NATURALISTA CAMPANO
ISSN: 1827-7160
Volume 28 Issue 1, 2024

A Study on Phytochemical Profiling of *Ocimum sanctum* Linn. Extracts from different locations of West Bengal, India

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Abstract
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. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often associated with adulterations and side effects. Hence, we aimed to study the phytochemical profiling of "*Ocimum sanctum* Linn" one of the most important 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. Results depicted that the methanol solvent extracts of *O. sanctum* yielded highest extractive value i.e., 8.20%. The ethanol solvent extract of *O. sanctum* yielded highest polyphenolic content of 14.30 mg GAE /g extract, and the flavonoid content was found to be highest of 9.20 mg QE/g extract in acetone extract. The highest quantity of total ash content (3.20%) and acid-insoluble ash content (0.90%) was observed acetone extract of *O. sanctum*. Whereas, water-soluble ash content was found to be highest (2.50%) in hexane extract of *O. sanctum*. The hexane solvent extract of *O. sanctum* yielded highest total essential oil i.e., 1.10%. The highest quantities of photosynthetic pigments *viz.* total carotenoids (0.30 mg/g), anthocyanin (0.15 mg/g), Chlorophyll (Chl)*a* (0.55 mg/g), Chl *b* (0.28 mg/g), and total Chl II (0.83 mg/g) were observed in hexane extract of *O sanctum*. In conclusion, our study findings on *O. sanctum* extracts phytochemical profiling may be established as an excipient to prepare various formulations, and these formulations may be explored for implications in foods, nutrition and human health.

Keywords: *Ocimum sanctum,* Phytochemical profiling, Polyphenol, Flavonoid, Essential oil

Introduction

India is well known as an "Emporium of medicinal plants". It possesses about 8% of the estimated biodiversity of the world with around 12600 species and is one of the 12 mega biodiversity centers with 2 hot spots of biodiversity in the Western Ghats and North-eastern region.[1] It's also rich in ethnic diversity, there are about 67.37 million tribal people belonging to 537 tribal groups living in different geographical locations with various subsistence patterns.[2] These tribal groups living in diverse, rich areas possess a wealth of knowledge and skills on the utilization and conservation of food and medicinal plants.[3] According to the World Health Organization (WHO), almost 65% of the world's population has incorporated the value of plants as a methodology of medicinal agents into their primary modality of health care. It is often noted that 25% of all drugs prescribed today come from plants.[4] This estimate suggests that plant derived drugs make up a significant segment of natural product-based pharmaceuticals.[5]

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.[6,7]*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). They are useful in cough, bronchitis, catarrh, halitosis, bacterial and viral infections, foul ulcers.[8]

Figure 1: Showing *Ocimum sanctum* whole plant

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients.[9] They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals.[10] Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been catalogued and are classified by protective function, physical characteristics and chemical characteristics, and about 150 phytochemicals have been studied in detail.[11] Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds. Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the

variety, processing, cooking and growing condition. Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals.[12]

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.[13,14] 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.[15] 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.[16] These phytoconstituents work with nutrients and fibers to form an integrated part of defense system against various diseases and stress conditions.[17]With this background, the present research investigation was designed to study the phytochemical profiling of "*Ocimum sanctum* Linn" one of the most important medicinally valued herb in West Bengal region of India.

Materials and Methods

Collection of plant material

The *O. sanctum* plantswere collected from different locations of West Bengal, India. The plant sample was identified and authenticated by Dr. Kamal Kant Patra, Associate 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 powered with an electric grinder.

Extraction

The fines powdered *O. sanctum* plantwas 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 solvent extractive values of *O. sanctum* extracts

About 5 g of correctly measured air-dried *O. sanctum* extract was taken in a conical flask, fitted with a cork, and macerated with 100 ml of 90% alcohol for 24 hrs. It was shaken constantly for 6 hr in an electrical shaker and kept constant for next 18 hr. Filter it carefully so that there should not be any loss of alcohol and 30 mL of the filtrate was evaporated to dryness at a temperature 105°C in a tarred flat-bottomed shallow China dish. Weight of extract was taken, and percentage was calculated.

The extractive values of *O. sanctum* extract for other solvents *viz.* methanol, acetone, chloroform, and hexane were determined following the above procedure.

