum ether, the defatted residue was obtained, that was dried in the sun light.
7. About 50gm of defatted dried powdered of plant material was uniformly packed into a thimble and was extracted with 150ml of ethanol (99.9%) and water solvent separately [14].
8. The process of extraction was kept continues for 24 hours or till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent get evaporated.
9. Dried extract was kept in refrigerator at 4ºC for their use in phytochemical analysis [15].

Phytochemical Screening

Qualitative examination of phytoconstituents [16][17][18]

1. Test for Alkaloids:

Dragendorff's Test: To 1 gm of the extract, add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

Ethanolicand water both extract of *Ocimum* species give positive result.

2. Test for Glycosides:

Extract is hydrolysed with dil. HCl, and then subjected to test for glycosides.

(A) Modified Borntrager's Test: Extract is treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-`pink colour in the ammonical layer indicates the presence of anthranol glycosides.

Ethanolic and water both extract of *Ocimum* species gives positive result.

(B) Legal's Test: Extract is treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

Ethanol and water both extract of *Ocimum*species gives negative result.

3. Test For Tannins:

To 1gm of the extract, add ferric chloride solution, formation of a dark blue or greenish black color product shows the presence of tannins.

Ethanolic and water both extract of *Ocimum* species gives positive result.

4. Test for phenols:

Ferric Chloride Test: Extract is treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Ethanolic and water both extract of *Ocimum* species gives positive result.

5. Test for Steroids:

Libermann-Burchard Test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green color shows the presence of sterols.

Ethanolic and water both extract of *Ocimum* species gives positive result.

6. Test for Triterpenoids:

Noller's Test: Dissolve two or three granules of tin metal in 2ml thionyl chloride solution. Then add 1gm of the extract into test tube and warm, the formation of pink color indicates the presence of triterpenoids.

Ethanolic and water both extract of *Ocimum* species gives positive result.

7. Test for Flavonoid:

Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappears on addition of an acid indicates the presence of Flavonoid.

Ethanolic and water both extract of *Ocimum* species gives positive result.

8. Detection of saponins:

(A) Froth Test: Extract is diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Ethanolic and water both extract of *Ocimum* species gives positive result.

9. Detection of proteins and aminoacids:

(A)Xanthoproteic Test: The extract is treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

Ethanolic and water both extract of *Ocimum* species gives positive result.

(B)Ninhydrin Test: To the extract, 0.25% w/v ninhydrin reagent is added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

Ethanolic and water both extract of *Ocimum* species gives positive result.

Analytical Parameter

Ash Values

The residues remaining after incineration is the ash content of the drug. Ash values are helpful in determining the quality and purity of crude drug, especially in the powdered form. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may also include inorganic matter added for the purpose of adulteration. Hence, an ash determination furnishes a basis for judging the identity and cleanliness of a drug and gives information regarding its adulteration with inorganic matter. Procedure given in Indian Pharmacopoeia was used to determine the different ash values such as total ash, acid insoluble ash, and water soluble ash.

(a) Determination of total ash value:

Accurately weighed about 3 gm of air dried powdered drug was taken in a tared silica crucible and incinerated by gradually increasing the temperature to make it dull red hot until free from carbon. Cooled and weighed, repeated for constant value. Then the percentage of total ash was calculated with reference to the air dried drug.

(b) Determination of acid insoluble ash value:

The ash obtained as directed under total ash value was boiled with 25 ml of 2NHCl for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water, ignited and weighed, then calculated the percentage of acid insoluble ash with reference to the air dried drug.

(c) Determination of water soluble ash value:

The total ash obtained was boiled with 25 ml of water for 5 minutes. The insoluble matter was collected on an ash less filter paper, washed with hot water and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of insoluble matter was subtracted from the weight of total ash [19] [20].

Extractive Values

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

(a) Determination of Alcohol Soluble Extractive Value:

10gms of the air-dried coarse powder of Ocimum specieswere macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drugs [21].

