***Original Research Article***

# Free Radicals Scavenging and Protein Protective Property of *Ocimum Sanctum* (L)

**Abstract**

*Ocimum sanctum* (L) is a well-known herbal plant used for the treatment of bronchitis, malaria, diarrhea, dysentery, skin disease, arthritis, eye diseases, insect bites. It is proposed to have anticancer, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic and antispasmodic action. In our present study, we have tried to explore its antioxidant, free radical scavenging and its ability to prevent damage to protein from free radicals. The antioxidant and free radical scavenging activity of *Ocimum sanctum* leaves was determined using ferric thiocyanate, thiobarbituric Acid, 2, 2-diphenyl-1–picrylhydrazyl, hydrogen peroxide, hydroxyl radical and superoxide radical scavenging methods. Further, its protein protective property was evaluated using UV- Vis spectrophotometer methods. The results of ferric thiocyanate and 2, 2-diphenyl-1–picrylhydrazyl methods were determined that *O. sanctum* have strong antioxidant activity (92.12 %) and free radicals scavenging property (50.74 %) respectively. Further, hydrogen peroxide, hydroxyl radical and superoxide radical scavenging methods determined that extracts (100 µg/ml) have hydrogen peroxide (20.12 %), hydroxyl radicals (12.68 %) and superoxide radicals (21.68 %) scavenging activity respectively. Hemoglobin is a standard protein which was damaged by hydrogen peroxide treatment and this damage was confirmed by UV-Vis spectrophotometer. The extract showed the significant pre and post protein protective action against hydrogen peroxide induced hemoglobin damage. Thus the study concluded that *O. sanctum* leaves have strong antioxidant, free radicals scavenging and pre and post proteins protective property.

**Keywords:** Oxidants, Antioxidants, FTC, DPPH, Hemoglobin, *Ocimum sanctum* (L).

## Introduction

In organisms, including humans, free radicals are generated during metabolic and immune system functions [1]. Reactive oxygen species (ROS) have beneficial role at certain concentration in phagocytosis, apoptosis, and necrosis. When concentration of ROS increases beyond a certain limits, it will damage the biomolecules such as DNA, RNA, proteins, lipids, carbohydrates and poses serious problems in humans [2]. In the humans various ROS such as hydroxyl radical, superoxide, and hydrogen peroxide are generated. Due to longer longevity and compartmentalisation of hydrogen peroxide it can react with biomolecules which contain transition metals ($Cu^{+}$ , $Fe^{++}$) and form hydroxyl radicals via Fenton's reaction [3-7].

$$Fe^{+2} + H_2O_2 \longrightarrow {}^{\cdot}OH + OH^{-} + Fe^{+3}$$ (Fenton's reaction) [1-3]

Antioxidants are those substances which neutralize the effect and action of free radicals [8]. In the humans, several endogenous antioxidants are present (superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, thioredoxin, Vitamin E, Vitamin C, carotenoids, flavonoids and related polyphenols, α-lipoic acid, and glutathione) which protect the biomolecules from free radical injuries [9-10]. It is present in natural sources such as leaves, fruits, and roots of various plants. Some Indian medicinal plants such as *Aegle marmelos, Allium cepa, Allium sativum, Aloe vera, Azadirchata indica, Camellia sinensis, Cinnamomum verum, Curcuma longa and Ocimum sanctum* have antioxidant properties [11] and therefore scavenge the free radicals from the human body and protect them from cell injuries [12].

Imbalance between production of free radicals and antioxidants causes oxidative stress [13] Some researches have proved that oxidative stress enhances or develops the disease in humans,

such as Parkinson's disease [14-15], atherosclerosis [16], Alzheimer's disease [17], major depression [18], diabetic nephropathy, end stage renal disease [19], cardiovascular disease [20], non ulcer dyspepsia [21] and so forth.

The plants of genus *Ocimum* belongs to family Labiatae. It has two species , *Ocimum sanctum* Linn. (Krishna Tulasi) and *Ocimum gratissium* (Ram Tulasi) and the leaves are black and green in color respectively [22]. *O. sanctum* (L) contains some important compounds such as linalool, eugenol, methyl charicol and cineole. It has the antioxidants property due to presence of eugenol [23]. Several studies have proved that *Ocimum sanctum* contains therapeutic property to treat the gastrointestinal [24, 25], cardiovascular [26-27], antitumor [28], antifertility [29] and anti-inflammatory [30] diseases. Thus in our present study, we tried to explore the antioxidant, free radical scavenging activity of *Ocimum sanctum* leaves and its protein protective property, pre and post the exposure to damage caused by hydrogen peroxide of protein. Hemoglobin was used as the standard protein.

