Pharmacognostic, Phytochemical and Antioxidant Study of *Ocimum sanctum* in Oxidative Stress Related Disorders

Miss Ghadge Punam Ramesh[1], Miss Shaikh Sanobar Illiyas[2]
[1,2]*Shri Laxmanrao Trymbakrao Nakade Pharmacy College Dhorjalgaon Ne*

Abstract- Oxidative stress is a major contributing factor in the development of several chronic and degenerative diseases such as diabetes, cardiovascular disorders, neurodegenerative diseases, cancer, and inflammatory conditions. It occurs due to an imbalance between the production of free radicals and the antioxidant defense system of the body. Medicinal plants rich in natural antioxidants play an important role in neutralizing free radicals and reducing oxidative damage.

***Ocimum sanctum* (Holy Basil), commonly known as Tulsi, is an important medicinal plant widely used in traditional medicine for the treatment of various disorders. It is known to possess antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, and adaptogenic properties. The present study was aimed to carry out pharmacognostic, phytochemical, and antioxidant evaluation of *Ocimum sanctum* leaves to scientifically validate its role in oxidative stress related disorders. Pharmacognostic studies included macroscopic and microscopic evaluation. Phytochemical screening was performed to identify major bioactive constituents. Antioxidant activity was evaluated using standard in vitro methods such as DPPH radical scavenging assay and hydrogen peroxide scavenging assay. The results revealed the presence of important phytoconstituents such as flavonoids, phenolics, tannins, alkaloids, and saponins along with significant antioxidant activity. The study concludes that *Ocimum sanctum* is a rich source of natural antioxidants and can be useful in the management of oxidative stress related disorders.**

Keywords-Ocimum sanctum, Tulsi, Pharmacognostic study, Phytochemical screening, Antioxidant activity, Oxidative stress

I.INTRODUCTION

Oxidative stress is a pathological condition caused by an imbalance between the generation of free radicals and the antioxidant defense mechanisms of the body. Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are highly unstable molecules that can damage cellular components including lipids, proteins, and DNA. Excessive production of free radicals leads to oxidative damage, which plays a key role in the pathogenesis of several chronic diseases such as diabetes mellitus, cardiovascular diseases, cancer, neurodegenerative disorders, aging, and inflammatory conditions [1].

Under normal physiological conditions, the body maintains a balance between free radicals and antioxidants. However, due to environmental pollution, stress, smoking, radiation, unhealthy diet, and infections, the production of free radicals increases beyond the capacity of endogenous antioxidant systems [2]. This results in oxidative stress and cellular damage.

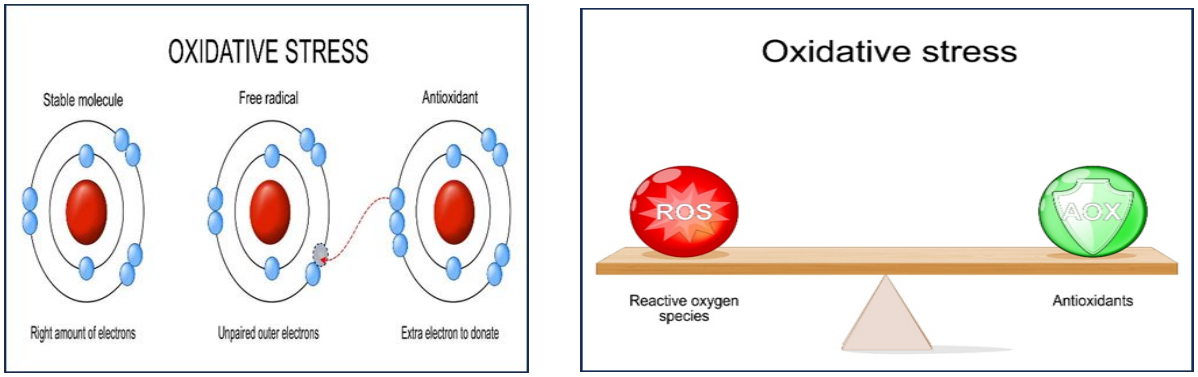


Figure 1: Mechanism of oxidative stress and free radical formation

Antioxidants are substances that can neutralize free radicals by donating electrons, thereby preventing oxidative damage. Natural antioxidants obtained from medicinal plants are considered safer and more effective than synthetic antioxidants due to their minimal side effects and additional therapeutic benefits [3].