Isolation of essential oil

The *O. sanctum* extractswere subjected to hydrodistillation for 4 h using a Clevenger-type apparatus.[18]The isolated essential oil was dried over anhydrous sodium sulfate, filtered and the percentage yield was calculated.

Quantitative estimation of phytochemicals

Total phenolics

The concentration of total phenolics in the *O. sanctum* extractswas determined by the Folin-Ciocalteu assay that involves reduction of the reagent by phenolic compounds, with concomitant formation of a blue complex, and its intensity at 725nm increases linearly with the concentration of phenolics in the reaction medium.[19]The phenolic

content of the extract was determined from calibration curve which was made by preparing gallic acid solution (0-0.8 mg/ml) in distilled water and was expressed in mg gallic acid equivalent/g of extract powder (mg GAE/g).

Total flavonoids

Aluminum chloride colorimetric method was used for flavonoids determination in *O. sanctum* extracts.[20]The flavonoid content was determined from extrapolation of calibration curve which was made by preparing quercetin solution (0-0.8 mg/ml) in distilled water. The concentration of flavonoid was expressed in terms of mg quercetin equivalent/g of extract powder (mg QE/g).

Determination of ash content

Determination of total ash

Specifically weighed 3 g of *O. sanctum* extractsdried in atmospheric air was retained in a pre-ignited and weighed silica crucible. The granules of *O. sanctum* extractswere layered in the crucible and incinerated in the muffle furnace by slowly evaluating the temperature to make it complete white free from traces of carbon. Then, crucible was kept out in air to cool down, weighed, and the steps were recapitulated till a steady weight was obtained.

Determination of acid-insoluble ash

Total ash was boiled with 25mL of 2M HCl for 4-5minutes. The matter which was not dissolved was deposited on ash less filter paper, followed by the washing with hot water. Transfer that insoluble ash to the pre-weighed silica crucible, incinerate it, cool it and measure its weight. The steps were again followed till a fixed weight was obtained.

Determination of water-soluble ash

Total ash was bubbled with 25 mL of water for 4-5 minutes. Undissolved matter was gathered on ash less Whatman filter paper and rinsed with little warm water. Place filter paper with insoluble ash in a crucible and allow to getting completely burn within 15 minutes at a temperature of 450°C and weigh it. Mass of undissolved matter was deducted from the weight of total ash obtained. The variation obtained represents water soluble ash.

Quantitative estimation of photosynthetic pigments

Estimation of chlorophyll (Chl)

The Chl (*a, b, II*) contents were determined from *O. sanctum*extracts. Solvent extracts of *O. sanctum* were placed in a test tube containing 10 mL of dimethylformamide (DMF) and stored for 24 h at 4°C. The absorbance of the supernatant was read at 480, 647 and 666 nm in a monochromator base multimode detector (BioTek, Snergy 2, USA) with DMF as a blank. The contents of Chl ***a,*** *Chl b, and Chl II* were calculated according to Moran and Porath (1980).[21]

Carotenoid assay

O. sanctum extracts(0.050 g) were mixed with 2.5 mL of 100% ethanol in the dark for 24 h at 4°C. Then the samples were centrifuged for 10 min at 7000 rpm. Lichtenthaler's equation was employed to analyze the concentration of carotenoid, based on absorbance at 649, 665, and 470 nm.[22]

Anthocyanin assay

Total anthocyanins were estimated using the AOAC method.[23] Two buffers with pH 1.0 (potassium chloride, 0.025 M) and pH 4.5 (sodium acetate, 0.4 M) were used to determine the total anthocyanin content. Test solutions were prepared to have 1 part of *O. sanctum* extracts and 4 parts of buffers. Absorptions were taken at 520 nm and 700 nm for each pH system. Total anthocyanins were estimated using the following formula:

$$\text{Anthocyanin pigment}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{\text{A} \times \text{MW} \times \text{DF} \times 1000}{\varepsilon \times \text{l}}$$

