



## **Assessment of Antioxidant Potential of *Ocimum sanctum* Linn. Extracts from West Bengal, India**

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### **ABSTRACT**

*Synthetic antioxidants are reported to have various side effects including carcinogenicity and potential risks to human health. Therefore, there is resurgence to search for natural antioxidants. We aimed to assess the various antioxidant activities *Ocimum sanctum* Linn extracts one of the most imperative medicinally valued herb in West Bengal region of India. The *O. sanctum* plants were collected from different geographical locations of West Bengal, India, and subjected to successive solvent extraction by continuous hot extraction (Soxhlet) method with different solvents viz. methanol, ethanol, acetone, chloroform, and hexane for the preparation of different polarity *O. sanctum* extracts. The antioxidant activities of *O. sanctum* extracts viz. DPPH radical scavenging assay, guaiacol peroxidase activity (GPx), catalase activity, and ascorbate peroxidase assay were evaluated using standardized assay methods. Results revealed that acetone extract of *O. sanctum* exhibited maximum DPPH antioxidant activity i.e., IC<sub>50</sub> value of 47.50 µg/mL, GPx activity of 2.50 U/mg protein, catalase activity of 4.60 U/mg protein, and ascorbate peroxidase activity of 3.50 U/mg protein than other solvent extractions of *O. sanctum*. In conclusion, the acetone extract of *O. sanctum* could be explored for the development of natural antioxidant agents.*

**Keywords:** *Ocimum sanctum*, Antioxidant activities, Catalase, DPPH, Guaiacol peroxidase, Ascorbate peroxidase

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### **INTRODUCTION**

Living plants produce a vast quantity of chemicals required for their performance and improvement. Some of these chemicals are primary metabolites, which consist of proteins (amino acids), carbohydrates, fats, nucleic acids, etc. but, besides these primary chemicals, the plants further provide just so-called secondary metabolites, which are specific to some taxonomic groups (families, genera). About 80% of the world's population still depends on the traditional system of medicine for curing several health hazards [1]. Despite the vast scientific development in contemporary medicine, Ayurvedic system of medicine is widely practiced and accepted by people not only in India but also in many developed countries. According to the World Health Organization (WHO), about 80% of patients in India still, rely on the practitioners of the traditional system of medicines. The therapeutic use of herbal crude medicines in different rural and urban communities is most of the time regulated by their traditional beliefs, and thus a majority of the herbal drugs are used as "folk" medicines and well-practiced since long past. Furthermore, increasing dependency on medicinal plants in the industrialized communities have been found to the extraction and improvement of several remedies and chemotherapeutics from these plants and from traditionally established rural herbal remedies [2].

Population-based epidemiological evidence has helped to clarify the role of diet in preventing and controlling morbidity and premature mortality resulting from chronic diseases [3]. Diet and nutrition are vital aspects in the endorsement and maintenance of good health throughout the entire life course. Poor diet is a major contributor to the leading causes of chronic and fatal diseases including coronary heart disease, cancer, and strokes. Research and development in food science is helping to identify many functional foods ingredients, because they can provide health benefits by reducing risk and enhancing the

body's ability to manage certain chronic diseases [4-5]. The functional properties of plants/foods depend on the presence of specific chemical compounds, mainly secondary metabolites such as phenolics and flavonoids [6-8]. The presence of such bioactive compounds promotes the therapeutic uses of medicinal plants also [7, 9].

Reactive oxygen species (ROS) are essentially produced as the intermediate metabolites in a number of physiological processes like energy production in mitochondria, phagocytosis, cell growth regulation, intracellular signaling and detoxification of xenobiotics in the human body. Exposure to long term UV irradiations, chemical pollutants, organic solvents, tobacco smoke and pesticides are some of the environmental factors contributing to ROS over production. Stress generated due to current life style further adds to the overproduction of free radicals and causing tissue damage leading to deteriorating health conditions. High and chronic stress is related to a large number of pathological conditions like atherosclerosis, arthritis, ischemia, cancer and injuries to many tissues including central nervous system [10]. Human health is the primary concern as it influences the efficiency and indirectly affects the economic status of individual, family, society and nation. Use of natural antioxidants to combat increasing oxidative stress under normal and pathological conditions is exponentially rising. Application of natural based antioxidants finds a new dimension in food technology to improve the shelf life of eatables. Synthetic antioxidants are reported to have various side effects including carcinogenicity and potential risks to human health, hence research on natural antioxidants and their applications is the need of the hour [11]. The phytochemicals and secondary metabolites of plants contribute to their medicinal values. Polyphenols absorb, quench and neutralize free radicals, act as reducing agents, metal chelators and can efficiently protect biological systems from degeneration under high oxidative stress [12]. Antioxidants from plant sources, especially polyphenolic compounds effectively impede rancidity due to lipid oxidation in foods as well as development of oxidative stress related diseases [13].