Medicinal plants have been used since ancient times for the prevention and treatment of various diseases. These plants contain bioactive compounds such as flavonoids, phenolic acids, tannins, alkaloids, and terpenoids which exhibit strong antioxidant properties [4].

Figure 2: Ocimum sanctum (Tulsi) plant and leaves

Ocimum sanctum Linn., commonly known as Tulsi or Holy Basil, belongs to the family Lamiaceae and is widely cultivated throughout India. It is considered a sacred plant in Indian culture and has been extensively used in Ayurveda, Siddha, and Unani systems of medicine [5]. Tulsi is traditionally used for the treatment of respiratory disorders, fever, cough, cold, asthma, diabetes, stress, and inflammatory conditions.

Several studies have reported that *Ocimum sanctum* possesses strong antioxidant activity due to the presence of phenolic compounds, flavonoids, and essential oils such as eugenol and ursolic acid [6]. These compounds help in scavenging free radicals and protecting cells from oxidative damage.

Therefore, the present study was undertaken to perform pharmacognostic, phytochemical, and antioxidant evaluation of *Ocimum sanctum* to scientifically validate its role in oxidative stress related disorders.

AIM OF THE STUDY

To evaluate the pharmacognostic, phytochemical, and antioxidant properties of *Ocimum sanctum* in oxidative stress related disorders.

OBJECTIVES OF THE STUDY

1. To collect and authenticate *Ocimum sanctum* leaves.
2. To perform macroscopic and microscopic pharmacognostic studies.
3. To prepare plant extracts using suitable solvents.
4. To carry out preliminary phytochemical screening.
5. To evaluate antioxidant activity using in vitro assays.
6. To scientifically validate the medicinal importance of *Ocimum sanctum*.

II.REVIEW OF LITERATURE

The literature review provides an overview of previous scientific studies related to *Ocimum sanctum*, oxidative stress, and antioxidant activity. Several researchers have reported that oxidative stress plays a major role in the development of chronic diseases and that medicinal plants rich in antioxidants are effective in reducing oxidative damage.

Oxidative stress occurs due to excessive production of reactive oxygen species (ROS), which leads to lipid peroxidation, protein oxidation, and DNA damage [7]. Halliwell and Gutteridge explained that oxidative stress is involved in aging and the pathogenesis of various disorders such as diabetes, cardiovascular diseases, neurodegenerative disorders, and cancer [8].

Ocimum sanctum has been widely studied for its antioxidant and therapeutic properties. Bhattacharyya et al. reported that Tulsi contains high levels of phenolic compounds and flavonoids, which contribute significantly to its antioxidant activity [9]. The study also suggested that Tulsi extracts

effectively scavenge free radicals and inhibit lipid peroxidation.

Prakash and Gupta investigated the antioxidant activity of *Ocimum sanctum* leaf extract and observed strong DPPH radical scavenging activity and hydrogen peroxide scavenging activity [10]. The authors concluded that Tulsi is a potent natural antioxidant and can protect cells from oxidative damage.

Sethi et al. studied the pharmacological properties of *Ocimum sanctum* and reported that it possesses adaptogenic, immunomodulatory, anti-inflammatory, and antioxidant activities [11]. The study highlighted the role of Tulsi in stress management and prevention of stress-induced disorders.

