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Original Article

A Comparative Evaluation of the Antioxidant and Phytochemical Properties of Various Extracts of *Ocimum Sanctum* Linn Grown in Jaffna District, Sri Lanka

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Abstract

Background and Aim: *Ocimum sanctum* Linn (*O. sanctum*) (Lamiaceae) is a valuable medicinal herb. It contains several therapeutic characteristics due to its bioactive components. This study aimed to compare the phytochemical and antioxidant properties of different extracts of the leaves and flowers of the *O. sanctum* plant, which grows in Sri Lanka.

Materials and Methods: Standard procedures were followed for the qualitative and quantitative phytochemical analyses and the DPPH as well as ABTS antioxidant activities.

Results: The findings demonstrated that the ethanolic and methanolic extracts had adequate concentrations of flavonoids, phenols, tannins, and alkaloids compared with the aqueous extracts of the flowers and leaves of *O. sanctum*. The supreme quantity of the flavonoid content existed in the ethanolic flower extracts (50.88±0.62 µg QE/g) and methanolic leaf extract (49.11±0.27 µg QE/g), while the higher amount of the phenol content (20.96±0.65 µg GAE/g) was found in the ethanolic leaf extract. The methanolic leaf extract had the highest tannin content (409.95±0.66 µg TAE/g) compared with other extracts. Moreover, the highest amount of alkaloid was observed in flowers (75.83±0.55 mg/g) and not in leaves (52.60±0.66 mg/g). Furthermore, the methanolic flower extract exhibited the greatest DPPH (IC_{50}: 0.68±0.19µg/mL) and ABTS (IC_{50}: 1.82±0.32µg/mL) activities compared with the Trolox.

Conclusion: The present study proved that although the leaf extracts contained higher concentrations of phytochemicals, the methanolic flower extract of *O. sanctum* had a greater potential for antioxidants than the methanolic leaf extract.

Keywords: Antioxidant, Comparative evaluation, Various extracts, *Ocimum sanctum,* Phytochemicals, Sri Lanka.

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Introduction

Therapeutic benefits of various diseases might be provided by chemical structures and bioactive composites (therapeutic

ingredients) present in medicinal plants (1). Approximately, 80% of people worldwide still use traditional medicines such as herbal remedies (2, 3). Herbal medicine is an efficient and safe method of treating diseases and infections. Many people worldwide, particularly those living in developing countries, continue getting their medical care from herbal remedies. Even though synthetic drugs are widely available and effective in treating a wide range of disorders in today's culture, some individuals choose to employ traditional folk remedies since they have less adverse effects (4). Nearly 13,000 secondary metabolites, or phytochemicals, including alkaloids, flavonoids, tannins, and phenolic compounds, have been found in medicinal plants. Secondary metabolites in plants act as defensive chemicals or fulfill specific roles, and have healing benefits (pharmacological actions), such as anti-diabetic, analgesic and antioxidant actions (5-8). These natural phytochemicals of many therapeutic plants can help reduce inflammation, protect against oxidative damage, and improve liver function. Moreover, they are mostly determined by the place in which they are grown, the environment of that region, and the technique as well as season of collection (9).

Mahmood (2012) states that *Ocimum sanctum Linn* (*O. sanctum*), often known as holy basil or *tulsi*, is an excellent exemplar of nature's pharmacy (10). *Tulsi* has received a lot of attention in traditional medicine for its rich phytochemical composition and various pharmacological actions in treating a variety of health problems. This perennial plant is highly appreciated in traditional medical systems for its antioxidant, antibacterial, anti-inflammatory, anti-asthmatic, immune-modulatory, anti-stress characteristics and anti-carcinogenic effects (10, 11). Its distinct parts have long been utilized in traditional medicine to manage the conditions, such as bronchitis, malaria, diarrhea, skin conditions, and rheumatism (12). *O. sanctum* has been linked to a number of therapeutic properties (13), and is commonly found in various Asian countries, including India, Sri Lanka, Himalaya, etc. (14, 15). Various chemical components resembling chavicol and linalool present in essential oil derived from *O. sanctum* leaves and flowers are efficiently used in herbal medicine (16).