India is well known as an "Emporium of medicinal plants". It possesses about 8% of the estimated biodiversity of the world with around 12,600 species and is one of the 12 mega biodiversity centers with 2 hot spots of biodiversity in the Western Ghats and North-eastern region [14]. Herbal plants or Folk medicines have been long used as a medication since classical times for the treatment of the majority of diseases. Folk plants used to enact a very significant role in world health [15, 16]. *Ocimum sanctum* (synonym *Ocimum tenuiflorum*), commonly known as holy basil, tulasi (sometimes spelled thulasi) or tulsi, is an aromatic perennial plant in the family Lamiaceae. An erect much branched softly pubescent undershrub, 30-60 cm height with red or purple sub quadrangular branches; leaves simple, opposite, elliptic, oblong, obtuse or acute, entire, serrate or dentate, pubescent on both sides, minutely gland dotted, petioles slender, hairy; flowers small, purplish, in terminal thyrsoid panicles; calyx purplish, 2-lipped, pubescent, upper lip orbicular, reflexed, lower lip 4-lobed; corolla 2-lipped; stamens 4, didynamous, filaments purple, anthers yellow; nutlets ellipsoid, smooth, mucilaginous when wetted. The leaves are acrid, thermogenic, aromatic, antibacterial, insecticidal, antiviral, appetizing, and deodorant (Figure 1).



**Figure 1:** Showing *Ocimum sanctum* whole plant

Literature reports revealed that the ethanolic extract of *Ocimum* species found to be potent in showing antimetastatic activity and in boosting antioxidant enzyme activity. Many herbs mentioned in Ayurveda for the treatment of tumors including cancer therapy and are also capable of preventing the side effects with

the complete healing power [17, 18]. Oxidative phosphorylation may cause the emergence of reactive oxygen species (ROS) that can destroy cellular DNA and other natural proteins. Higher amount of ROS may break double stranded DNA and causes the ageing of the cell, cardiac disorders, mutation with in the cells, and cancerous cell growth [19, 20]. Furthermore, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often associated with adulterations and side effects. Therefore, there is need to search for alternative drugs from plant sources [21, 22]. The primary benefits of using plants derived medicines are that they are relatively safer than synthetic alternatives offering profound therapeutic benefits and more affordable treatment [23]. Phytoconstitutens are the natural bioactive compounds found in plants. Phytochemicals have antioxidant or hormone-like effect which helps in fighting against many diseases including cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues.<sup>24</sup> With this scenario, the present research study was carried out to assess the various antioxidant activities *O. sanctum* extracts one of the most imperative medicinally valued herb in West Bengal region of India.

## **MATERIAL AND METHODS**

### **Collection of plant material**

The *O. sanctum* plants were collected from different locations of West Bengal, India. The plant sample was identified and authenticated by Dr. Veermani Kumar, Assistant Professor, Department of Botany, YBN University, Ranchi, Jharkhand, India. The collected *O. sanctum* plants were thoroughly washed with tap water to avoid dusts and other unwanted materials accumulated on the leaves from their natural environment. The dust free *O. sanctum* plants were shade, dried at room temperature, and then finely powdered with an electric grinder.

### **Extraction**

The finely powdered *O. sanctum* plant was subjected Soxhlet extraction with different solvents viz. methanol, ethanol, acetone, chloroform, and hexane for the preparation of different polarity *O. sanctum* extracts. Extracts were continuously stirred for 6 h and kept at room temperature up to 18 h. The process was repeated up to complete extraction. The extract was filtered and concentrated under vacuum in a rotatory evaporator (Buchi Rotavapor, Switzerland) at 40°C. The extract was finally freeze-dried and stored at 4°C for further use.