Where,

A = (A520 nm − A700 nm) pH1.0 – (A520 nm – A700 nm) pH4.5

MW (molecular weight) =449.2 g/mol of cyanidin-3-glucoside

DF=dilution factor;
l=path length in cm
ε=26,900 molar extinction coefficient

Results and Discussion

The drugs derived from natural resources have a significant contribution in the traditional and modern system of medicines. Relevant steps have been taken by the World Health Organization WHO to carry out the research with the aim of finding new and effective medicinal agents from plants. The development of science of phytopharmaceuticals and the hopes for remedy in chronic diseases generated new enthusiasm in the research work to develop herbal medicines. Plants used in traditional medicines can serve as source of novel therapeutic agents directly or as model compounds for synthetic or semi-synthetic structural modifications.[24] The genus *Ocimum* (Lamiaceae) is distributed all over the world and can be found in many environments. *Ocimum species* is a rich source of various phytochemicals including tannins, phenolic acids, anthocyanins, phytosterols, and policosanols. These phytochemicals have the potential to significantly impact human health.[25] Hence in the present we aimed to study the phytochemical profiling of *O. sanctum* one of the most important medicinally valued herb in West Bengal region of India.

The results of extractive value of *O. sanctum* extractswas represented in Table 1 and plotted in Figure 2. Results depicted that methanol solvent extract of *O. sanctum* yielded highest extractive value i.e., 8.20% followed by ethanol, acetone, chloroform, and hexane with extractive values 7.50%, 6.80%, 5.90%, and 4.30% respectively. These results also inferred that lowest extractive yield (%) of *O. sanctum* was observed with hexane (4.30%) as compared to other selected solvents indicating *O. sanctum* extracts, extracted with different solvents were having different polarities.

Table 1: Extractive value of different solvent extracts of *O. sanctum*

Solvent Extracts of *O. sanctum*	Extractive Value (%)
Methanol	8.20
Ethanol	7.50
Acetone	6.80
Chloroform	5.90
Hexane	4.30

Values are expressed mean; n=3

The extractive value of different solvent extracts of *O. sanctum* were comparable with that of findings reported by Sharma et al., wherein authors reported water soluble extractive value, alcohol soluble extractive value, and ether soluble extractive value of *O. sanctum* were found to be 3.6%, 2.6%, and 3.9%, respectively.[24]