**Materials and Methods:**

All the reagents were purchased from Sigma Aldrich India.

**Plant material:**

Fresh *O. sanctum* leaves were obtained from Botanical garden of Banaras Hindu University, Varanasi. Leaves were dried under shade at room temperature for one week and powdered using the grinder. The powder was stored in the desiccator for further use.

**Preparation of plant extract:**

5 % aqueous extract of *O. sanctum* was prepared by adding 5 grams leaves powder in the Erlenmeyer flask containing 100 ml deionized water and this mixture was heated for 2 hours on magnetic stirrer at 70 $^{o}$C. Extracts were obtained by centrifuging the filtrates at 5000 rpm for 15 min followed by filtrations using whatman No. 1 filter paper. This filtrate was concentrated by evaporation at 40°C and stored at 4 $^{0}$C for further use [31].

**Antioxidant Assay**

The total antioxidant activity of extract was tested by two methods, ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. FTC method was used to determine the peroxide level in the beginning of lipid peroxidation whereas TBA method used to determine the free radicals present after peroxide oxidation.

**Ferric thiocyanate (FTC) method**

FTC method was performed according to Kikuzaki and Nakatani methods (1993) [32]. 4 mg *O. sanctum* leaves was dissolved in 4 ml absolute ethanol and mixed with 4.1 ml linoleic acid (2.5 % in absolute ethanol), 8 ml of phosphate buffer (50 mM, $P^{H}$ 7.0), and 3.9 ml of distal water to make final volume 20 ml and stored in dark oven at 40 $^{0}$C. 100 µl of this mixture was added to 9.7 ml ethanol (75 %), 100 µl ammonium thiocyanate (30 %), 100 µl ferrous chloride (20mM in 3.5 % HCl). Exactly after 3 min the absorbance of red color was determined at 500 nm using UV- Vis spectrophotometer (Systronics, AU-2701). The mixture was stored in dark oven at 40 $^{0}$C and the absorbance of mixture was determined after every 24 h for 7 days.

Butylated Hydroxy Toluene (BHT) was used as positive control while the mixture without leaves powder used as negative control and absolute ethanol used as blank. The lipid peroxidation inhibition was calculated as follows:

$$Percentage\ inhibition = 100 - [\frac{ZA}{ZS} \times 100]$$

Where ZA and ZS represent the absorbance of sample and control respectively.

**Thiobarbituric Acid (TBA) method**

TBA method was used according to Ottolenghi (1959) [33]. 1 ml of sample solution (as prepared in FTC method) was mixed to 2 ml of trichloroacetic acid (20 %) and 2 ml of thiobarbituric acid (0.67 %). This mixture was placed in water bath at 100 $^0$C for 10 min followed by centrifugation at 3000 rpm for 20 min and the absorbance of supernatant were determined at 552 nm using UV-Vis spectrophotometer (Systronics, AU-2701). The antioxidant activity was based on the absorbance on the final day of FTC method (i.e. for 7 days).

**Free radical scavenging activity**

**2, 2-diphenyl-1–picrylhydrazyl (DPPH) method**

The method was performed according to Burits and Bucar methods (2000) [34]. Free radicals scavenging activity of *O. sanctum*, BHT and Vitamin C was determined with the help of stable radical DPPH in terms of hydrogen atom or electron or radical scavenging activity. 0.1 ml of different fraction at different concentration of leaves powder (25-500 µg/ml in ethanol) was mixed with 4 ml DPPH (0.004 % in methanol) solution.

Incubate at room temperature for 30 minutes and the absorbance was determined at 517 nm using UV- Vis spectrophotometer (Systronics, AU-2701) against blank. Lower the absorbance of sample indicates the higher free radical scavenging activity. BHT and Vitamin C was used as

standard while methanol was used as blank solution. The scavenging activity of *O. sanctum* was determined (based on % of DPPH radical scavenged) by the following formula:

$$Percentage\ scavenging = \frac{Zp - Zq}{Zp} \times 100$$

Where Zp is absorption of control (all the reagent except the test sample) while Zq absorption of test/ standard sample.