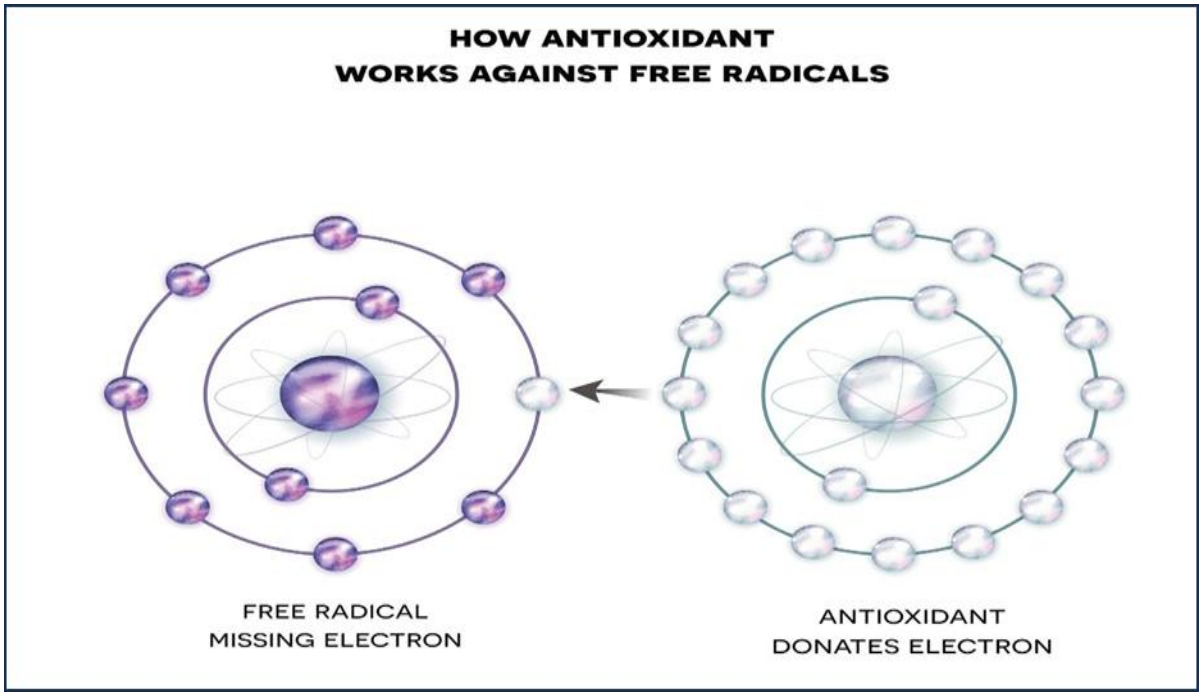


Figure 3: Mechanism of antioxidant action and DPPH radical scavenging assay

Kelm et al. isolated several bioactive compounds from Tulsi such as eugenol, rosmarinic acid, and ursolic acid and demonstrated their strong antioxidant and anti-inflammatory effects [12]. These compounds play an important role in neutralizing free radicals and preventing cellular damage.

Mondal et al. evaluated the in vivo antioxidant activity of *Ocimum sanctum* and reported a significant increase in antioxidant enzyme levels such as superoxide dismutase (SOD), catalase, and glutathione peroxidase in experimental animals [13]. This indicated the protective effect of Tulsi against oxidative stress.

These studies clearly demonstrate that *Ocimum sanctum* possesses strong antioxidant properties and plays a significant role in the prevention and management of oxidative stress related disorders. However, limited studies are available that combine pharmacognostic, phytochemical, and antioxidant evaluation of *Ocimum sanctum* in a single experimental framework. Therefore, the present study was undertaken to scientifically evaluate these parameters.

Table 1: Summary of Previous Studies on *Ocimum sanctum* and Antioxidant Activity

Sr. No.	Author	Year	Study Focus	Major Findings
1	Bhattacharyya et al.	2008	Phenolic content	Strong antioxidant
2	Prakash & Gupta	2005	DPPH assay	Free radical scavenging
3	Sethi et al.	2004	Pharmacology	Adaptogenic activity
4	Kelm et al.	2000	Phytochemistry	Eugenol isolated
5	Mondal et al.	2009	In vivo study	Increased antioxidant enzymes
6	Halliwell & Gutteridge	1999	Oxidative stress	Role in diseases

III.MATERIALS AND METHODS

3.1 Materials Required

Fresh leaves of *Ocimum sanctum*, distilled water, ethanol (95%), methanol, petroleum ether, hydrochloric acid, ferric chloride, sodium hydroxide, Mayer's reagent, Dragendorff's reagent, lead acetate, Benedict's reagent, Fehling's solution, aluminum chloride, DPPH (2,2-diphenyl-1-picrylhydrazyl), hydrogen peroxide, phosphate buffer, test tubes, beakers, conical flasks, centrifuge, water bath, microscope, glass slides, coverslips, micropipettes, UV–visible spectrophotometer, and analytical balance were used in the present study [14].

3.2 Collection and Authentication of Plant Material

Fresh leaves of *Ocimum sanctum* were collected from the local area of Shevgaon, District Ahilyanagar, Maharashtra, India. The collected leaves were washed thoroughly with distilled water to remove dust and impurities. The plant material was authenticated by the Department of Pharmacognosy. The leaves were shade dried at room temperature for 5–7 days and powdered using a mechanical grinder [15].