(b) Determination of Water Soluble Extractive Value:

Weigh accurately the 10 gm of coarsely powdered drug and macerate it with 100 ml of water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tared flat-bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug [21].

Loss on Drying

Loss on drying is the loss in weight in % w/w determined by means of the procedure given below. It determines the amount of volatile matter of any kind (including water) that can be driven off under the condition specified (Dessicator or hot air oven). If the sample in the form of large crystals, then reduce the size by quickly crushing to a powder [22].

Procedure

About 1.5 gm, of powdered drug was weighed accurately in a porcelein dish which was previously dried at 105°C in hot air oven to constant weight and then weighed.

IN-VITRO ANTIOXIDANT METHOD

DPPH Radical Scavenging assay

Principle

The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. Antioxidants in extract react with DPPH and convert it to 2, 2-diphenyl-1-picrylhydrazine that bears the yellow colour. The degree of discoloration indicates the scavenging potential of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a

measure of antioxidant potential of extract. The resulting decolourization is sticheometric with respect to number of electrons captured [23].

Method

1. A solution of the radical was prepared by dissolving 2.4 mg DPPH in 100 ml methanol.
2. A test solution (5 µl) was added to 3.995 ml of methanolic DPPH.
3. The mixture was shaken vigorously and kept at room temperature for 30 min in the dark.
4. Absorbance of the reaction mixture was measured at 517 nm spectrophotometrically.
5. Absorbance of the DPPH radical without antioxidant, i.e. blank was also measured.
6. All the determinations was performed in triplicate.
7. The capability to scavenge the DPPH radical was calculated using the following equation.

DPPH Scavenged (%) = $((A_B - A_A)/A_B) \times 100$

where, A_B is absorbance of blank at t= 0 min;

A_A is absorbance of the antioxidant at t= 30 min.

8. A calibration curve was plotted with % DPPH scavenged versus respective concentrations used and IC_{50} was calculated from the graph. IC_{50} value is the effective concentration of sample at which the antioxidant activity is 50%.

RESULTS

YIELD OF CRUDE EXTRACT

After extraction highest yield of extract was obtained for Ocimum sanctum in ethanol solvent

Table-1 Yield of crude extract

Species name	Solvent used	Yield of crude extract (gm/100gm)
Ocimum sanctum	Ethanol	8.14
	Water	6.78
Ocimum gratisimum	Ethanol	6.06
	Water	6.98

PHYTOCHEMICAL TEST

The phytochemical analysis of the extracts showed the presence of alkaloids,anthraquinone, glycosides, gum mucilage, proteins, amino acids, tannins, phenoliccompounds, triterpenoidss, steroids, sterols, saponins, flavonoidswhere as cardiac glycoside is absent [24].

Table-2 Qualitative analysis of the two *Ocimum* species

Phytochemicals	*Ocimum Sanctum*	*Ocimum grattisimum*
Alkaloids	+	+
Cardiac glycosides	-	-
Anthroquinone glycosides	+	+
Phenolic compounds	+	+
Triterpenoids	+	+
Steroids	+	+
Sterols	+	+
Saponins	+	+
Flavanoids	+	+
Protein	+	+
Amino acid	+	+
Tanins	+	+

(+) = Present and (-) = Absent

PHENOLIC CONTENT

In the quantitative analysis of antioxidative components, the total phenolic contents in ethanol and water extracts of two *Ocimum* species are as follows: ***Ocimum sanctum*** (308µg/ml, 141µg/ml), ***Ocimum gratissimum*** (214µg/ml, 93µg/ml) respectively [25] [26].