**Hydrogen peroxide ($H_2O_2$) scavenging activity**

$H_2O_2$ scavenging activity was determined according to the method by Ruch et al (1989) [35]. 2 mM $H_2O_2$ solution was prepared in phosphate buffer (50mM, pH 7.4). 100 µl of different concentration (25- 250µg/ml in 50mM phosphate buffer, pH 7.4) of *O. sanctum* solution was added to 300µl phosphate buffer (50 mM, pH 7.4). 600 µl $H_2O_2$ solution was added to it and the mixture was vortexed. The absorbance was determined at 230 nm against blank using UV- Vis spectrophotometer (Systronics, AU-2701) after 10mins. Vitamin C was used as standard and phosphate buffer (50mM, pH 7.4) used as blank. The $H_2O_2$ scavenging activity was calculated as:

$$Percentage\ scavenging\ = \frac{As - Az}{As} \times 100$$

Where As is the absorbance of control (all the reagent except the test sample) and Az absorbance of test sample/ standard.

**Hydroxyl radical ($OH^-$) scavenging activity**

The effect of *O. sanctum* on $OH^-$ radicals was determined according to the Khan et al; method (2012) [31]. 75µl *O. sanctum* solution (50-250 µg/ml in methanol), 450µl sodium phosphate

buffer (200mM, PH 7.0), 150µl 2-deoxyribose (10mM), 150µl FeSO4-EDTA (10mM), 150µl $H_2O_2$ (10mM) and 525 µl distilled water were mixed. This mixture was incubated at 37 $^0$C for 4 hour. The reaction was stopped by the addition of 750 µl trichloroacetic acid (2.8 %) and 750 µl TBA (1 % in 50 mM NaOH solution). The mixture was placed in boiling water bath for 10 min and cooled by tap water. The absorbance of solution was determined at 520 nm. Vitamin C was used as standard while methanol was used as blank. The hydroxyl radical scavenging activity was calculated as:

$$Percentage\ scavenging = \frac{Zr - Zs}{Zr} \times 100$$

Whereas Zr is the absorbance of control (all the reagent except the test sample) and Zs, absorbance of test/standard sample.

**Superoxide ($O_2^-$) radical scavenging activity**

Superoxide scavenging activity of *O. sanctum* was determined according to the Liu et al; method (1997) [36]. Superoxide radicals are generated in Nicotinamide adenine dinucleotide (NADH) – phenazinemethosulphate (PMS) system by oxidation of NADH and it was determined by the reduction of Nitro Blue tetrazolium (NBT). 200 µl of *O. sanctum* (100-500µg/ml in methanol), 1ml of 16 mM Tris- HCl buffer (pH-8), 1ml of 50 µM NBT, 1 ml of 78µM NADH, and 1ml of 10 µM PMS solution were mixed. The reaction mixture was kept at 25 $^0$C for 5 min. absorbance was determined at 560 nm using UV- Vis spectrophotometer (Systronics, AU-2701). Vitamin C was used as standard antioxidant. Percentage inhibition of superoxide generation was calculated as:

$$\% \, Inhibition = \frac{Zs - Zt}{Z1} \times 100$$

Whereas Zs is the absorbance of control (the entire reagent except the sample), Zt is absorption of test sample/standard.

**Determination of Total phenolic content:**

Total phenolic content of leaves powder was determined according to the Khan et al; method (2012) [31]. Calibration curve was prepared by mixing 1ml Gallic acid at different concentration (25-400 µg/ml) with 5ml Folin-Ciocalteu reagent (1:10 dilution). The mixture was kept for 5 min at room temperature and then 4ml sodium carbonate (115µg/ml) was added. The absorbance was determined at 765 nm. 1 ml of *O. sanctum* solution of different fraction at different concentration (0.5- 5 mg/ml) was mixed with reagents described above. After 2 hour the absorbance was measured at 765 nm to determine the total plant phenolic content. Total phenolic content in *O. sanctum* was expressed as Gallic acid equivalent (GAE) mg/g of the dry extract.

**Protein protective property of *O. sanctum*:**

**Spectral analysis:**

Protein protective property of a substance was determined according to Ali et al; method (2010) [37]. Hemoglobin solution, *O. sanctum* extract and mixture (hemoglobin, $H_2O_2$ and *O. sanctum)* were prepared followed by spectral analysis to determine the normal hemoglobin peaks. Effect of *O. sanctum* on hemoglobin damaged by $H_2O_2$ was measured in the range of 350 – 500 nm using UV-Vis spectrophotometer (Systronics, AU-2701) in time dependent manner (1-60 min).

## Results

**Antioxidant assay:**

FTC and TBA results proved the antioxidant activity of *O. sanctum* leaves extracts (Table-1). The extract showed the strong antioxidant activity which represented by lower absorbance value. *O. sanctum* showed the higher antioxidant property against the standard antioxidant BHT as 92.12 % and 95.36 % respectively (Fig 1 and Fig 2).

**Free radicals scavenging:**

**DPPH scavenging activity**

The result of DPPH determined the total free radicals scavenging activity of *O. sanctum* and standard BHT was 50.74 % and 49.94 % respectively.