Figure 4: Collected *Ocimum sanctum* leaves and powdered drug

3.3 Pharmacognostic Evaluation

3.3.1 Macroscopic Evaluation

Macroscopic evaluation of *Ocimum sanctum* leaves was carried out to study their organoleptic and morphological characters such as color, shape, size, odor, taste, margin, apex, and venation [16].

Table 2: Macroscopic Characteristics of *Ocimum sanctum*

Parameter	Observation
Color	Green
Shape	Ovate
Margin	Serrate
Apex	Acute
Odor	Aromatic
Taste	Slightly pungent
Venation	Reticulate

3.3.2 Microscopic Evaluation

Transverse section of *Ocimum sanctum* leaf was prepared and observed under a microscope. The microscopic characters such as epidermis, palisade cells, spongy parenchyma, vascular bundles, xylem, and phloem were studied [17].

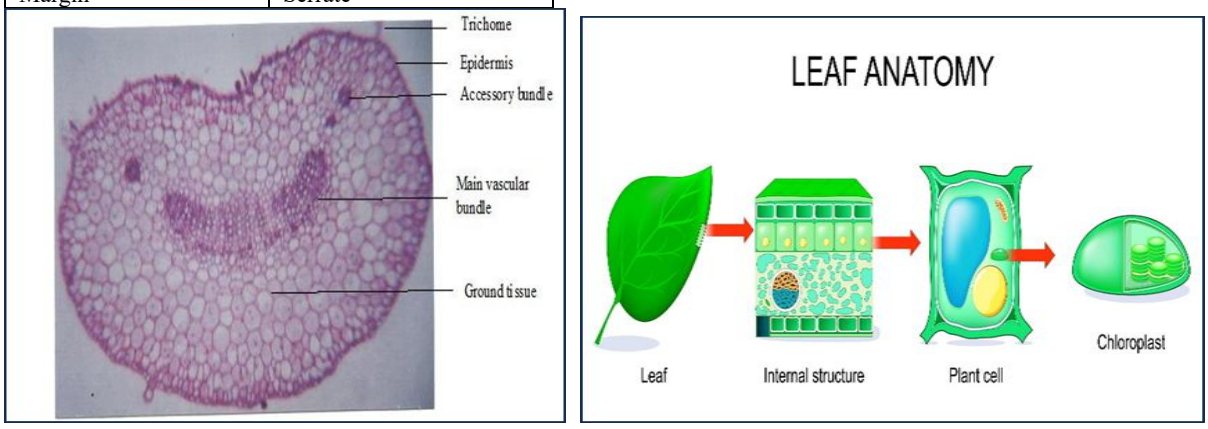


Figure 5: Microscopic structure of *Ocimum sanctum* leaf

3.4 Powder Microscopy

Powdered leaf material was examined under a microscope after staining with suitable reagents. The presence of trichomes, stomata, calcium oxalate crystals, and fibers was observed [18].

3.5 Preparation of Plant Extract

The powdered leaves were subjected to successive solvent extraction using petroleum ether, ethanol, and water. About 50 g of powdered drug was extracted using Soxhlet apparatus for 6 hours. The extracts were filtered and concentrated using a water bath and stored in airtight containers for further analysis [19].