Table-3 Total phenol content

Species name	Solvent used	Phenol content (ug/ml)
Ocimum sanctum	Ethanol	308
	Water	141
Ocimum gratisimum	Ethanol	214
	Water	93

ANTIOXIDANT ACIVITY AND IC_{50} VALUE

In vitro antioxidant studies of the two *Ocimum* extracts, the extent of DPPH radical scavenging at different concentration (40-200µg/ml) of ***Ocimum sanctum*** and ***Ocimum gratissimum*** extracts were measured, with ascorbic acid as standard.

The free radical scavenging effects were found to increase with increasing concentration of crude extracts (µg/ml). Maximum activities have been observed in ***Ocimum sanctum*** (95.67±0.10%) and minimumin ***Ocimum gratissimum*** (68.87±0.02%) with IC50 values of 33.20µg/ml and 92.10µg/ml [28]. The mean values of ***Ocimum sanctum*** were found to be closer to the control and indicated that it had more radical scavenging activity than ***Ocimum gratisimum*** [29] [30]**.**

Table-4 Antioxidant activities of two *Ocimum* Species in Ethanolic extracts

Conc. of extracts (ug/ml)	Antioxidant activity %	
	Ocimum sanctum	***Ocimum gratisimum***
40	77.54±0.45	54.4±0.34
80	80.67±0.23	57.54±0.26
120	85.54±0.75	61.24±0.45
160	91.87±0.21	64.63±0.14
200	95.34±0.30	68.57±0.22

Table-5 Antioxidant activities of two *Ocimum* Species in Aqueous extracts

Conc. of extracts (ug/ml)	Antioxidant activity %	
	Ocimum sanctum	***Ocimum gratisimum***
40	58.43±0.35	53.31±0.36
80	64.86±0.63	55.45±0.15
120	67.53±0.23	58.22±0.44
160	71.30±0.19	65.57±0.11
200	75.56±0.12	63.30±1.35

Table-6 IC_{50} value of both *Ocimum* species

Species name	Solvent used	IC_{50} value (ug/ml)
Ocimum sanctum	Ethanol	33.20
	Water	81.43
Ocimum gratisimum	Ethanol	88.54
	Water	92.10

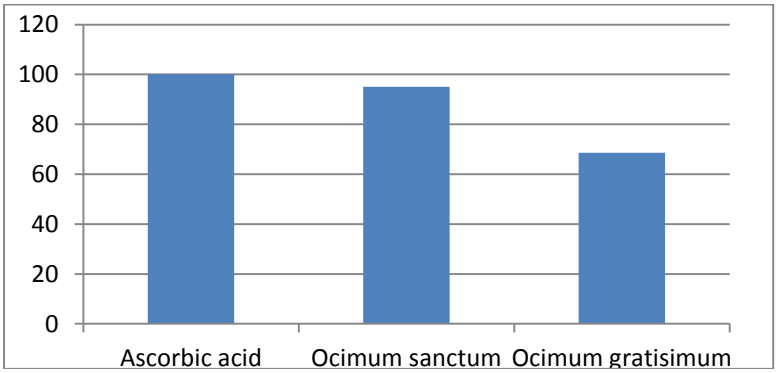

Figure 2 DPPH Scavenging activity (%) of Ethanolic extracts of two *Ocimum* species

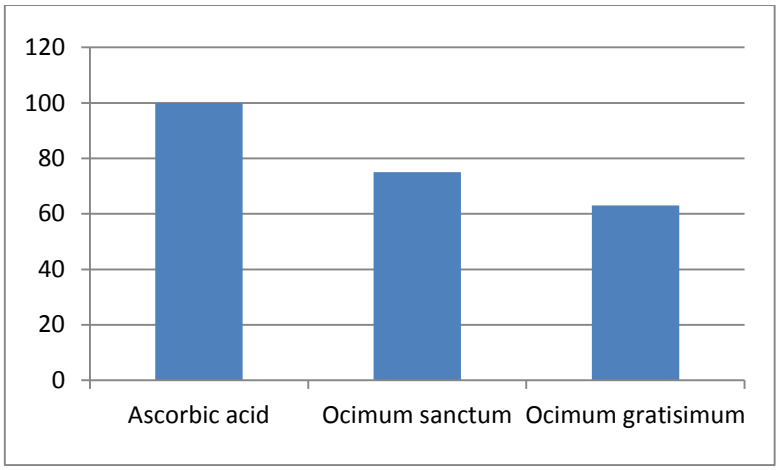

Figure 3 DPPH Scavenging activity (%) of Aqueous extracts of two *Ocimum* species