**Hydroxyl radical scavenging assay**

*O. sanctum* and BHT (100 µg/ml) has 12.68 % and 9.55% hydroxyl radicals scavenging activity respectively.

**Hydrogen peroxide scavenging activity**

*O. sanctum* and Vitamin C (100 µg/ml) determined the 20.12 % and 10.51 hydrogen peroxide scavenging. (Table and Fig 3)

**Superoxide radicals**

*O. sanctum* and Vitamin C (100 µg/ml) have 21.95 % and 19.51 superoxide radicals scavenging activity (Table & Fig 3).

**Phenolic content:**

The result proved that *O. sanctum* leaves extract contained the total phenolic content was 200 mg GAE/g dry extract (Table).

**Spectral analysis:**

The results of spectral analysis are represented in the Figure-4 showing the spectra of normal hemoglobin (Fig: 4A) and hemoglobin treated with $H_2O_2$ (Fig: 4B). Figure-5 shows normal peaks of aqueous extract of *O. sanctum* leaves (Fig: 5A) and the effect of $H_2O_2$ on *O. sanctum* extract (Fig: 5B). Figure-6 shows the scavenging (Fig: 6A), and protective effect of *O. sanctum* leaves (Fig: 6B) against $H_2O_2$ damaging.

**Discussion:**

Oxidative stress develops with the imbalance between the oxidants and antioxidants. The major oxidative factors are free radicals ($H_2O_2$, $^{\cdot}OH$, $O_2^{-}$) which damage the biomolecules such as DNA, proteins and lipids [1]. In the present study we have observed that the aqueous extract of *Ocimum sanctum* (L) leaves have strong antioxidant activity as compared with BHT on the final day of reaction ($7^{th}$ days) (Figure 1 & 2 ). *O. sanctum* has more antioxidant activity as compared with BHT from starting day to fourth day of reaction after that negligible changes were observed. The percentage of free radicals scavenging property of O. sanctum and vitamin C were 50.74 and 49.94 respectively. This scavenging property of *O. sanctum* was slightly greater than vitamin C (Table1). The percentage of hydrogen peroxide scavenging activity of O. sanctum and vitamin C was 20.12 and 10.52 respectively. The percentage of superoxide scavenging activity of O. sanctum and vitamin C was 21.95 and 19.51 respectively. The percentage of hydroxyl radicals scavenging activity of O. sanctum and vitamin C was 12.68 and 9.55 respectively. These values confirmed that the *O. sanctum* have greater free radicals scavenging activity as compared with vitamin C (Table1 and Figure 3). Spectral analysis confirmed that the normal hemoglobin has peak at 410 nm while the peak was quenched and shifted from 410 to 420 nm when

damaged by hydrogen peroxide (Figure 4) while the extract property was not affected by hydrogen peroxide (Figure 5). Further, the pre addition of extract have more protein protective role against hydrogen peroxide while post addition of extract have less protein protective activity and they were sifted peaks towards their original place (Figure 6). This was confirmed that the pre addition of extract (before the treatment of hydrogen peroxide to hemoglobin) given better protein protective as compared to post addition of extract (after the treatment of hydrogen peroxide to hemoglobin). It is well accepted in literature that the phytocompounds have medicinal value that having protecting properties against free radicals injuries [35], anti cancer [36] induced apoptosis in precancerous and cancerous cells [37-38], hypoglycemic [39], and prevent liver, kidney and brain injury and protect against the genetic, immune and cellular damaged caused by pesticides, pharmaceuticals and industrial chemicals waste [40]. Thus, it seems that this antioxidants, free radicals and pre and post proteins protective properties of *O. sanctum* leaves might be due to presence of phytocompounds, but it needs more explorative study for better clarification.

**Conclusion**

Hydrogen peroxide is an oxidant with its free radical and reactive nature. Due to these properties it is able to denature the hemoglobin protein. Present study revealed that the *O. sanctum* leaves extracts diminished the hydrogen peroxide hemoglobin denaturation and having free radical scavenging property. Thus, it is concluded that the leaves extract of *O. sanctum* have antioxidant and hemoglobin protective property.