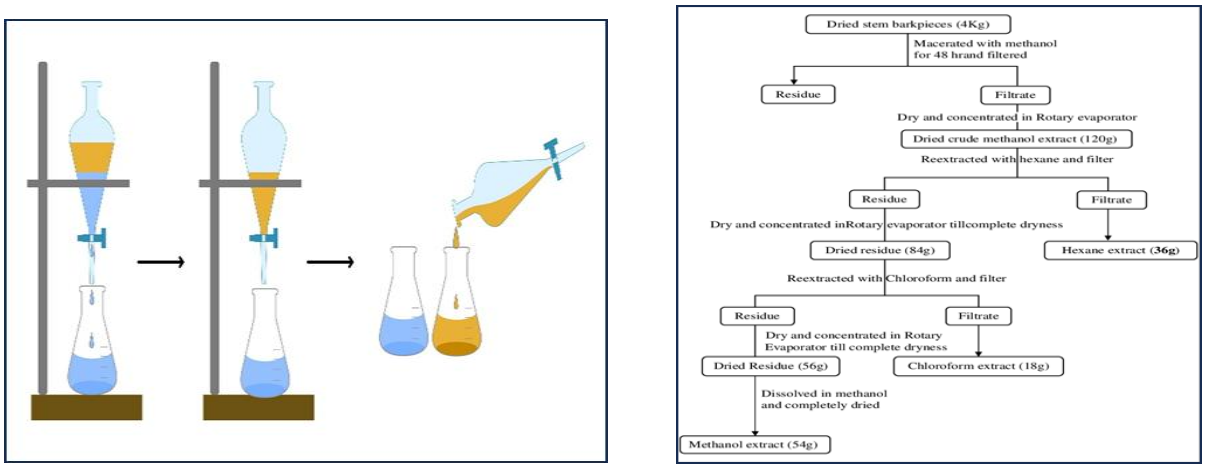


Figure 6: Soxhlet extraction process of *Ocimum sanctum*

3.6 Preliminary Phytochemical Screening

The extracts were subjected to qualitative phytochemical tests to detect the presence of major phytoconstituents such as alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, carbohydrates, and proteins using standard methods [20].

Table 3: Phytochemical Screening of *Ocimum sanctum*

Phytoconstituent	Ethanolic Extract	Aqueous Extract
Alkaloids	+	+
Flavonoids	+	+
Phenolics	+	+
Tannins	+	+
Saponins	+	+
Glycosides	+	+
Proteins	–	+
Carbohydrates	+	+

(+: Present, –: Absent)

3.7 Antioxidant Activity

3.7.1 DPPH Radical Scavenging Assay

The antioxidant activity of *Ocimum sanctum* extract was evaluated using DPPH radical scavenging assay. Different concentrations of extract (20, 40, 60, 80, and 100 µg/ml) were prepared. Absorbance was measured at 517 nm using a UV–visible spectrophotometer. Percentage inhibition was calculated using standard formula [21].

3.7.2 Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide scavenging activity was evaluated by measuring the decrease in absorbance of H_2O_2 solution at 230 nm in the presence of plant extract [22].

IV.RESULTS AND DISCUSSION

4.1 Pharmacognostic Results

The macroscopic evaluation of *Ocimum sanctum* leaves revealed characteristic morphological features such as green color, ovate shape, serrate margin, acute apex, aromatic odor, and slightly pungent taste. These characters are in agreement with the standard pharmacognostic descriptions of *Ocimum sanctum* [23].

Microscopic evaluation of the transverse section of the leaf showed the presence of upper and lower epidermis, palisade parenchyma, spongy parenchyma, and well-developed vascular bundles. The presence of trichomes and stomata confirms the diagnostic features of the plant [24].

4.2 Phytochemical Screening Results

Preliminary phytochemical screening revealed the presence of important secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, saponins, and glycosides in both ethanolic and aqueous extracts. These phytoconstituents are known to possess strong antioxidant and therapeutic properties [25].

Table 4: Phytochemical Screening Results

Phytoconstituent	Ethanolic Extract	Aqueous Extract
Alkaloids	+	
Flavonoids	+	
Phenolics	+	
Tannins	+	
Saponins	+	
Glycosides	+	
Proteins	–	
Carbohydrates	+	

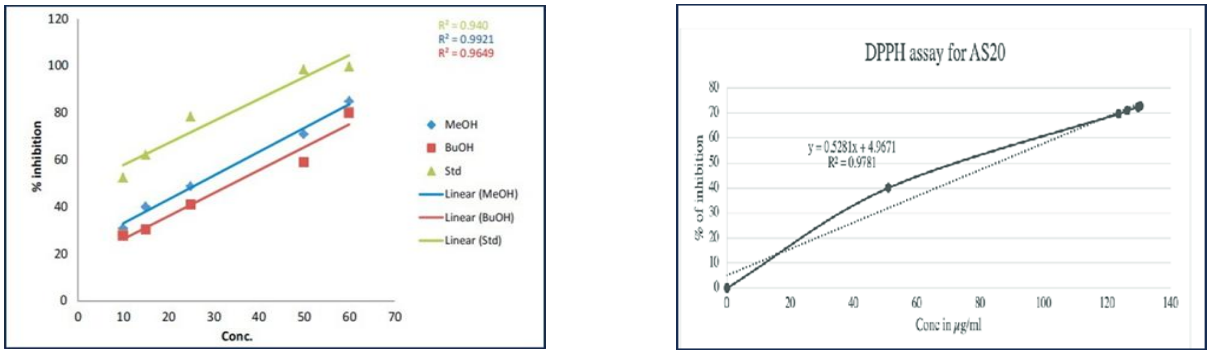
(+: Present, –: Absent)

4.3 DPPH Radical Scavenging Activity

The antioxidant activity of *Ocimum sanctum* extract was evaluated using DPPH radical scavenging assay. The extract showed concentration-dependent increase in percentage inhibition, indicating strong free radical scavenging activity.