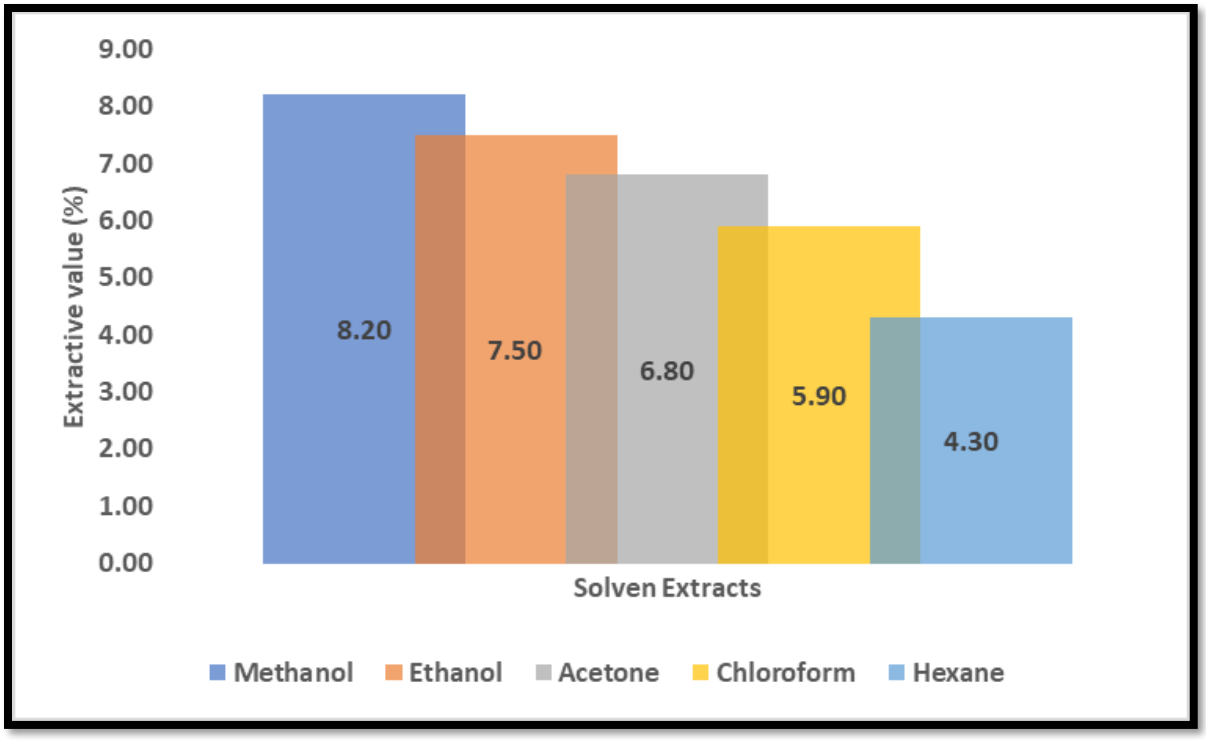

Figure 2: Extractive value of different solvent extracts of *O. sanctum*

The quantitative estimation of polyphenolic and flavonoid contents in different solvent *O. sanctum* extractswas represented in Table 2 and plotted in Figure 3. Results depicted that ethanolic solvent extract of *O. sanctum* yielded highest polyphenolic content of 14.30 mg GAE/g extract and lowest polyphenolic content of 11.80 mg GAE/gextract in acetone extracts. Similarly, the flavonoid content was found to be highest of 9.20 mg QE/g extract and lowest of 7.90 mg QE/g extract in acetone and ethanol extracts respectively. These findings implied that the major phytochemicals of *O. sanctumviz.* polyphenols and flavonoids and found to be maximally soluble in ethanol and acetone respectively.

Table 2: Quantitative estimation of major phytochemicals in different solvent extracts of *O. sanctum*

Solvent Extracts of *O. sanctum*	**Phenolic Content (mg GAE/g)**	**Flavonoid Content (mg QE/g)**
Methanol	12.50	8.70
Ethanol	14.30	7.90
Acetone	11.80	9.20
Chloroform	13.20	8.50
Hexane	12.60	8.00