**Results**

**Table1:** Table shows the FTC, TBA, DPPH, percentage of $H_2O_2$, $O_2^-$, $OH^-$ scavenging activity of sample (*O. sanctum*) and standard (ascorbic acid, BHT) and total phenolic content of *O. sanctum.*

| S.N | Sample/ Standard | FTC (%) | TBA (%) | DPPH (%) | Percentage of $H_2O_2$ scavenging (100µg/ml) | Percentage of $O_2^-$ scavenging (100µg/ml) | Percentage of $OH^-$ scavenging (100µg/ml) | Total Phenolic Content |
|---|---|---|---|---|---|---|---|---|
| *1* | *O. sanctum* | 92.12 | 92.90 | 50.74 | 20.12 | 21.95 | 12.68 | 200 mg GAE/g dry extract |
| 2 | Ascorbic Acid | ---- | ---- | 49.94 | 10.52 | 19.51 | 9.55 | ---- |
| 3 | BHT | 95.36 | 95.68 | ---- | ---- | ---- | ---- | ---- |

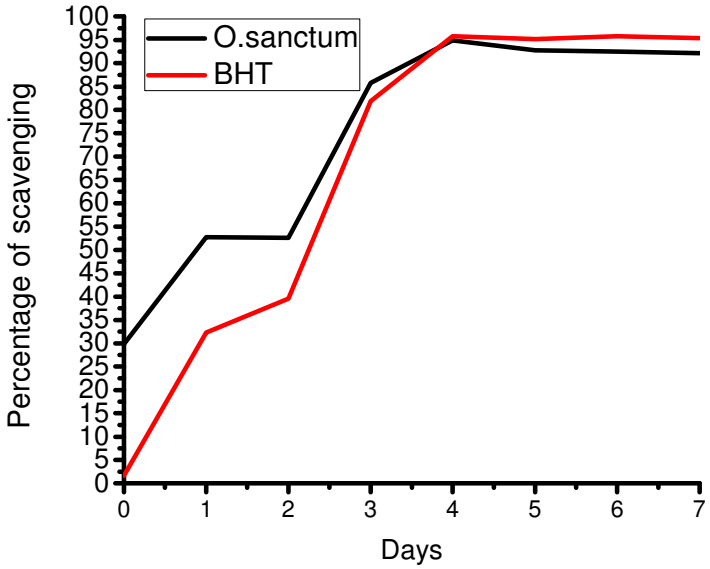



Figure 1: Antioxidant property of *O. sanctum* and BHT by FTC method.

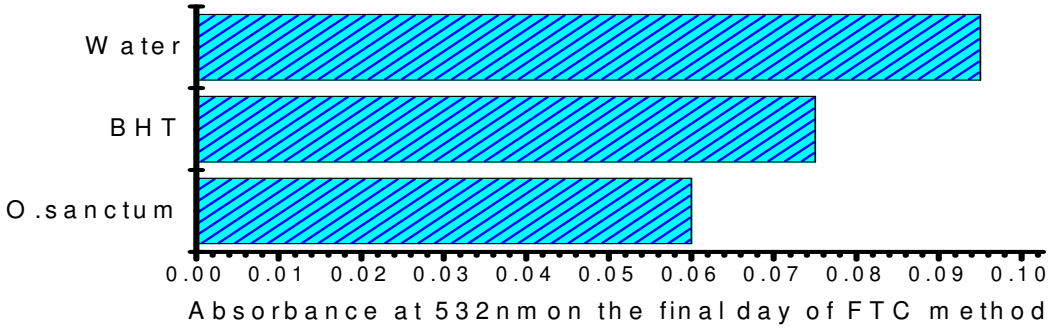



Figure 2: Antioxidant activity of *O. sanctum* and BHT by TBA method.