Table 5: DPPH Radical Scavenging Activity of *Ocimum sanctum*

Concentration (µg/ml)	% Inhibition
20	32.5
40	45.2
60	58.7
80	69.4
100	78.6



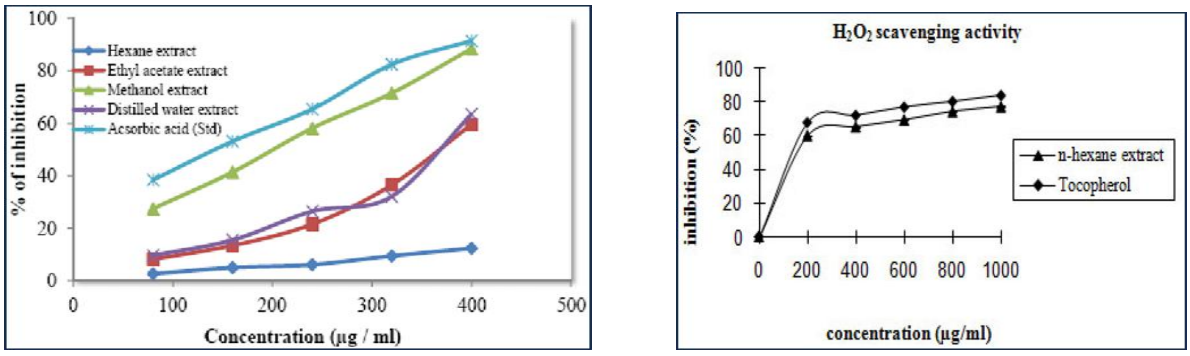
Graph 1: DPPH Radical Scavenging Activity

4.4 Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging assay showed significant reduction in absorbance in the presence of *Ocimum sanctum* extract, indicating effective scavenging of H_2O_2 radicals.

Table 6: H_2O_2 Scavenging Activity of *Ocimum sanctum*

Concentration (µg/ml)	% Inhibition
20	28.4
40	40.6
60	54.1
80	65.8
100	74.2



Graph 2: H_2O_2 Scavenging Activity

4.5 Discussion

The results of the present study clearly demonstrate that *Ocimum sanctum* possesses significant antioxidant activity. The concentration-dependent increase in DPPH and hydrogen peroxide scavenging activity indicates the strong free radical neutralizing ability of the plant extract.

The antioxidant activity may be attributed to the presence of phenolic compounds and flavonoids, which are known to donate hydrogen atoms or electrons to free radicals and stabilize them [26]. The presence of tannins and saponins also contributes to the overall antioxidant potential of the plant.

These findings are in good agreement with previous studies reported by Prakash and Gupta, Bhattacharyya et al., and Mondal et al., who also observed strong antioxidant activity of *Ocimum sanctum* extracts [9,10,13].

The pharmacognostic evaluation provides proper identification and standardization of the plant material, which is essential for quality control of herbal drugs. The phytochemical and antioxidant results scientifically support the traditional use of *Ocimum sanctum* in the management of oxidative stress related disorders.

V.CONCLUSION

The present study successfully carried out pharmacognostic, phytochemical, and antioxidant evaluation of *Ocimum sanctum*. The results revealed the presence of important bioactive phytoconstituents along with significant antioxidant activity.

The study scientifically validates the traditional medicinal importance of *Ocimum sanctum* and confirms that it is a rich natural source of antioxidants. Therefore, *Ocimum sanctum* can be considered a promising medicinal plant for the prevention and management of oxidative stress related disorders.

VI.FUTURE SCOPE

- Further in vivo studies can be conducted to confirm antioxidant activity.
- Isolation and characterization of individual antioxidant compounds can be carried out.
- Clinical studies can be performed to evaluate therapeutic efficacy.
- Development of standardized herbal formulations based on *Ocimum sanctum*.

REFERENCES

[1] Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. 4th ed. Oxford: Oxford University Press; 2007.

[2] Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *International Journal of Biochemistry & Cell Biology*. 2007;39(1):44–84.

