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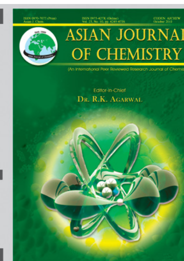

Investigation of Standardized Ethanolic Extract of *Ocimum sanctum* Linn. (Holy Basil) Leaves for its *in vitro* Antioxidant Potential and Phenolic Composition

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Antioxidant activities of standardized ethanolic (90 %) extract of *Ocimum sanctum* (EEOS) leaves was studied for its free radical scavenging property on different *in vitro* models *e.g.*, 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH), superoxide anion radical, nitric oxide radical, hydroxyl radical scavenging and reducing power assays. The extract showed significant activities in all antioxidant assays and measurement of total phenolic compounds by Folin-Ciocalteu phenol reagent indicated that the extract (1 mg) contained 56.7 µg pyrocatechol equivalent of phenolics. Moreover, the reductive power of ethanolic extract of *Ocimum sanctum* and butylated hydroxy toluene found to be 1.155 and 0.771 at 500 µg/mL, respectively. The antioxidant activities of ethanolic extract of *Ocimum sanctum* increased in a concentration dependent manner. The IC_{50} values were found to be 31.37, 31.83, 29.17 and 400.90 mg/mL, respectively, in DPPH radical scavenging, nitric oxide radicals generated from sodium nitroprusside, scavenging the superoxide generated by phenazine methosulphate (PMS)/ nicotinamide adenine dinucleotide (NADH)-nitroblue tetrazolium (NBT) system and hydroxyl radical generated by Fenton's reaction. The results obtained in the present study indicate that the antioxidant property of ethanolic extract of *Ocimum sanctum* may be due to high content of phenolic compounds and justify the therapeutic applications of the plant in the indigenous system of medicine, augmenting its therapeutic value.

Key Words: *Ocimum sanctum*, Antioxidant, Free radicals, ROS, Reductive ability.

INTRODUCTION

Free radicals are generally very reactive molecules possessing an unpaired electron. They are produced continuously in cells either as by-products of metabolism, or for example, by leakage from mitochondrial respiration. The most important reactions of free radicals in aerobic cells involve molecular oxygen and its radical derivatives (superoxide anion and hydroxyl radicals), peroxides and transition metals. Cells have developed a comprehensive set of antioxidant defense mechanisms to prevent free radical formation and to limit their damaging effects. These mechanisms include enzymes to inactivate peroxides, proteins to sequester transition metals and a range of compounds to scavenge free radicals. Reactive free radicals formed within cells can oxidize biomolecules and this may lead to cell death and tissue injury. Establishing the involvement of free radicals in the pathogenesis of a disease, however, is extremely difficult, due to the short lifetimes of these species, but also due to the lack of sufficiently sensitive technology to detect radicals directly in biological systems[1,2].

Reactive oxygen species (ROS), which consist of free radicals such as superoxide anion ($O_2^{\cdot -}$) and hydroxyl ($HO^{\cdot}$) radicals and non-free radical species such as H_2O_2 and singled oxygen ($^1O_2$), are different forms of activated oxygen[3-5]. Reactive oxygen species are produced by all aerobic organisms and can easily react with most biological molecules including proteins, lipids, lipoproteins and DNA. Thus, ample generation of ROS proceed to a variety of pathophysiological disorders such as arthritis, diabetes, inflammation, cancer and genotoxicity[6,7]. Therefore, living organisms possess a number of protective mechanisms against the oxidative stress and toxic effects of ROS.

Antioxidants regulate various oxidative reactions naturally occurring in tissues and are evaluated as a potential anti-aging agent. Hence, antioxidants can terminate or retard the oxidation process by scavenging free radicals, chelating free catalytic metals and also by acting as electron donors. Antioxidants have been widely used as food additives to provide protection from oxidative degradation of foods and oils. Hence, antioxidants are used to protect food quality mainly by the prevention of oxidative deterioration of constituents of lipids. The most extensively used synthetic antioxidants are propylgallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and *tert*-butylhydroquinone (*t*BHQ)[8]. However BHT and BHA have been suspected of being responsible for liver damage and carcinogenesis[9-11]. Natural antioxidants are able to protect from ROS as well as other free radicals and retard the progress of many chronic diseases and lipid oxidative rancidity in foods[12-15]. Polyphenols are widely distributed in plants and phenolic antioxidants have been found to act as free radical scavengers as well as metal chelators[16,17].