O. sacntum is commonly utilized in herbal medications in the management of various ailments in the Sri Lankan Traditional System of Medicine. It includes exceptional elements with great biological activities. Nonetheless, more scientific research is required to establish additional therapeutic potentials and pharmacological effects. No research has been published in Sri Lanka, on the efficiency of this plant's phytochemicals and antioxidants utilizing comparable laboratory and scientific methodologies to date. Thus, the purpose of this study was to evaluate the phytochemical and antioxidant potentials of several leaf and flower extracts from the *O. sanctum* plant, which grows in Sri Lanka.

Figure 1. Leaves and Flowers of the *Ocimum Sanctum* L. Plant.

Materials and Methods

Collection of Samples

The *O. sanctum* plant's leaves and flowers were collected in December 2022 from the Jaffna District in Sri Lanka's Northern Province. The created herbarium underwent botanical authentication, and then the voucher specimen was placed in the Department of Botany, Faculty of Science, University of Jaffna.

Preparation of the Samples

To prevent the direct loss of phytoconstituents from sunshine, the samples were carefully allowed to air-dry at room temperature for three weeks after being frequently cleaned with tap water to remove dust and grime (Figure 1-3). The materials that had been shade-dried were ground into a fine powder using a pulverizer and sieved through as many as 80 meshes. For further examination, it was stored separately in sealed bottles at 31±3°C.

Extraction Procedure

Figure 2. The Plant Herbarium of *Ocimum sanctum* L.

Figure 3. Powder Samples of the Leaves and Flowers of the Ocimum Sanctum.

Each sample was extracted with ethanol, methanol and water separately using the cold extraction technique (17). A total of 50 g of powered materials of each sample was separately weighed and placed in 500 ml of culture bottles. 150 ml of 100 % absolute ethanol/ methanol/ water (1:3) was added to it and mixed well separately. Lids of the bottles were covered with parafilm. The solution was kept for 5 days with occasional shaking using shaker at 150 rpm for 15 minutes in every morning and evening. Subsequently, they were filtered through Whatman No.1 filter paper. The part of the filtered content was concentrated using rotatory evaporator (Buchi), and the other part was kept in refrigerator at 4°C for further uses. The analysis was done for three replicates of each extract for both samples.

Qualitative and Quantitative Phytochemical Analysis

The ethanol, methanol, and aqueous extracts of every sample of *O. sanctum* were individually screened for phytochemicals such as flavonoids, phenols, tannins, alkaloids, terpenoids, saponins, glycosides, anthraquinones, etc., using the standard laboratory techniques mentioned by Rajkumar *et al*. 2021, 2022, and 2023 (17-19).

For quantification purposes, total phenol (µg GAE/g) and tannin (µg TAE/g) contents were estimated based on the Folin-Ciocalteu colorimetric method, (20, 21) total flavonoid content (µg QE/g) based on the aluminum colorimetric method, (22) and total alkaloid contents (mg/g) based on Edeoga *et al*. (2005) (23) and Aliyu *et al*. (2008) (24) in each filtered sample of *O. sanctum* using a spectrophotometer (17).

Evaluation of the Antioxidant Activity

The DPPH (1, 1-diphenyl-2-picryl-hydrazyl) and ABTS (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) complementing tests were used to measure the in vitro antioxidant activity of each filtered sample of *O. sanctum* using a spectrophotometer. Trolox served as the standard for comparison. Utilizing the graph and the standard equation, the free radical scavenging activity (%) and IC_{50} value were determined.