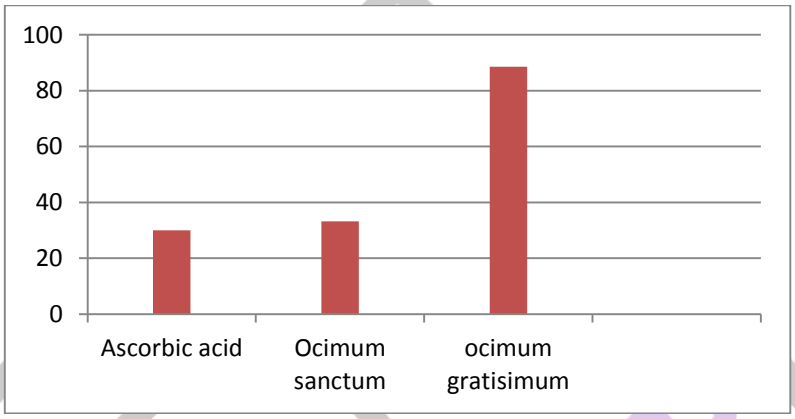

Figure 4 IC_{50} Values of Ethanolic extracts of two *Ocimum* species

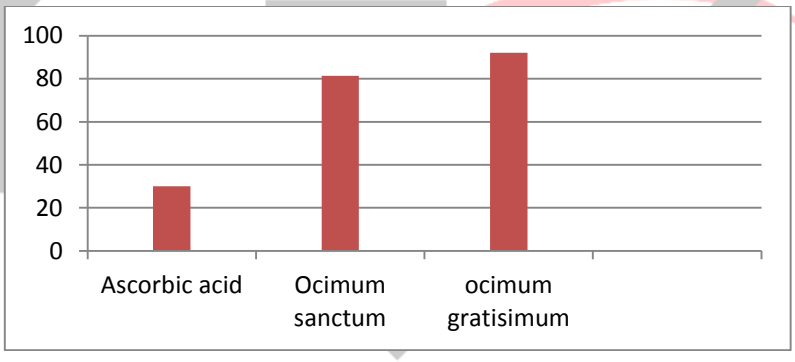

Figure 5 IC_{50} Values of Aqueous extracts of two *Ocimum* species

CONCLUSION

Brief introductions of *Ocimum* species with their uses and therapeutic importance and introduction of antioxidant activity have been summarized. The ethanolic and aqueous extract of ***Ocimum sanctum*** and ***Ocimum gratisimum*** were subjected for the preliminary phytochemical test. The results showed the presence of the alkaloids, anthraquinone, glycosides, gum mucilage, proteins, amino acids, tannins, phenolic compounds, triterpenoidss, steroids, sterols, saponins, flavones, flavonoids where as cardiac glycoside was absent. The ethanolic and aqueous extract of both the Ocimum species ie. Ocimum sanctum and ***Ocimum gratisimum*** were subjected for invitro antioxidant activity by using the DPPH method. The result showed that ***Ocimum sanctum*** bear the highest antioxidant activity in Ethanolic extract among both the species under study. From the study it is concluded that 200µg/ml of ethanolic extract of ***Ocimum sanctum*** is better antioxidant than ***Ocimum gratissiumas*** observed DPPH scavenging assay. These findings suggest thatthe rich phytochemical contents of ***Ocimum sanctum*** and its good antioxidant activity may be responsible for its popular and traditional use.

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