Ocimum sanctum Linn. (*O. sanctum*), commonly known as Holy Basil or "Tulsi" belonging to the family Labiatae (Lamiaceae), is considered a sacred plant in India and grown in every rural household. Traditionally, fresh juice or decoction of *O. sanctum* leaves is used to promote health and in treatment of various disorders as advocated in Ayurveda, the Indian System of Medicine. Indian Materia Medica describes the use of aqueous, hydroalcoholic and methanolic extract of *O. sanctum* leaves in a variety of disorders, like bronchitis, rheumatism and pyrexia[18,19]. Several recent investigations using these extracts have indicated that *O. sanctum* possesses significant antiinflammatory[20], antioxidant[21], immunomodulatory[22] and antistress[23] properties. In addition, it has been reported to have radioprotective and anticarcinogenic property[24].

Epidemiological studies have suggested positive associations between the consumption of phenolic-rich foods or beverages and the prevention of diseases[25]. These effects have been attributed to antioxidant components such as plant phenolics, flavonoids and phenylpropanoids among others[26]. Basils (*Ocimum* spp., Lamiaceae) contain a wide range of essential oils rich in phenolic compounds[27] and a wide array of other natural products including polyphenols such as flavonoids and anthocyanins. *O. sanctum* is mentioned in the oldest Sanskrit Ayurvedic text-Charak Samhita and Rig Veda. It is classified as 'Rasayan', an herb that nourishes health and promotes long-life. Medicinal, religious and culinary uses of *O. sanctum* have been documented for centuries in Asia, China, the Middle East, North Africa and Australia. It is evident that the plant has great potentials in treating a number of ailments where the free radicals have been implicated to be the major factors contributing to the disorders. The consideration of tremendous health benefits of *O. sanctum* has prompted us to investigate and evaluate the antioxidant activity of ethanolic extract of *O. sanctum* (EEOS) leaves.

EXPERIMENTAL

Ammonium thiocyanate was purchased from Merck, Darmstadt, Germany. Ferrous chloride, ferric chloride, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), nicoti-namide adenine dinucleotide (NADH), EDTA, butylated hydroxy toluene (BHT), butylated hydroxy anisole (BHA), α-tocopherol, ascorbic acid, quercetin, catechin, pyrocatechol, curcumin, nitroblue tetrazolium (NBT), thiobarbituric acid (TBA), 2-deoxy-2-ribose, trichloroacetic acid (TAA), phenazine methosulfate (PMS) and potassium ferricyanide were purchased from Sigma, St. Louis, MO, USA. All other chemicals and reagents used were of analytical grade.

Plant material and extraction: The aerial parts of the plant *O. sanctum* was collected from Bhavani, Erode district, Tamil Nadu, India. It was taxonomically identified by Survey of Medicinal Plants and Collection Unit, Ooty, Tamilnadu, India and a herbarium of the plant is preserved in Department of Pharmacognosy, J.S.S. College of Pharmacy, Ooty, for reference. The whole plant was washed and leaves were separated from other aerial parts, freed from earthy material and shade dried with occasional stirring at room temperature. Dried leaves were coarsely powdered (1.9 kg ± 0.5 dry basis) and subjected to extraction by cold maceration with 90 % ethanol (17.38 % yield) at room temperature with continuous stirring (300 rpm) for 7 days, after defatting with petroleum ether (60-80 ºC). The solvents were evaporated with rotary vacuum and stored in a desiccator and then made in to a fine suspension using 0.5 % Tween 80[28]. The principle chemical constituents (rosmarinic acid and ursolic acid) of the extract were identified and quantified using HPLC (Shimadzu LC system equipped with a LC-10AT-vp solvent delivery system, SPD M-10 AVP photo diode array detector and Rheodyne 7725i injector with 5 µL loop; analysis was performed on a reverse phase Phenomenex C_{18} column [25 cm × 4.6 mm i.d, 5 µ] as stationary phase) and LC-MS (Shimadzu VP-ODS consisting of a LC connected to a single quadrupole MS analyzer, with atmospheric pressure chemical ionization interface) methods and validated.