### **Determination of antioxidant activities of *O. sanctum* extracts**

#### **DPPH radical scavenging assay**

The free radical scavenging activity of the *O. sanctum* extracts was tested by appraising their capacity to scavenge 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) stable radicals. The DPPH assay was performed as described previous reports.<sup>8,25</sup> Briefly, the samples (from 0.5 to 500.0 µg/ mL) mixed with 1 mL of 90 µM DPPH solution, were made up with 95% MeOH to a final volume of 4 mL. Butylated hydroxytoluene (BHT), a synthetic antioxidant, was used as a positive control. After 1 h incubation at room temperature, the absorbance was read at 515 nm. Percent radical scavenging concentration was worked out using the following formula:

$$\text{Radical scavenging (\%)} = 100 \times (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}})$$

Where,

$A_{\text{blank}}$ : Absorbance of the control (containing all reagents except the *O. sanctum* extracts)

$A_{\text{sample}}$ : Absorbance of the test *O. sanctum* extracts

IC<sub>50</sub> values: The concentration of *O. sanctum* extracts that caused 50% scavenging of DPPH radicals, and which was calculated from the curve drawn by plotting percent scavenging versus concentration

#### **Guaiacol peroxidase activity (GPX)**

The GPX activity was determined according to the modified method of Shevyakova et al., (2002).<sup>26</sup> The activity of GPX in the extract was determined spectrophotometrically by measuring the increase in absorbance at 470 nm. The reaction mixture contained 80 mmol/L guaiacol and 10 mmol/L H<sub>2</sub>O<sub>2</sub> in 0.066 mol/L phosphate buffer, pH=7.4. The enzymatic reaction was started by adding 0.1 mL of the extract to 3 mL of reaction mixture. Enzyme activity was calculated as the increase in absorbance (ΔE) min<sup>-1</sup> g<sup>-1</sup> extract.

#### **Catalase activity**

Catalase activity was assayed as described by Chandlee and Scandalios (1984).<sup>27</sup> Briefly, 500 mg of extract was homogenized in 5 mL of ice cold 50 mM sodium phosphate buffer (pH 7.5) containing in 1mM PMSF. The extract was centrifuged at 4°C for 20 min at 12,500 rpm. The supernatant was used for the enzyme assay. The assay mixture contained 2.6 mL of 50 mM potassium phosphate buffer (pH 7.0) 0.4 mL, 15 mM H<sub>2</sub>O<sub>2</sub> and 0.04 mL of enzyme extract. The decomposition of H<sub>2</sub>O<sub>2</sub> was followed by the decline in

absorbance at 240 nm. The enzyme activity was expressed in units of 1 mM of H<sub>2</sub>O<sub>2</sub> reduction per minute per mg extract.

#### Ascorbate peroxidase assay

Ascorbate peroxidase was extracted and estimated by the modified method of Asada and Takahashi (1987).<sup>28</sup> Five hundred milligrams of *O. sanctum* extract was grounded in a pestle and mortar using liquid nitrogen and 10 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1% PVP and 1 mM ascorbic acid. The homogenate was filtered through a double layered cheese cloth and centrifuged at 15,000 rpm for 20 min at 4°C. The supernatant was used as the source for enzymes. One mL of reaction mixture contained a 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM H<sub>2</sub>O<sub>2</sub> and 200 µL of enzyme extract. The absorbance was read as a decrease at 290 nm against the blank, correction was done for the low, non-enzymatic oxidation of ascorbic acid by H<sub>2</sub>O<sub>2</sub> (extinction coefficient 2.9 mM<sup>-1</sup> cm<sup>-1</sup>). The enzyme activity was expressed in units of per mg of extract.