Values are expressed mean; n=3

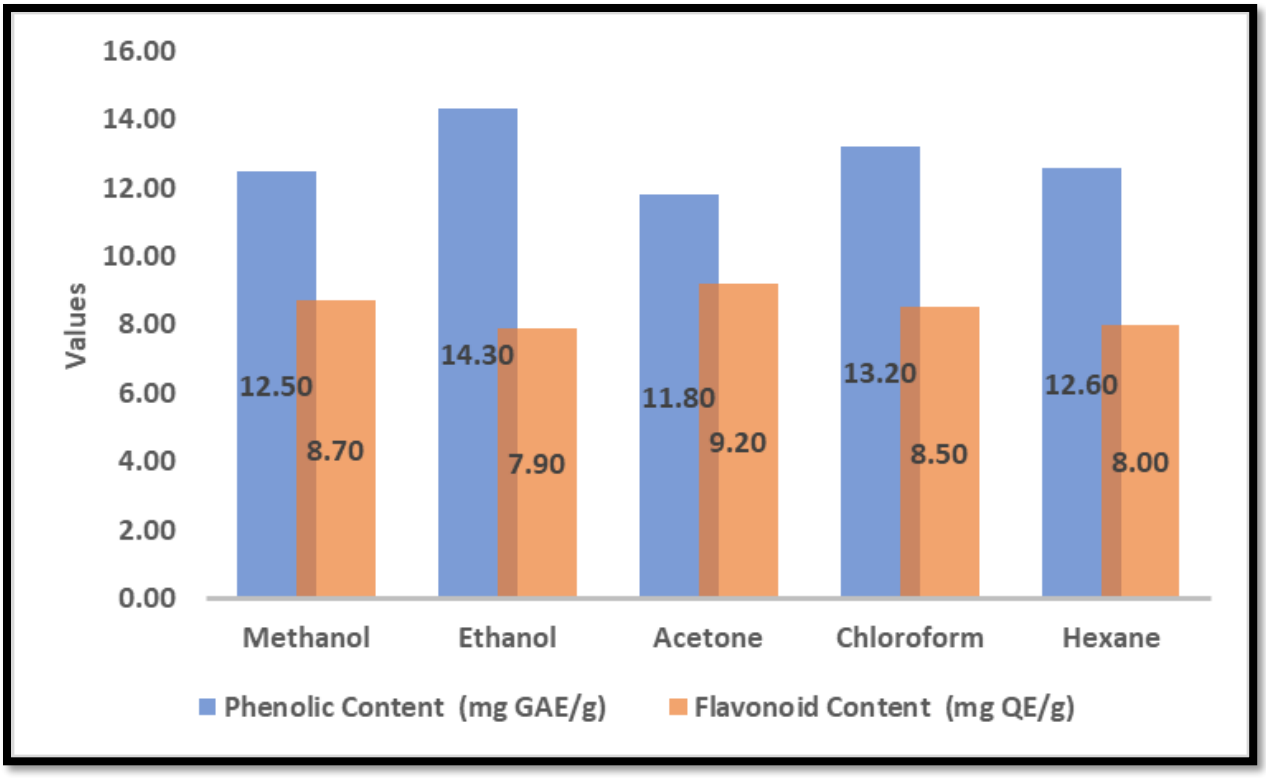

Figure 3: Quantitative estimation of major phytochemicals in different solvent extracts of *O. sanctum*

The results of quantitative estimation of total ash, acid-insoluble ash, and water-soluble ash content was represented in Table 3. Results revealed that highest quantity of total ash content (3.20%) and acid-insoluble ash content (0.90%) was observed acetone extract of *O. sanctum*. Whereas, water-soluble ash content was found to highest (2.50%) in hexane extract of *O. sanctum*. These findings depicted that the total minerals contents of *O. sanctum* were maximally extracted in acetone indicating their optimum solubility in acetone.

Table 3: Quantitative estimation of ash contents in different solvent extracts of *O. sanctum*

Solvent Extracts of *O. sanctum*	Total Ash Content (%)	Acid-insoluble Ash Content (%)	Water-soluble Ash Content (%)
Methanol	2.90	0.80	2.10
Ethanol	2.60	0.70	1.90
Acetone	3.20	0.90	2.30
Chloroform	2.60	0.60	2.00
Hexane	3.60	1.10	2.50

Values are expressed mean; n=3

The results of total ash, acid-insoluble ash, and water-soluble ash content in our study were comparable with the findings of Shamra et al., wherein, total ash value, acid insoluble ash value, and water-soluble ash value were found to be 8.80%, 0.40%, and 3.90%, respectively.[24]

The results of total essential oil yield of different solvent extract of *O. sanctum* was represented in Table 4 and plotted in Figure 4. Results delineated that hexane solvent extract of *O. sanctum* yielded highest total essential oil i.e., 1.10% followed by acetone, methanol, ethanol, and chloroform with total essential oil yield of 0.90%, 0.80%, 0.70%, and 0.60% respectively.

Table 4:Total essential oil yield of different solvent extracts of *O. sanctum*

Solvent Extracts of *O. sanctum*	Total Essential Oil Yield (%)
Methanol	0.80
Ethanol	0.70
Acetone	0.90
Chloroform	0.60
Hexane	1.10

Values are expressed mean; n=3

The results of total essential oil yield of our study were comparable with those reported by Kicel et al., wherein authors reported 0.59–0.83% essential oil yield from *O. sanctum* at different growth stages.[26] However, Zheljazkov et al., reported 0.07–0.34% (% of oil in air-dry herbage) essential oil contents from *O. sanctum* cv. local United States.[27,28] Whereas, Joshi reported 1.20% yield of essential oil from *O. sanctum* from Belgaum. Such variations in the essential oil content of *O. sanctum* across countries might be attributed to the varied agroclimatic conditions of the regions.[29]