Total phenolic compounds: Total soluble phenolic compounds in ethanolic extract of *Ocimum sanctum* were determined with the Folin-Ciocalteu reagent according to the method of Slinkard and Singleton[29]. A 0.1 mL aliquot of a suspension of 1 mg ethanolic extract of *Ocimum sanctum* in water was totally transferred to a 100 mL Erlenmeyer flask and the final volume was adjusted to 46 mL by the addition of distilled water. Next 1 mL Folin-Ciocalteu reagent was added to this mixture, followed by 3 mL 2 % Na_2CO_3 3 min later. Subsequently, the mixture was shaken for 2 h at room temperature and absorbance measured at 760 nm. All tests were performed in triplicate. The concentration of total phenolic compounds in ethanolic extract of *Ocimum sanctum* was determined as µg pyrocatechol equivalents using the following equation obtained from a standard pyrocatechol graph:

$$\text{Absorbance} = 0.001x \text{ pyrocatechol } (\mu g) + 0.0033$$

Total antioxidant activity: The antioxidant activity of ethanolic extract of *Ocimum sanctum* was determined according to the method of Mistuda *et al.*[30]. About 10 mg of ethanolic extract of *Ocimum sanctum* was dissolved in 10 mL water. Various concentrations (50, 100, 250 and 500 mg/mL) of ethanolic extract of *Ocimum sanctum* were added to linoleic acid emulsion (2.5 mL, 0.04 M, pH 7.0) and phosphate buffer (2 mL, 0.04 M, pH 7.0). The linoleic acid emulsion was prepared by mixing 0.2804 g of linoleic acid, 0.2804 g of

Tween 20 as emulsifier and 50 mL phosphate buffer mixture and was homogenized. The final volume was adjusted to 5 mL with potassium phosphate buffer (0.04 M, pH 7). Then the mixed samples were incubated at 37 ºC in a glass flask for 60 h to accelerate the oxidation process. Each 12 h, 1 mL of the incubated sample was removed and 0.1 mL of $FeCl_2$ (0.02 M) and 0.1 mL of ammonium thiocyanate (30 %) were added to the 1 mL aliquot that was removed from the sample.

DPPH radical scavenging activity: The free radical scavenging activity of ethanolic extract of *Ocimum sanctum* was measured by DPPH using the method of Blois[31]. 0.1 mM solution of DPPH in methanol was prepared and 1 mL of this solution was added to 3 mL of various concentrations of ethanolic extract of *Ocimum sanctum* and reference compound (50, 100, 150, 200 and 250 mg). After 0.5 h, absorbance was measured at 517 nm. BHA was used as a reference material. All the tests were performed in triplicate and the graph was plotted with the mean value. The percentage of inhibition was calculated by comparing the absorbance values of reference compound (BHA) and test samples.

Nitric oxide radical scavenging activity: Nitric oxide (NO) generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which can be measured by Griess reaction[32]. The reaction mixture (3 mL) containing sodium nitroprusside (10 mM) in phosphate buffered saline and ethanolic extract of *Ocimum sanctum* and reference compound (curcumin) at different concentrations (10, 25, 50, 75 and 100 µg) were incubated at 25 ºC for 150 min. Each 0.5 h, 0.5 mL of the incubated sample was removed. 0.5 mL of Griess reagent (1 % sulphanilamide, 0.1 % naphthylethylene diamine dihydrochloride in 2 % H_3PO_4) was added to the 0.5 mL aliquot of the sample removed. The absorbance of the chromophore formed was measured at 546 nm. All the tests were performed in triplicate and the results averaged. The percentage inhibition of NO generated was measured by comparing the absorbance values of curcumin and test.

Superoxide anion radical scavenging activity: Measurement of superoxide anion scavenging activity of ethanolic extract of *Ocimum sanctum* was done based on the method described by Nishimiki *et al.*[33] with modifications. About 1 mL 156 µM NBT solution in phosphate buffer (100 mM, pH 7.4), 1 mL 468 µM NADH in phosphate buffer (100 mM, pH 7.4) and 0.1 mL of various concentration of ethanolic extract of *Ocimum sanctum* and reference compounds (10, 25, 50, 75 and 100 µg) were mixed and the reaction was started by adding 100 mL 60 mM PMS in phosphate buffer (100 mM, pH 7.4). The reaction mixture was incubated at 25 ºC for 5 min and the absorbance at 560 nm was measured against reference compounds (BHT and quercetin). BHT and quercetin were used as reference compounds. All the tests were performed in triplicate and the results averaged. The percentage inhibition was calculated by comparing the results of reference compounds and test samples.