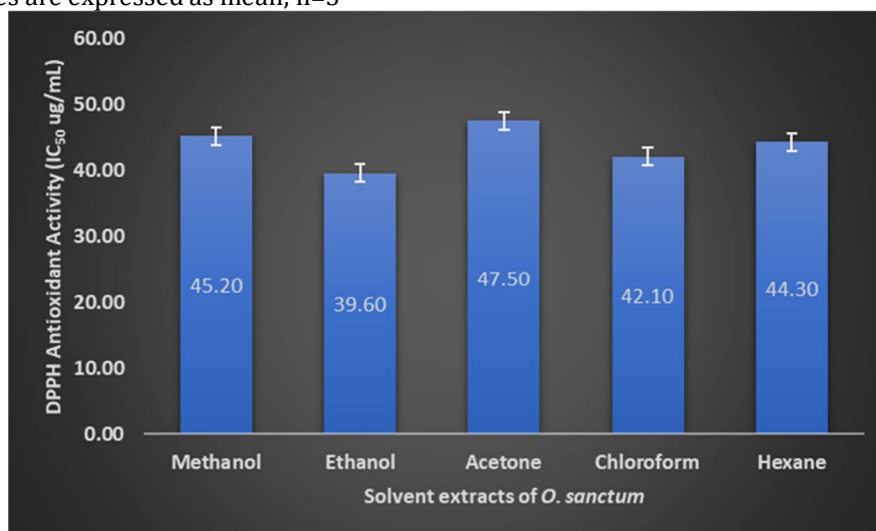
## RESULTS AND DISCUSSION

The results of DPPH radical scavenging assay of *O. sanctum* extracts was represented in Table 1 and plotted in Figure 2. Results depicted that acetone extract of *O. sanctum* exhibited maximum DPPH antioxidant activity i.e., IC<sub>50</sub> value of 47.50 µg/mL followed by methanol, hexane, chloroform, and ethanol with IC<sub>50</sub> values of 45.20 µg/mL, 44.30 µg/mL, 42.10 µg/mL, and 39.60 µg/mL respectively. These findings implied that the optimum extraction of secondary phytochemicals mainly polyphenols and flavonoids responsible for DPPH radical scavenging activity was observed in acetone.

**Table 1:** DPPH radical scavenging assay of different solvent extracts of *O. sanctum*

Solvent Extracts of <i>O. sanctum</i>	DPPH Antioxidant Activity (IC <sub>50</sub> µg/mL)
Methanol	45.20
Ethanol	39.60
Acetone	47.50
Chloroform	42.10
Hexane	44.30

Values are expressed as mean; n=3



**Figure 2:** DPPH radical scavenging assay of different solvent extracts of *O. sanctum*

*O. sanctum*'s antioxidant qualities confer a wide range of health advantages. A typical technique for assessing the antioxidant activity of natural substances like plant extracts is the DPPH (2, 2-diphenyl-1-picrylhydrazyl) test. In this test, antioxidants in the sample diminish DPPH radicals, which are stable free radicals, causing a purple color to turn yellow[29]. The results of DPPH radical scavenging assay of *O. sanctum* extracts were comparable with the previous findings reported by various other research investigators in the literature. Chaudhary et al., reported the strongest antioxidant activity in methanol extract of *O. sanctum* with IC<sub>50</sub> value of 20.31 g/mL, Furthermore, significant antioxidant activity was also demonstrated by the ethanol and water extracts, with IC<sub>50</sub> values of 26.36 and 32.74 g/mL, respectively

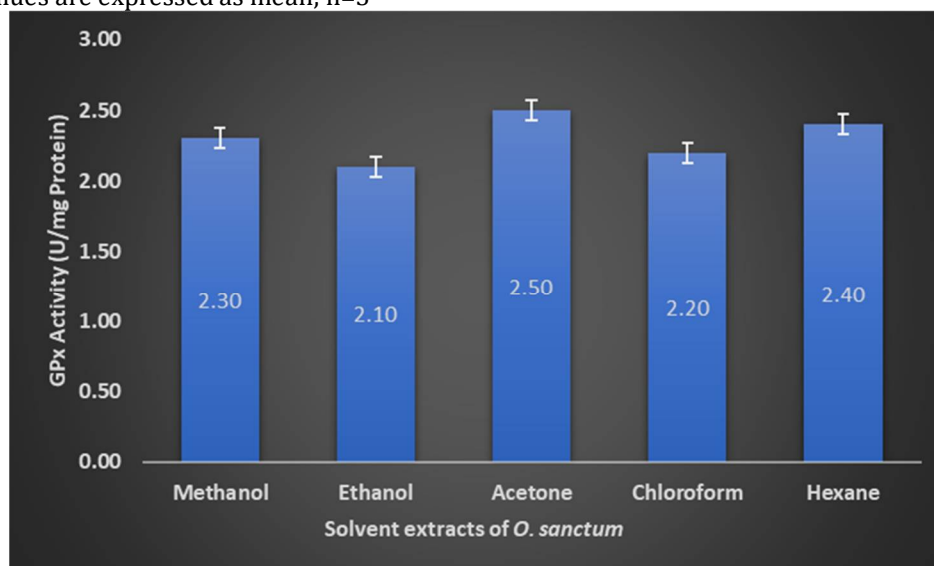
[30]. Accordingly, these results indicate that *O. sanctum* plant extracts have considerable DPPH radical scavenging activity, which may explain some of its health advantages.