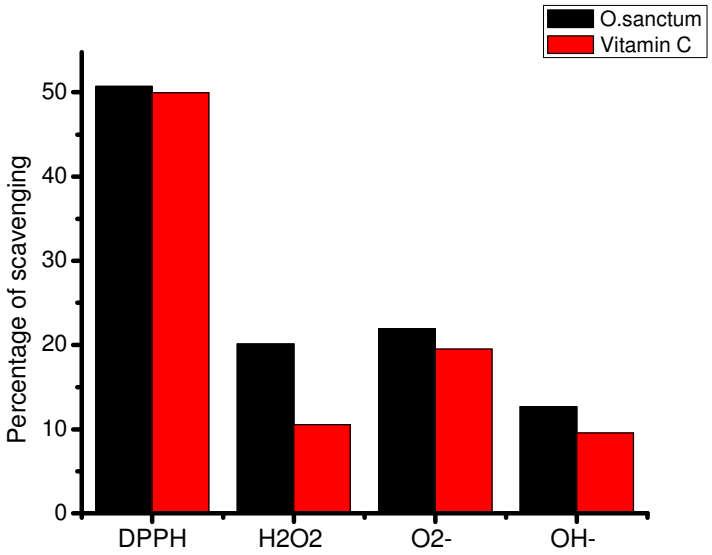
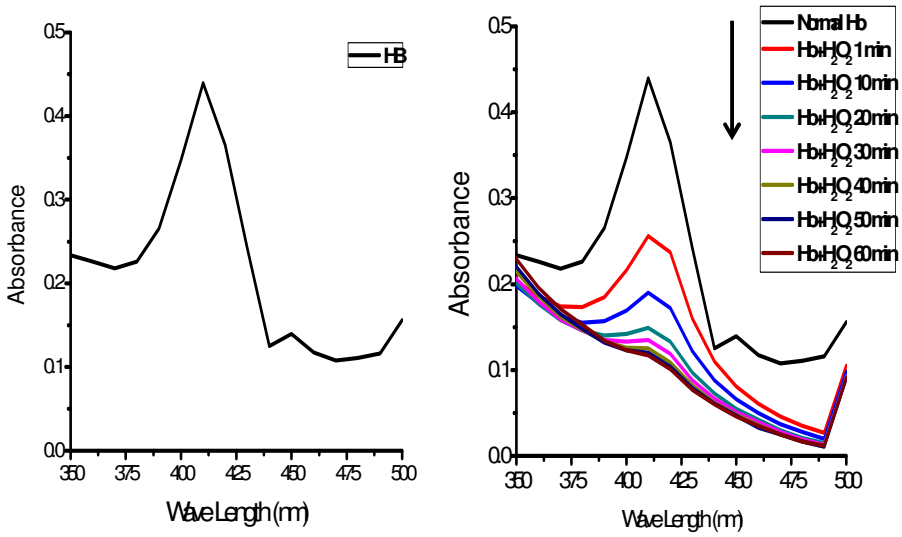
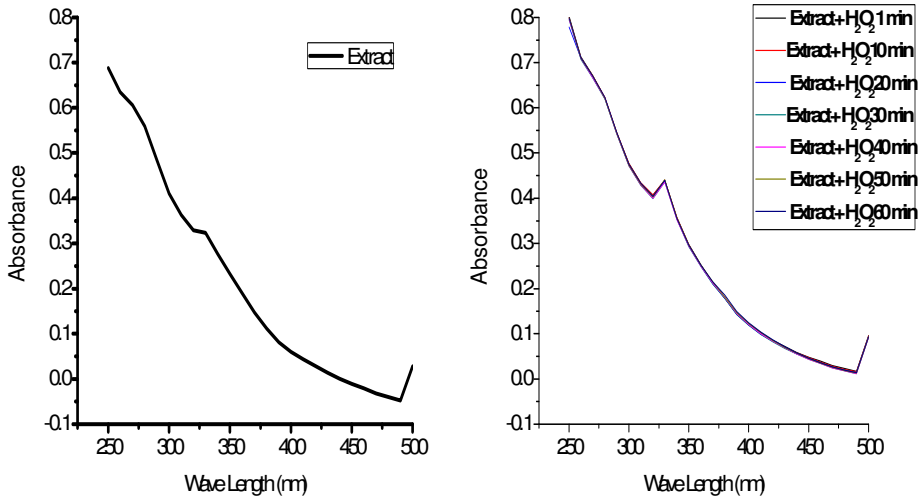



Figure 3: Total free radicals and individual free radicals ($H_2O_2$, $O_2^{\cdot-}$, $OH^-$) scavenging property of *O. sanctum* and vitamin C.