Hydroxyl radical scavenging activity: Hydroxyl radical scavenging activity was measured by studying the competition between deoxyribose and test compound (ethanolic extract of *Ocimum sanctum*) for hydroxyl radical generated by Fe^{3+}-ascorbate-EDTA-H_2O_2 system (Fenton reaction) according to the method of Kunchandy and Rao[34]. The reaction mixture contained, in a final volume of 1 mL, 100 mL of 2-deoxy-2-ribose (28 mM in KH_2PO_4-KOH buffer, 20mM, pH 7.4), 500 µL of the various concentrations of ethanolic extract of *Ocimum sanctum* and reference compound (1, 100 and 1000 µg) in KH_2PO_4-KOH buffer (20 mM, pH 7.4), 200 mL of 1.04 mM EDTA and 200 µM $FeCl_3$ (1:1 v/v), 100 µL of 1.0 mM H_2O_2 and 100 µL of 1.0 mM ascorbic acid was incubated at 37 ºC for 1 h. 1.0 mL of TBA (1 %) and 1.0 mL of TAA (2.8 %) were added to the test tubes and were incubated at 100 ºC for 20 min. After cooling, absorbance was measured at 532 nm against control containing deoxyribose and buffer. Reactions were carried out in triplicate. The percentage inhibition was determined by comparing the results of the test and reference compound (catechin).

Reducing power: The reducing power of ethanolic extract of *Ocimum sanctum* was determined by the method of Oyaizu[35]. Ten mg of ethanolic extract of *Ocimum sanctum* in 1 mL distilled water was mixed with 2.5 mL 200 µM potassium phosphate buffer, pH 6.6 and 2.5 mL 1 % potassium ferricyanide. The mixture was incubated at 50 ºC for 20 min. A 2.5-mL aliquot of 10 % TAA was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL distilled water and 0.5 mL 0.1 % $FeCl_3$ and absorbance was measured at 700 nm. BHT was used as a reference material. All tests were performed in triplicate and the graph was plotted with the average of the three determinations.

Statistical analysis: Experimental results were mean ± SD of three parallel measurements. Statistical analysis was performed according to the student's *t*-test. IC_{50} values for all the above experiments were determined by linear regression method. $p < 0.05$ were regarded as significant.

RESULTS AND DISCUSSION

Estimation of total phenolic compounds: Phenols exhibit significant antioxidant and free radical scavenging ability[36] due to the presence of hydroxyl groups[37] and effective hydrogen donating ability[38]. 1 mg of ethanolic extract of *Ocimum sanctum* was found to contain 56.7 µg pyrocatechol equivalent of phenolics. The result indicates a strong association between antioxidative activities of phenolic compounds ($r^2 = 0.9983$), suggesting that phenolic compounds are probably responsible for the antioxidative activities of ethanolic extract of *Ocimum sanctum*[39]. Thus therapeutic properties of *O. sanctum* may possibly be attributed to the antioxidant property of phenolic compounds present in ethanolic extract of *Ocimum sanctum*. It is known that plant phenolics and terpenoids constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Therefore, it was reasonable to determine their total phenolic amount in the selected plant extract.

Total antioxidant activity: Fig. 1 illustrates the antioxidative activities of various concentrations (50, 100, 250 and 500 µg/mL) of ethanolic extract of *Ocimum sanctum* and the antioxidant activity (% inhibition), 61.33, 66.21, 72.04 and 76.83 %, respectively, on the lipid peroxidation of linoleic

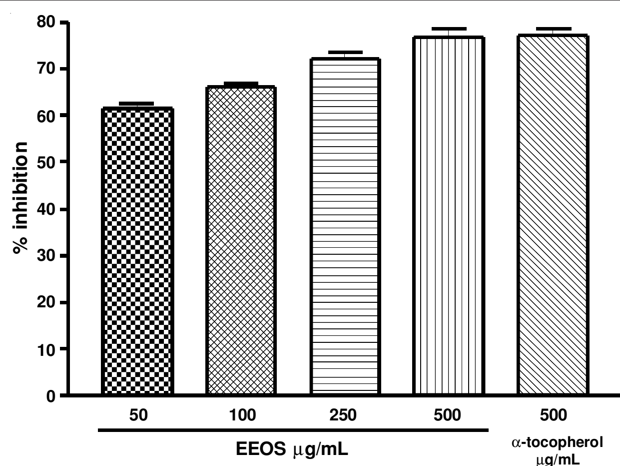

Fig. 1. Inhibition of lipid peroxidation of α-tocopherol and different doeses of ethanolic extract of *Ocimum sanctum* in the linoleic acid emulsion