***DPPH Free Radical Scavenging Assay*:**

The method defined by Yamaguchi *et al*. 1998 (25) with a few adjustments has been used for this determination (26). A series of different solvent extracts of plant parts (100 µl, 200 µl, 300 µl, 400 µl, 500 µl) were prepared separately. 1 ml of DPPH (0.002g/50 ml) in methanol was added in each sample, and the absorbance was measured at 517nm after incubation for 15 min.

$$\text{Inhibition \% radical DPPH activity} = \frac{Absorbance\ of\ control\ -\ Absorbance\ of\ the\ sample}{Absorbance\ of\ control} x\ 100$$

***ABTS Free Radical Scavenging Assay*:**

The procedure outlined by Re *et al*. (1999) (27) was used to measure this assay. A mixture of ABTS (7 mM) and potassium persulfate (2.5 mM) was prepared and kept at room temperature for 16 hours in a dark room to produce ABTS+. Different solvents of the extracts were mixed with diluted ABTS solution, and the absorbance was measured at 734 nm up to 6 min.

$$\text{Inhibition \% radical ABTS activity} = \frac{(\text{Absorbance of ABTS + methano mixture}) - \text{Absorbance of the sample}}{\text{Absorbance of the sample}} \text{X } 100$$

Statistical Analysis

ANOVA and Tukey's multiple comparisons at probability value ($p \leq 0.05$) were used in the data analysis, which was carried out using Minitab 17 software. Every analysis was carried out in triplicate, and the mean ± standard deviation (SD) was reported as the result of the analysis.

Results and Discussion

Plants are highly significant basis of potentially bioactive elements for the advance of new chemotherapeutic drugs. In the first stage to achieve this intention, leaves and flowers of *O. sanctum* were subjected to systematic qualitative and quantitative analyses by different extracts to determine the phytochemicals and their total contents.

Identification of the Phytochemicals

The *O. sanctum* was subjected to a preliminary phytochemical screening in order to identify its phytochemicals from its various extracts of the leaves and flowers. Table 1 shows that there were more phytochemicals in the methanolic and ethanolic extracts of *O. sanctum* than in the aqueous extract which might be due to the fact that some chemicals may not be properly soluble in the aqueous solvent. Notably, the leaves contained a more diverse range of phytochemicals than the flowers. Different extracts of *O. sanctum* ensure the richness of phenols, flavonoids, tannins, alkaloids, etc. Anthraquinones were consistently absent in all extracts of both leaves and flowers. These constituents might be possibly accountable for the biological activities of *O. sanctum*.

Table 1. Identification of the Phytochemicals in the *O. sanctum*.

Phytochemical	Leaves			Flowers		
	Ethanol	Methanol	Aqueous	Ethanol	Methanol	Aqueous
Flavonoids	++	+++	++	+++	+	+
Phenols	+++	++	-	++	+	-
Tannins	++	+++	+	++	+	-
Alkaloids	++	+	+	+++	+	+
Terpenoids	+	+	+	++	+	++
Saponins	+	-	+	+	-	+
Glycosides	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-
Xanthoproteins	+	+	+	+	-	-
Quinones	+	-	+	-	-	+
Coumarins	-	-	-	+	-	-
Carboxylic acids	+	-	+	-	-	-
Reducing sugars	-	-	+	+	+	+
Proteins and amino acids	++	+	-	++	++	+

The leaves of *O. sanctum* have been a subject of numerous scientific investigations due to their therapeutic potential. The present research is almost comparable to several studies which have focused on the phytochemical composition of the leaves, revealing the occurrence of various secondary metabolites (28-36). The flowers contain essential oils, which contribute to their fragrance and therapeutic properties as antioxidant, and antimicrobial agents (37-39).

Determination of Phytochemicals

Table 2 compares the quantities of four distinct phytochemicals—phenol, flavonoid, tannin, and alkaloid—in the ethanolic, methanolic, and aqueous extracts of *O. sanctum*'s leaf and flower parts. The total content of phytochemicals was attained by using the standard curve for each of these phytochemicals (Figures 4, 5 and 6).

Table 2. Determination of Phytochemicals in the *O. sanctum*.