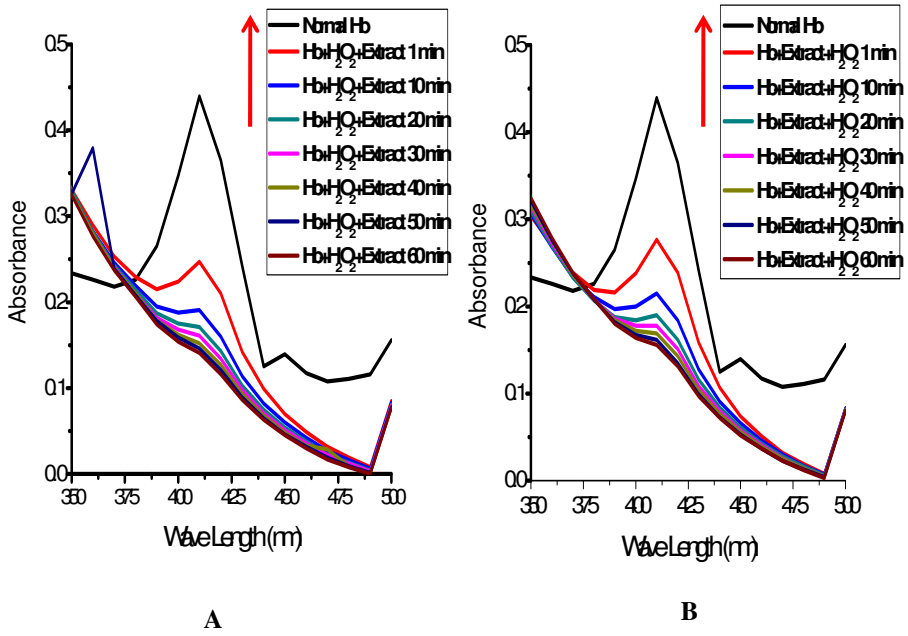

A B

**Figure 4**: Spectra showing normal hemoglobin peaks (Fig-4A) and hemoglobin treated with $H_2O_2$ in 1-60 min (every 10 minute of intervals, Fig-4B).




A B

**Figure 5:** Spectra showing normal aqueous extract of *O. sanctum* peaks (Fig-5A) and effect of $H_2O_2$ on aqueous extract of *O. sanctum* (Fig-5B) in 1-60 min of every 10 minutes of intervals.




**A** **B**

**Figure 6:** Spectra showing scavenging effect of *O. sanctum* extract against $H_2O_2$ (Fig-6A) and protective effect of *O. sanctum* extract against $H_2O_2$ (Fig-6B) in 1-60 min of per 10 minutes of intervals.

**References**

1. Keshari AK, Verma AK, Kumar T, Srivastava R. Oxidative stress: A Review. The International Journal of science &Tehnoloedge. 2015; 3, 1-7.

2. Keshari AK, Farooqi H. Evaluation of the Effect of Hydrogen Peroxide ($H_2O_2$ ) on hemoglobin and the protective effect of glycine. 2014; 2, 1-2.

3. Thomson DL. The effect of hydrogen peroxide on the permeability of the cell. 1927; 1, 252-257.

4. Wu HC, Chen HM, Shiau CY. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (Scomberaustriasicus). Food Research International. 2003; 36, 949–957.

5. Apel K , Hirt H. Reactive oxygen species : Metabolism, Oxidative Stress, and Signal Transduction. Annual Review of Plant Biology. 2004; 55, 373–99.

6. Halliwell B, Clement MV, Long LH. Hydrogen peroxide in the human body. Minireview. FEBS Letters. 2000; 486,10-13.

7. Stedman ER, oliver CN. Metal catalyzed oxidation of protein: Physiological consequence, minireview. Journal of Biological chemistry. 1991; 266, 2005-2008.

8. Halliwell B. Free Radicals and other reactive species in Disease. Encyclopedia of life science. Nature Publishing Group. 2001;1-7.

9. Gulcin I, Kufrevioglu OI, Oktay M, Buyukokuroglu ME. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (*Urticadioica* L.). Journal of Ethnopharmacology . 2004; 90, 205–215.

10. Rahal A, Kumar A, Singh V, et al. Oxidative Stress, Prooxidant, and Antioxidants: The Interplay. BioMed Research International. 2014;7, 1-19.

11. Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD. Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. J Assoc Physicians India. 2004; 52, 794-804.

12. Pal RS, Ariharasiva kumar G, Girhepunje K, Upadhyay A. In Vitro antioxidative activity of Phenolic and Flavonoids compounds extracted from seeds of abrus precarorius. International journal of pharmacy and pharmaceutical science. 2009; 1,136-140.

13. Ruch RJ, Cheng SJ, Klaunig JE.Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989 ;10, 1003-1008.

14.Verma AK, Raj J, Sharma V, Singh TB, Srivastava S, and Srivastava R, "Plasma Prolidase Activity and Oxidative Stress in Patients with Parkinson's Disease," Parkinson's Disease. 2015; 1-6.

**15.**Verma AK, Keshari AK, Raj J, et al. ProlidaseAssociated Trace Elements (Mn, Zn, Co, and Ni) in the Patients with Parkinson's Disease. Biological Trace Element Research. 2016; 171, 48-53.

**16.** Upston JM, Kritharides L, Stocker R "The role of vitamin E in atherosclerosis" Progress in Lipid Research. 2003; 42, 405-422.

**17.** Atwood CS, Perry G, Zeng H, et al. Copper mediates dityrosine cross-linking of Alzheimer's amyloid-beta" Biochemistry. 2004; 43, 560-568.

18. Bajpai A, Verma AK, Srivastava M, Srivastava R. Oxidative Stress and Major Depression. Journal of Clinical and Diagnostic Research.. 2014; 8, 4-7.