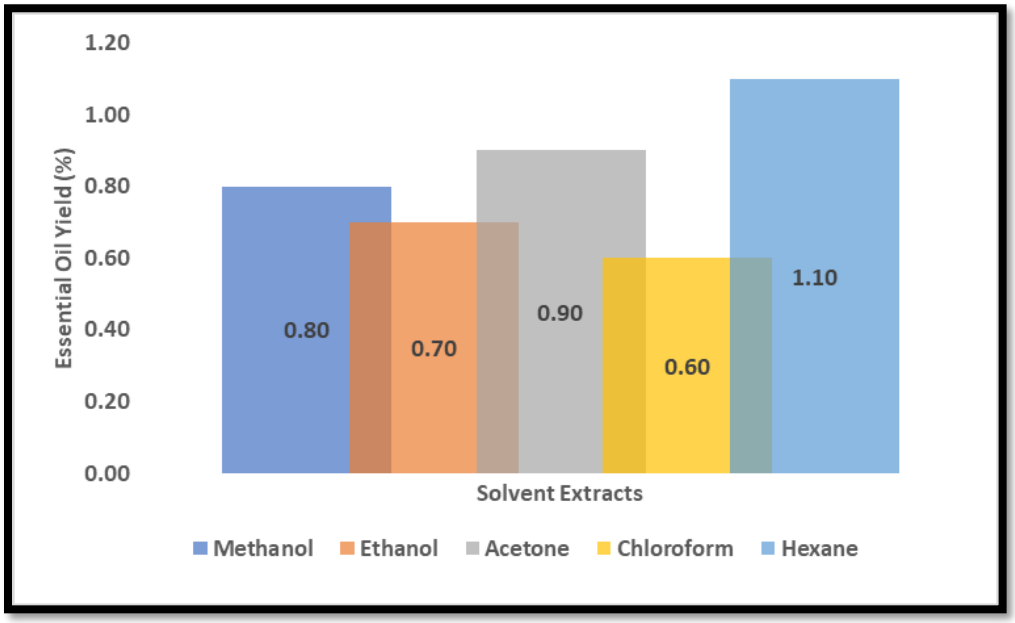

Figure 4: Total essential oil yield of different solvent extracts of *O. sanctum*

Chlorophylls and carotenoids are the two important bioactive molecules in plants. These two bioactive compounds have great applications in herbal medicine.[30]The results of quantitative estimation of photosynthetic pigments *viz.* total carotenoids, anthocyanin, Chlorophyll (Chl)*a*, Chl *b*, and total Chl II was represented in Table 5. Results delineatedthose highest quantities of photosynthetic pigments *viz.* total carotenoids (0.30 mg/g), anthocyanin (0.15 mg/g), Chl *a* (0.55 mg/g), Chl *b* (0.28 mg/g), and total Chl II (0.83 mg/g) were observed in hexane extract of *O sanctum*. Whereas, lowest quantities of photosynthetic pigments *viz.* total carotenoids (0.21 mg/g), anthocyanin (0.10 mg/g), Chl *a* (0.42 mg/g), Chl *b* (0.20 mg/g), and total Chl II (0.62 mg/g) were observed in ethanol extract of *O sanctum*. These findings inferred that the photosynthetic pigments *viz.* total carotenoids, anthocyanin, Chl *a*, Chl *b*, and total Chl II of *O. sanctum* were maximally soluble in hexane.

Table 5: Quantitative estimation of photosynthetic pigments in different solvent extracts of *O. sanctum*

Solvent Extracts of *O. sanctum*	Total Carotenoids (mg/g)	Anthocyanin (mg/g)	Chl *a* (mg/g)	Chl *b* (mg/g)	Total Chl II (mg/g)
Methanol	0.25	0.12	0.48	0.24	0.72
Ethanol	0.21	0.10	0.42	0.20	0.62
Acetone	0.28	0.14	0.52	0.26	0.78
Chloroform	0.23	0.11	0.45	0.22	0.67
Hexane	0.30	0.15	0.55	0.28	0.83

Values are expressed mean; n=3

The concentration of chlorophyll and carotenoids may vary with region, season and leaf conditions. Furthermore, plant pigments concentration can vary depending on different species as well as by local environmental, bio-geological and bio-geochemical factors.[31]

Conclusion

The findings of our study on phytochemical profiling of *O. sanctum* collected from different regions ofWest Bengal, Indiarevealed maximum extraction yield with methanol. The major phytochemicals *viz.* polyphenols and flavonoids were found to be maximally extracted in ethanol and acetone respectively. The total minerals contents and essential oil of *O. sanctum* were maximally extracted in acetone and hexane respectively. The findings of our study depicted that phytochemical profiling of *O. sanctum* extracts may be established as an excipient to prepare various formulations, and these formulations may be explored for implications in foods, nutrition and human health.

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.

Acknowledgments

The authors wish to thank to Director, CSIR-National Botanical Research Institute for providing logistics and research facility for successful conduction of the research work. One of the authors (Siddhartha Sankar Ghosh) thanks VC, YBN University- Ranchi for official work support.

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