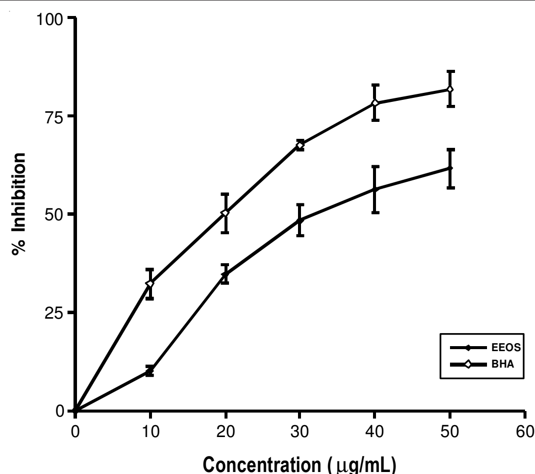

Fig. 2. Free radical scavenging activity of ethanolic extract of *Ocimum sanctum* and BHA by DPPH radicals

acid system. The inhibitory activity of ethanolic extract of *Ocimum sanctum* (500 μg/mL) was found to be equivalent to that of α-tocopherol (77.13 %) at 500 μg/mL. The IC_{50} of ethanolic extract of *Ocimum sanctum* was found to be 40.76 μg/mL. The results indicate that, ethanolic extract of *Ocimum sanctum* significantly ($p < 0.05$) inhibits the linoleic acid peroxidation. The antioxidative activity of ethanolic extract of *Ocimum sanctum* was measured using the ammonium thiocyanate method, which measured the level of peroxides, during the initial stages of lipid oxidation. The antioxidant activity of ethanolic extract of *Ocimum sanctum* might be due to the reduction of hydroperoxide, inactivation of free radicals or both. This antioxidant activity of ethanolic extract of *Ocimum sanctum* might be attributed to the presence of flavonoids and biflavones[40].

Inhibition of DPPH radical: Ethanolic extract of *Ocimum sanctum* had significant scavenging effects on the DPPH radical and the effect was found to be enhanced with an increase in the concentration between 10 and 50 μg/mL of the extract (Fig. 2). Compared with that of BHA, the scavenging effect of ethanolic extract of *Ocimum sanctum* was significantly less. The IC_{50} of ethanolic extract of *Ocimum sanctum* was found to be 31.37 μg/mL ($p < 0.01$). The DPPH radical is considered to be a model for a lipophilic radical chain reaction initiated by the lipid auto oxidation and has been widely used to evaluate the free radical scavenging capacity of antioxidants[41,42]. DPPH is relatively stable free radical and the assay determines the ability of ethanolic extract of *Ocimum sanctum* to reduce DPPH radical to the corresponding hydrazine by converting the unpaired electrons to paired ones. In addition, antioxidants are known to interrupt the free radical chain of oxidation and to donate hydrogen from phenolic hydroxyl groups, thereby, forming stable free radicals, which do not initiate or propagate further oxidation of lipids[43]. The determination of scavenging stable DPPH was robust method to evaluate the antioxidant activity of the extracts. With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm. The dose dependent inhibition of DPPH radical indicates that ethanolic extract of *Ocimum sanctum* caused reduction of DPPH radical in a stoichometric manner[44].

Inhibition of nitric oxide radical: It is known that NO has an important role in various types of inflammatory processes. The concentration of ethanolic extract of *Ocimum sanctum* needed to produce 50 % inhibition of NO release was found to be 31.83 μg/mL, whereas 7.48 μg/mL was needed for curcumin, used as a reference compound. The results were found to be statistically significant ($p < 0.05$). Fig. 3 illustrates the percentage inhibition of NO generation by ethanolic extract of *Ocimum sanctum*. It is well known that NO has an important role in various types of inflammatory processes. NO is an important chemical mediator generated by endothelial cells, macrophages, neurons, toxins, stress, *etc.* and involved in the regulation of various physiological processes[45]. Excess concentration of NO is associated with several diseases[46,47]. Oxygen reacts with the excess NO, to generate nitrite and peroxynitrite anions. Moreover, NO is implicated in several neurological disorders[48]. The extract competes with oxygen for NO and thus inhibits the generation of the anions and hence has an important protective role against neurological diseases.