Samples	Extracts/ Sample	Phenols (µg GAE/g)	Flavonoids (µg QE/g)	Tannins (µg TAE/g)	Alkaloids (mg/g)
Leaves	Ethanolic extract	20.96 ±0.65	41.19±0.24	286.08±0.37	-
	Methanolic extract	14.89±14.89	49.11±0.27	409.95±0.66	
	Aqueous extract	8.53±0.48	26.54±0.49	242.12±0.34	
	Powder sample	-	-	-	52.60±0.66
Flowers	Ethanolic extract	15.53±0.44	50.88±0.62	297.16±0.56	-
	Methanolic extract	7.53±7.53	23.88±0.76	261.00±0.48	
	Aqueous extract	4.59± 0.13	18.05±0.39	140.54±0.61	
	Powder sample	-	-	-	75.83±0.55

The maximum amount of flavonoid content was present in the ethanolic flower extracts (50.88± 0.62 µg QE/g) and methanolic leaf extract (49.11±0.27 µg QE/g), while the higher amount of phenol content (20.96 ± 0.65 µg GAE/g) was found in the ethanolic leaf extract. The methanolic leaf extract showed the highest tannin content (409.95±0.66 µg TAE/g) compared with other extracts. Moreover, the highest amount of alkaloid was present in flowers (75.83±0.55 mg/g) and not in leaves (52.60±0.66 mg/g). The present study proved that most of the phytochemicals as phenols, flavonoids and tannins were higher in leaves than flowers of *O. sanctum* plant.

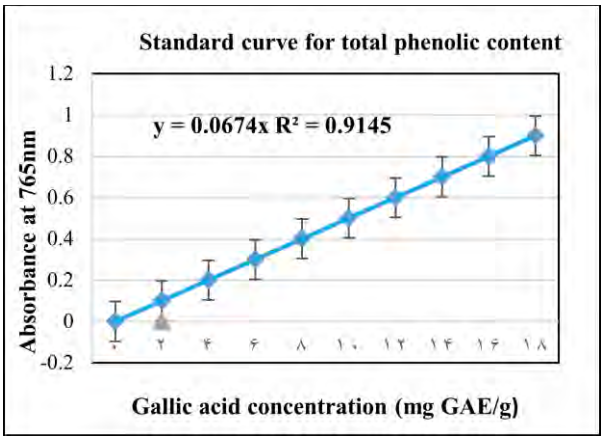

Figure 4. Standard Curve for Total Phenolic Content.