The results of GPx activity of *O. sanctum* extracts was represented in Table 2 and plotted in Figure 3. Results revealed that acetone extract of *O. sanctum* possess maximum GPx activity i.e., 2.50 U/mg protein followed by hexane, methanol, chloroform, and ethanol extracts of *O. sanctum* with GPx activities of 2.40 U/mg protein, 2.30 U/mg protein, 2.20 U/mg protein, and 2.10 U/mg protein respectively. These findings depicted that active protein molecules responsible for GPx activity were maximally extracted in acetone.

**Table 2:** Guaiacol peroxidase activity (GPx) of different solvent extracts of *O. sanctum*

Solvent Extracts of <i>O. sanctum</i>	GPx Activity (U/mg protein)
Methanol	2.30
Ethanol	2.10
Acetone	2.50
Chloroform	2.20
Hexane	2.40

Values are expressed as mean; n=3



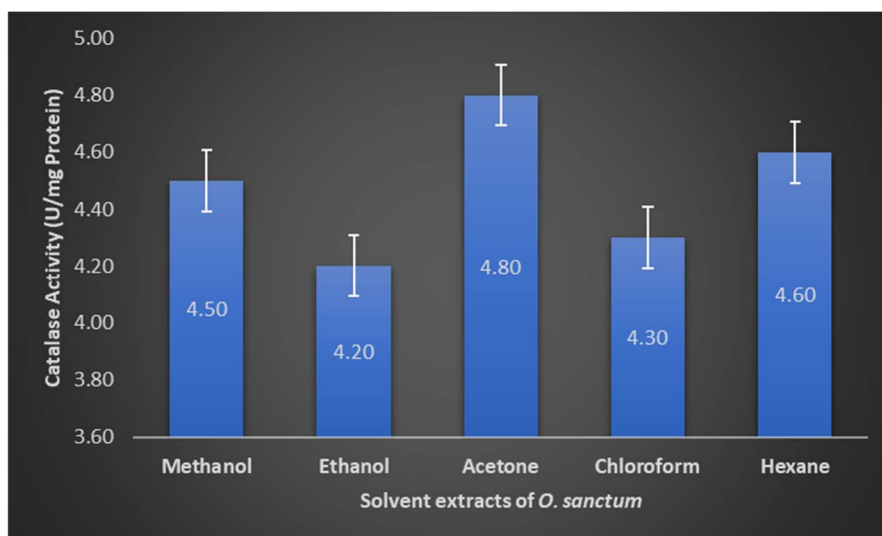
**Figure 3:** Guaiacol peroxidase activity (GPx) of different solvent extracts of *O. sanctum*

The results of catalase activity of *O. sanctum* extracts was represented in Table 3 and plotted in Figure 4. Results delineated that highest catalase activity of 4.80 U/mg protein was observed in acetone extract of *O. sanctum* followed by 4.60 U/mg protein, 4.50 U/mg protein, 4.30 U/mg protein, and 4.20 U/mg protein in *O. sanctum* extracts of hexane, methanol, chloroform, and ethanol respectively. These findings indicated that phytoactives ascribed to catalase activities were optimally extracted in acetone.

**Table 3:** Catalase activity of different solvent extracts of *O. sanctum*

Solvent Extracts of <i>O. sanctum</i>	Catalase Activity (U/mg protein)
Methanol	4.50
Ethanol	4.20
Acetone	4.80
Chloroform	4.30
Hexane	4.60