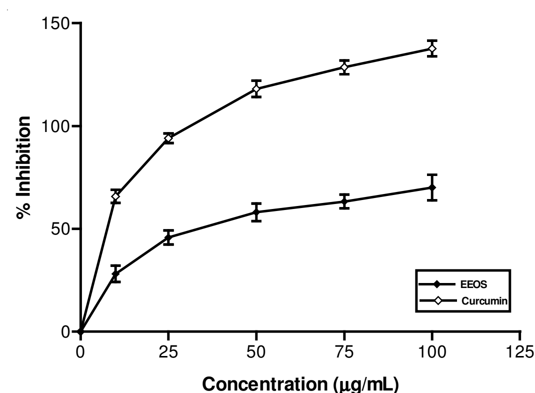

Fig. 3. Inhibition of nitrocoxide radicals by ethanolic extract of *Ocimum sanctum* and curcumin

Inhibition of superoxide anion radical: Superoxide anions indirectly initiate lipid oxidation due to superoxide and hydrogen peroxide, serving as precursors of singlet oxygen and hydroxyl radicals[49]. Robak and Glyglewski[50] reported that the antioxidant properties of flavonoids are effective, mainly

via the scavenging of superoxide anion. ethanolic extract of *Ocimum sanctum* at concentrations from 10-100 µg/mL inhibited the production of superoxide anion radicals by 39.35-75.55 % and showed a strong superoxide radical scavenging activity ($p < 0.01$). The IC_{50} of ethanolic extract of *Ocimum sanctum*, BHT and quercetin was found to be 29.17, 9.52 and 7.68 µg/mL, respectively (Fig. 4). Superoxide anions, the one-electron reduced form of molecular oxygen, is a precursor to active free radicals that have potential of reacting with biological macromolecules and thereby inducing tissue damage[51]. Dahl and Richardson[52] noted that superoxide decomposes to form stronger oxidative species, such as singlet oxygen and hydroxyl radicals, which initiate peroxidation of lipids. Meyer and Isaksen[53] reported that superoxide anion indirectly initiates lipid oxidation as a result of superoxide and hydrogen peroxide serving as precursor of singlet oxygen and hydroxyl radicals. The probable mechanism of scavenging the superoxide anions may be due to the inhibitory effect of ethanolic extract of *Ocimum sanctum* towards generation of superoxide in *in vitro* reaction mixture, supposed to be one of the major mechanisms contributing to their antioxidant capacity. Recent studies have shown that phenolic compounds, particularly flavonoids and terpenoids, are important antioxidants and superoxide scavengers. Their scavenging efficiency depends on the concentration of phenolics and the numbers of locations of the hydroxyl groups[54].

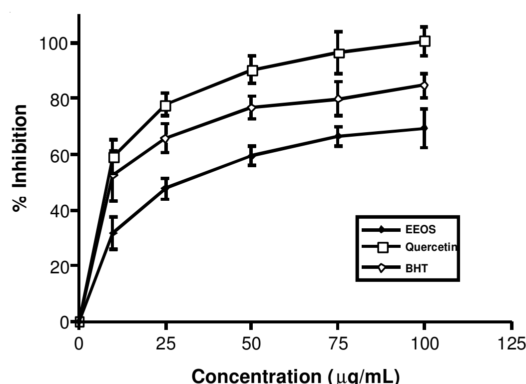

Fig. 4. Superoxide anion scavenging activity of ethanolic extract of *Ocimum sanctum*, quercetin BHT

Inhibition of hydroxyl radical: Ethanolic extract of *Ocimum sanctum* was found to exhibit dose dependent hydroxyl radical scavenging activity (Fig. 5) and the IC_{50} was found to be 400.90 µg/mL ($p < 0.05$). Epicatechin (IC_{50}: 24.5 µg/mL) used as a reference compound, is highly effective in inhibiting the oxidative DNA damage. The Fenton reaction generates hydroxyl radicals (OH^{-}) which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products. Attack at a sugar ultimately leads to sugar fragmentation, base loss and strand break with a terminal fragmented sugar residue[55]. Addition of low concentration of transition metal ions such as iron to DNA causes degradation of the sugar into MDA and other related compounds, which from a pink chromogen with TBA reactive substances[56,57]. When ethanolic extract of *Ocimum sanctum* and epicatechin, were added to the reaction