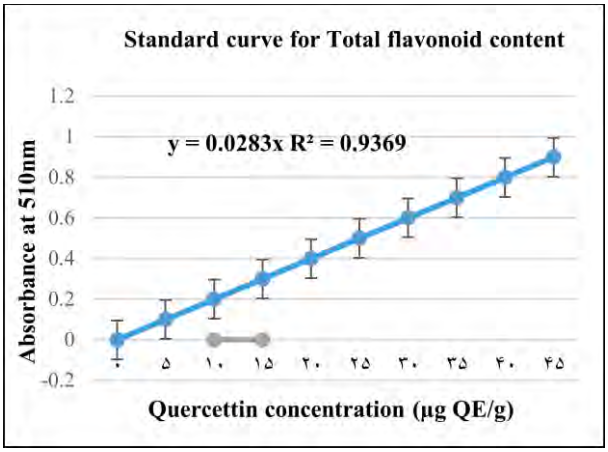

Figure 5. Standard Curve for Total Flavonoid Content

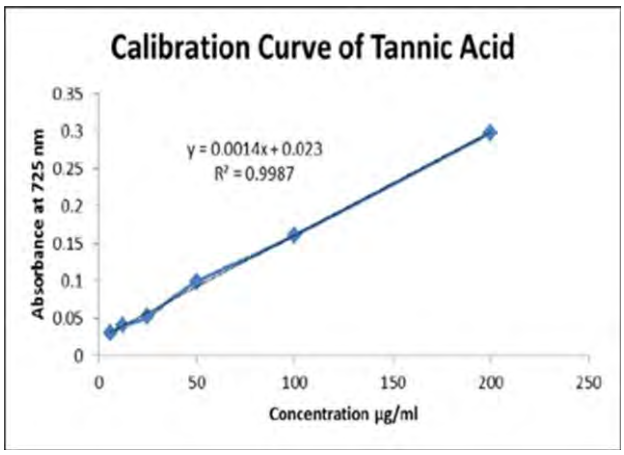

Figure 6. Standard Curve for Total Tannin Content

As it has been indicated in Table 3, the results of the present study for total phenol and flavonoid contents for the methanolic leaf extract of *O. sanctum* were not consistent with the results of previous studies conducted by Singh *et al*., 2014; Pathak & Niraula, 2019; Chaudhary *et al*., 2020 and Singh *et al*., 2020 (34, 35, 40, 41). Variances in the preparation process, sample storage technique, and seasonal or geographic variances in the environment from which plant materials were taken, or the standardization method employed could all be the causes of these discrepancies.

A class of metabolites known as phenolic compounds is produced by plants' secondary pathways. Flavonoids, phenolic acids, tannins, and coumarins are naturally occurring materials that can be found in a variety of plant products (42, 43). According to recent research, phenolic compounds may protect the body against diseases caused by oxidative stress (42-45).

Table 3. A Summary of Phytochemicals in the Methanolic Leaf Extract of *O. sanctum*.

Phytochemical	The present study	Singh *et al*., 2020	Chaudhary et al., (2020)	Pathak and Niraula, 2019	Singh et al., 2014
Total phenols (µg GAE/g)	14.89±14.89	703.12±0.00	87.13±4.6	180.21±0.89	4.49 - 9.31
Total flavonoids (µg QE/g)	49.11±0.27	100±0.0056	221.97±4.6	67.11±0.43	14 - 225

Epidemiological studies maintain that extensive period consumption of diets rich in plant polyphenols offer protection against the progress of chronic illness (46-48). Flavonoids are a significant class of natural compounds that are derived from plants and have an extensive array of positive health effects. They are efficiently used as pharmacological, therapeutic and nutraceutical agents. This is explained by their ability to modify the activities of important cellular enzymes as well as their antioxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic qualities (49). Plant polyphenols, called tannins, are bitter and astringent agents. They can precipitate or bind to proteins as well as alkaloids (50-52). This tannin-protein complex can provide persistent antioxidant activity (53-55). Alkaloids are predominantly found in plants, with some families of flowering plants having higher concentrations than others (56). Alkaloids generated from plants also have antioxidant properties (57).

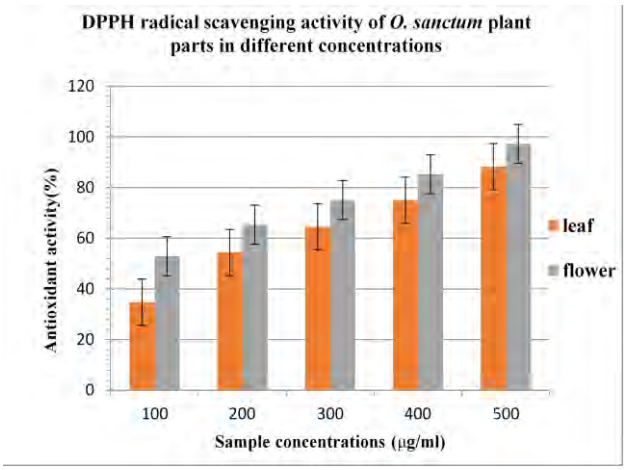

Figure 7. DPPH Radial Scavenging Activity of Different Parts

In Vitro Antioxidant Activity

Variations in the antioxidant activity of the methanolic extract of leaves and flowers of *O. sanctum* based on DPPH and ABTS assays have been indicated in Table 4 and Figures 7 and 8.

Table 4. DPPH and ABTS Antioxidant Activity of the Methanolic Extract of *O. Sanctum*.