Values are expressed as mean; n=3



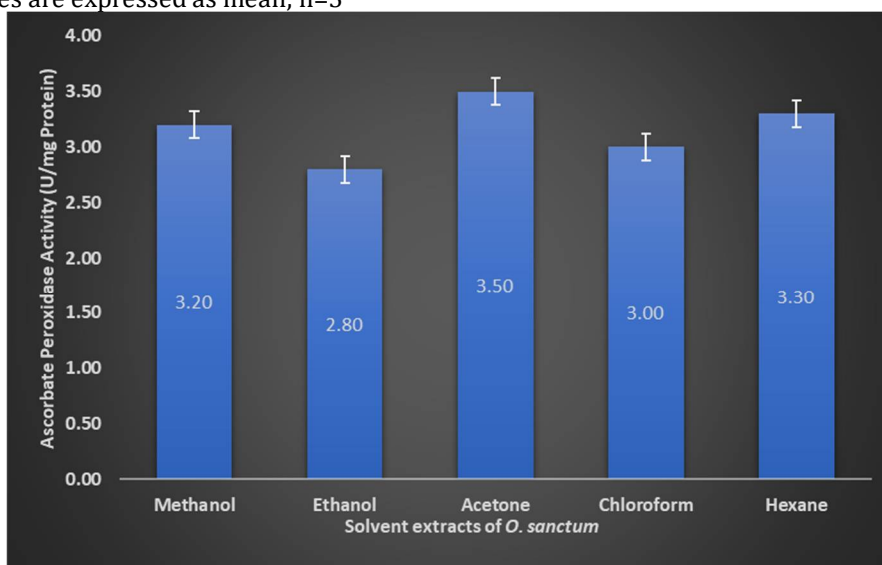
**Figure 4:** Catalase activity of different solvent extracts of *O. sanctum*

The results of ascorbate peroxidase activity of *O. sanctum* extracts was represented in Table 4 and plotted in Figure 5. Results revealed that acetone extract of *O. sanctum* exhibited maximum ascorbate peroxidase activity i.e., 3.50 U/mg protein followed by hexane, methanol, chloroform, and ethanol extracts of *O. sanctum* with ascorbate peroxidase activities of 3.30 U/mg protein, 3.20 U/mg protein, 3.00 U/mg protein, and 2.80 U/mg protein respectively. These findings depicted that active protein molecules responsible for ascorbate peroxidase activity were maximally dissolvable in acetone.

**Table 4:** Ascorbate peroxidase activity of different solvent extracts of *O. sanctum*

Solvent Extracts of <i>O. sanctum</i>	Ascorbate Peroxidase Activity (U/mg protein)
Methanol	3.20
Ethanol	2.80
<b>Acetone</b>	3.50
Chloroform	3.00
Hexane	3.30

Values are expressed as mean; n=3



**Figure 5:** Ascorbate peroxidase activity of different solvent extracts of *O. sanctum*

Ascorbate is one of the most extensively studied antioxidant and has been detected in the majority of plant cell types, organelles and apoplast. Ascorbic acid is readily oxidised to monodehydro ascorbic acid as part of its antioxidant function. Ascorbic acid prevents or reduces oxidative damage as reported in uniconazole

treated tomato seedlings [31]. An increase in catalase activity was noted in barley leaves and there by effective scavenging of H<sub>2</sub>O<sub>2</sub> to provide antioxidant defense mechanism [32]. The H<sub>2</sub>O<sub>2</sub> scavenging system represented by ascorbate peroxidase and catalase are more important in imparting tolerance than super oxide dismutase [33, 34].

The antioxidant property of plant extracts depends on the amount of flavonoids and phenolic acids of extracts [35]. The release of secondary metabolites including phenolic acids and flavonoids from plants may vary from plant to plant and species to species. Some previous reports in the literature revealed that the variation in the antioxidant potential of different *O. sanctum* around the world [36-38]. The *O. sanctum* analyzed in the present study exhibited comparable antioxidant activity with that reported in the literature.<sup>37</sup> The variation in the antioxidant activity might be ascribed to the varied phenolic profiles of *O. sanctum* species native to West Bengal state of India.

## CONCLUSION

The results of this study clearly demonstrated that acetone extract of *O. sanctum* possess highest antioxidant potential than other extracts of *O. sanctum* viz. methanol, ethanol, chloroform, and hexane. Hence, the acetone extract of *O. sanctum* could be explored for the development of natural antioxidant agents.

## AUTHOR CONTRIBUTION STATEMENT

Siddhartha Sankar Ghosh and Kamal Kant Patra were involved in the execution of all the field collection & laboratory work and data analysis. Veermani Kumar was contributed his valuable guidance as plant identification. Keshamma E contributed her guidance on laboratory skills for research work and in preparation of the current manuscript. All authors have read and approved the final manuscript before its submission.

## CONFLICT OF INTEREST

None to declare.

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