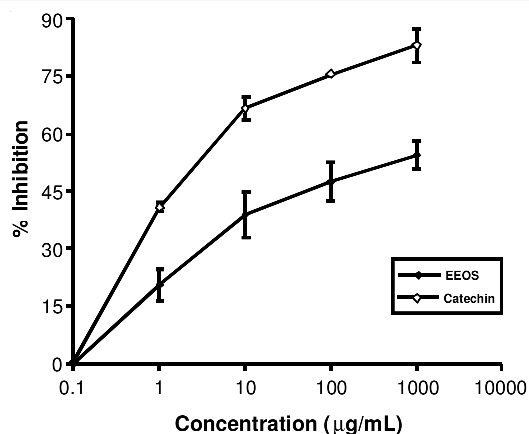

Fig. 5. Hydroxyl radical scavenging activity of ethanolic extract of *Ocimum sanctum* and epicatechin on deoxyribose damage

mixture they removed hydroxyl radicals from the sugar and prevented degradation. Hydroxyl radicals have been implicated as highly damaging species in free radical pathology. Ferrous salts can react with H_2O_2 and form hydroxyl radical *via* Fenton's reaction. The iron required for this reaction is obtained either from the pool of iron or the heme-containing-proteins[58]. The hydroxyl radical thus produced may attack the sugar of DNA base causing sugar fragmentation, base loss and DNA strand breakage[55], which contributes to cytotoxicity, mutagenesis, carcinogenesis, *etc*. In addition, this species is considered to be one of the initiators of lipid peroxidation process.

Reducing power: It has been demonstrated that the power of certain antioxidant is associated with the reducing power of their atoms, which is associated with the presence of reductones. During the reducing power assay, the presence of reductants (antioxidants) in the tested samples would result in reducing Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}). The Fe^{2+} can therefore be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The reducing power of ethanolic extract of *Ocimum sanctum* and reference compound (BHT) increased with an increase in the concentration and it was found to be 1.155 and 0.771 at 500 µg/mL, respectively (Fig. 6). All doses of ethanolic extract of *Ocimum sanctum* showed higher activities than BHT and the differences were significant ($p < 0.01$). The antioxidant activity of the herbal preparations or phenolic compounds may be attributed to concomitant reducing power[59,60] and may serve as a significant indicator of its potential antioxidant activity[61]. The reducing property of ethanolic extract of *Ocimum sanctum* implies that it is capable of donating hydrogen atom in a dose dependent manner. A substance may act as an antioxidant due to its ability to reduce ROS by donating hydrogen atom[62]. The high content of phenolic compounds in the extract may be a contributing factor towards antioxidant activity due to the presence of hydroxyl groups, which can function as hydrogen donor[63,64].

Conclusion

The results revealed potent antioxidant activity, reducing power and free radical scavenging activities against DPPH radical, nitric oxide radical, superoxide anion and hydroxyl radical of ethanol extract of *O. sanctum* (EEOS). Using the optimized chromatographic conditions (HPLC and LC-MS), the quantity of rosmarinic and ursolic acid in ethanolic extract

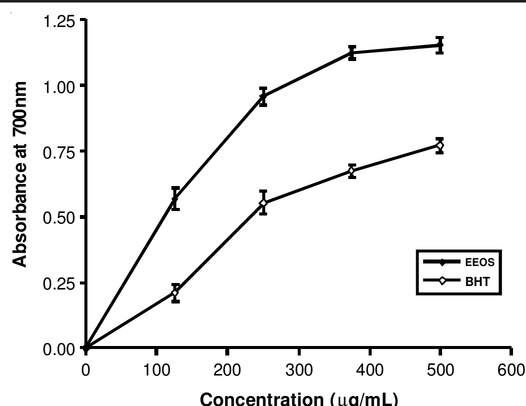

Fig. 6. Reducing power of ethanolic extract of *Ocimum sanctum* and BHT

of *Ocimum sanctum* was found to be 0.27 and 0.40 % w/w, respectively. Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals[58,65]. It is reported that phenolic compounds are natural products which have been shown to possess various biological properties related to antioxidant mechanisms. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals. Thus, in the present study, the antioxidant potential of standardized ethanolic extract of *Ocimum sanctum* may be attributed to the presence of abundant quantities of phenolic compounds like rosmarinic acid and ursolic acid.

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