19.Verma AK, Chandra S, Singh RG, Singh TB, Srivastava S, Srivastava R. Serum Prolidase Activity and Oxidative Stress in Diabetic Nephropathy and End Stage Renal Disease: A Correlative Study with Glucose and Creatinine. Biochemistry Research International. 2014; 8, 1-7.

**20.** Singh U, Jialal I. "Oxidative stress and atherosclerosis"Pathophysiology. 2006; 13, 129-42.

21. Kumari S, Verma AK, Rungta S, Mitra R, Srivastava R, Kumar N. Serum Prolidase Activity, Oxidant and Antioxidant Status in Nonulcer Dyspepsia and Healthy Volunteers. ISRN Biochemistry. 2013; 1-6 .

22. Prakash P, Gupta N. Therapeutic use of *Ocimum sanctum Linn* (Tulasi) with a note on eugenol and its Pharmacological action: A short Review" Indian J PhysiolPharmacol.2005; 49, 125–131.

23. Gupta RK, Chawla P, Tripathi M, Shukla AK, Pandey A. Synergisticantioxidant activity of tea with ginger, black paper, and Tulasi" International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6, 477-479.

24. Shukla A, Kaur K, Ahuja P. Tulsi the Medicinal Value. Online International Interdisciplinary Research Journal.2013; 3, 9-14.

25. Pattanayak P, Behera P, Das D. Panda SK. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacognosy Review. 2010; 4, 95–105.

26. Sarkar A, Pandey DN, Pant MC. Changes in blood lipid profile level after administration of *Ocimum sanctum* (Tulasi) leaves in normal albino rabbits, Indian J Physiology Pharmacology. 1994; 38, 311-312.

27. Lokhande PD, Jagdale SC & Chabukswar AR. Natural remedies for heart diseases. Indian Journal of Traditional Knowledge. 2006; 5, 420-427.

28. Prakash J, Gupta SK, chemo preventive activity of *Ocimum sanctum* seed oil. J Ethanopharmacol 2000; 72, 29-34.

29. Batta SK, Santha Kumari G, The anti-fertility effect of *Ocimum sanctum* and *Hibiscus rosasinensis*. Indian Journal of Medical Research. 1970; 59, 777-781.

30. Gupta SK, Prakash J, Srivastava S. Validation of claim of Tulasi, *Ocimum sanctum* Linn as a medicinal plant. Indian J Experimental Biology 2002; 40, 765-773.

31. Khan RA, Khan MR, Sahreen S, Ahmed M. Evaluation of phenolic contents and antioxidant activity of various solvent extracts of Sonchusasper (L.) Hill. Chemistry Central Journal. 2012; 6, 1-7.

32.Kikuzaki H, Nakatani N. Antioxidant effects of some ginger constituents. Journal of Food Science 1993; 58, 1407-1410.

33. Ottolenghi A. Interaction of ascorbic acid and mitochondria lipides. Archives of Biochemistry and Biophysics.1959; 79, 355-363.

34. Burits M, Bucar F. Antioxidant activity of *Nigella sativa*essential oil. Phytotherapy Research. 2000; 14, 323-328.

35. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechin isolated from Chinese green tea. Carcinogenesis. 1989; 10, 1003-1008.

36. Nishikimi M, Rao NA, Yagi K: The occurrence of superoxide anion in thereaction of reduced phenazine methosulfate and molecular oxygen. Biochemical and Biophysical Research Communications.1972; 46,849-854.

37. Ali S, Farooqi H, Prasad R, Naime M, et al. Boron stabilizes peroxide mediated changes in the structure of heme proteins" International Journal of Biological Macromolecules . 2010;47, 109–115.

38. Panda VS, Naik SR. Evaluation of cardio protective activity of Ginkgo biloba and *Ocimum sanctum* in rodents. Altern Med Rev. 2009; 14, 161–71.

39. Sethi J, Sood S, Seth S, Talwar A. Evaluation of hypoglycemic and antioxidant effect of *Ocimum sanctum. Indian Journal of Clinical Biochemistry; 2004; 19, 152-155.*

40. Siddique YH, Ara G, Beg T, Afzal M. Anti-genotoxic effect of *Ocimum sanctum* L. extract against cyproterone acetate induced genotoxic damage in cultured mammalian cells. Acta Biol Hung. 2007; 58, 397–409.

40. Jha AK, Jha M, Kaur J. Ethanolic extracts of *Ocimum sanctum*, *Azadirachtaindica* and *Withania somnifera* cause apoptosis in SiHa cells. Res J Pharm Biol Chem. 2012; 3, 57–62.