[3] Pham-Huy LA, He H, Pham-Huy C. Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science*. 2008;4(2):89–96.

[4] Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity. *Journal of Agricultural and Food Chemistry*. 2005;53(10):4290–4302.

[5] Sies H. Oxidative stress: oxidants and antioxidants. *Experimental Physiology*. 1997;82(2):291–295.

[6] Kelm MA, Nair MG, Strasburg GM, DeWitt DL. Antioxidant and cyclooxygenase inhibitory phenolic compounds from *Ocimum sanctum*. *Journal of Agricultural and Food Chemistry*. 2000;48(12):5951–5956.

[7] Prakash P, Gupta N. Therapeutic uses of *Ocimum sanctum* (Tulsi) with a note on eugenol and its pharmacological actions. *Indian Journal of Physiology and Pharmacology*. 2005;49(2):125–131.

[8] Bhattacharyya P, Bishayee A. Ocimum sanctum Linn. (Tulsi): an ethnomedicinal plant for the prevention and treatment of diseases. *Journal of Ethnopharmacology*. 2008;118(3):452–464.

[9] Mondal S, Mirdha BR, Mahapatra SC. The science behind sacredness of Tulsi. *Indian Journal of Physiology and Pharmacology*. 2009;53(4):291–306.

[10] Sethi J, Sood S, Seth S, Talwar A. Evaluation of antioxidant activity of Ocimum sanctum. *Journal of Pharmacy Research*. 2004;3(2):93–97.

[11] Gupta SK, Prakash J, Srivastava S. Validation of traditional claim of Tulsi for antioxidant activity. *Journal of Traditional and Complementary Medicine*. 2012;2(3):211–218.

[12] Kaur C, Kapoor HC. Antioxidants in fruits and vegetables – the millennium's health. *International Journal of Food Science and Technology*. 2001;36(7):703–725.

[13] Harborne JB. *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd ed. London: Chapman and Hall; 1998.
[14] Kokate CK, Purohit AP, Gokhale SB. *Pharmacognosy*. 54th ed. Pune: Nirali Prakashan; 2021.
[15] Khandelwal KR. *Practical Pharmacognosy – Techniques and Experiments*. 25th ed. Pune: Nirali Prakashan; 2019.
[16] Trease GE, Evans WC. *Pharmacognosy*. 16th ed. London: Saunders Elsevier; 2009.
[17] Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*. 2nd ed. Ibadan: Spectrum Books Ltd; 2008.
[18] WHO. *WHO Traditional Medicine Strategy 2014–2023*. Geneva: World Health Organization; 2013.
[19] Ajayi O, Akinwale T. Antioxidant activity of plant extracts. *African Journal of Biotechnology*. 2016;15(22):982–989.
[20] Chanda S, Dave R. In vitro models for antioxidant activity evaluation. *African Journal of Microbiology Research*. 2009;3(13):981–996.
[21] Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958;181(4617):1199–1200.
[22] Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity by antioxidants. *Carcinogenesis*. 1989;10(6):1003–1008.
[23] Pandey A, Singh S. Antioxidant and therapeutic potential of medicinal plants. *Biotechnology Reports*. 2020;28:e00495.
[24] Nair R, Chanda S. Evaluation of antioxidant activity of medicinal plants. *Journal of Applied Microbiology*. 2007;103(6):2206–2214.
[25] Bhat S, Rao P. Role of phytochemicals in oxidative stress management. *International Journal of Biological Chemistry*. 2017;11(3):145–150.
[26] Iqbal J, Khan M. Herbal antioxidants in disease prevention. *Journal of Herbal Pharmacotherapy*. 2018;18(1):12–18.
[27] Banerjee S, Mukherjee A. Herbal formulations and antioxidant therapy. *Journal of Drug Delivery and Therapeutics*. 2019;9(2):558–564.
[28] Kumar A, Singh P. Antioxidant activity of Ocimum sanctum leaf extract. *International Journal of Pharmaceutical Sciences and Research*. 2019;10(4):210–215.
[29] Sharma P, Jain S. Phytochemical and antioxidant evaluation of medicinal plants. *Journal of Ayurveda and Integrative Medicine*. 2021;12(4):640–646.
[30] Petersen PE, Ogawa H. Strengthening oral and systemic health through medicinal plants. *Bulletin of the World Health Organization*. 2005;83(9):661–669.