Sample	DPPH (IC_{50}µg/ml)	ABTS (IC_{50} µg/ml)
Leaf	1.95 ± 0.82	2.59 ± 0.44
flower	0.68 ± 0.19	1.82 ± 0.32

Mean ± Standard Deviation

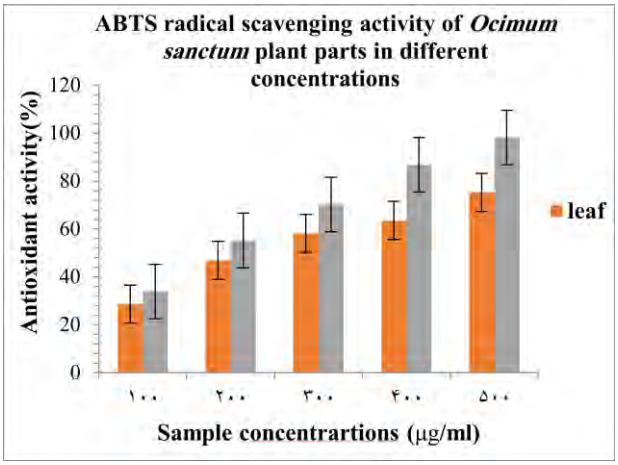

Figure 8. ABTS Radial Scavenging Activity of Different Parts of *O. Sanctum*

Although the leaves and flowers of *O. sanctum* have been documented to possess significant antioxidant potentials, a comparative assessment of their antioxidant activities is essential for understanding their beneficial value.

As stated in Table 4, the maximum radical scavenging activity (as characterized by the lowest IC_{50}) was observed in *O. sanctum* flowers followed by leaves in both DPPH and ABTS assays. Regarding the DPPH test, the methanolic extract of the flowers (IC_{50}: 0.68±0.188 µg/ml) exhibited the best radical scavenging activity in comparison with the methanolic extract of the leaves (IC_{50}: 1.95±0.82 µg/ml) when tested with Trolox. In case of ABTS assay, the flower methanolic extract (IC_{50}: 1.82±0.32 µg/ml) exhibited the best radical scavenging activity in comparison with the leaf methanolic extract (IC50: 2.59±0.44 µg/ml). According to the results, both the leaves and the flowers had significant quantities of antioxidant activity, even though the antioxidant activity of the flowers was higher. Since methanol dissolves many antioxidant compounds efficiently, these assays were performed against methanol extracts. According to the study's findings, the plant's leaves and flowers might provide a valuable supply of natural antioxidants that could be used to treat

degenerative disorders associated with oxidative stress.

Many studies have shown that the *O. sanctum* extract has greater DPPH antioxidant activity than ascorbic acid (58, 59), and that commercially available *O. sanctum* powder demonstrates higher degrees of DPPH radical scavenging activity than vitamin C (60). According to Chaudhary *et al*. (2020) (35), the methanolic leaf extract of *O. sanctum* exhibited antioxidant activity as demonstrated by the DPPH and ABTS assays. The main way that antioxidants work is by scavenging free radicals, which either prevents or delays cellular damage. Natural substances found in dietary supplements and medicinal plants are increasingly being used as therapeutic antioxidants (61). An *in vivo* study indicated that the potential antistressor activity of *O. sanctum* could be partially attributable to its antioxidant properties (62).

Conclusion

The findings of the present study revealed that the ethanolic and methanolic extracts had greater concentrations of flavonoids, phenols, tannins, and alkaloids than the aqueous extracts of the flowers and leaves of *O. sanctum*. The extreme quantity of flavonoid content was found in the ethanolic flower extract and methanolic leaf extract, while the advanced quantity of phenol content was found in the ethanolic leaf extract and the highest tannin content was present in the methanolic leaf extract. Moreover, the highest amount of alkaloid was present in flowers than in leaves, and the methanolic flower and leaf extracts exhibited considerable DPPH and ABTS radical scavenging activities related to the Trolox. This comparative study proved that despite the fact that the leaf extracts contained higher concentrations of phytochemicals, the methanolic flower extract of *O. sanctum* had a better potential for having antioxidant properties than the methanolic leaf extract. Thus, it could be used as a potential therapeutic ingredient in the development of functional medications and its utilization would be a viable alternative to combat various non-communicable diseases.

Acknowledgment

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

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