Exploring the Phytochemical Constituents and Bioactivities of *Ocimum sanctum* (Tulsi)

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Abstract

Ocimum sanctum is a medicinal plant that has been used in traditional Chinese medicine for treating oxidative stress-related conditions and microbial infections. This study aims to investigate the variation in secondary metabolite profiles of *Ocimum sanctum* plants grown under field conditions at Trai region of elevated land. Phytochemical screening was performed to detect alkaloids, amino acids, carbohydrates, phenols, flavonoids, terpenoids, coumarins, and glycosides. The ethanol extract (TE) was tested for antioxidant activity using the DPPH radical scavenging assay. Antimicrobial activity was evaluated against *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP), *Salmonella sonai* (SS), and *Escherichia coli* using the agar well diffusion method. Phytochemical analysis revealed several bioactive compounds. The TE extract showed concentration-dependent antioxidant activity but was less potent than Quercetin. TE exhibited moderate antimicrobial activity against all tested bacterial strains, suggesting potential use in managing certain infections. Overall, the findings from this study support the traditional use of *Ocimum sanctum* in herbal medicine, particularly in treating infections.

Keywords: Antimicrobial, DPPH Assay, *Ocimum sanctum*, Phytochemicals, Secondary Metabolite

Introduction

Nepal is celebrated for its unparalleled biodiversity, offering a rich tapestry of medicinal plants that has been integral to traditional health practices for centuries. Among these, *Ocimum sanctum*, commonly known as Holy Basil, stands out due to its profound cultural and medicinal significance (Pooja & Kumar, 2023). This revered plant belongs to the Lamiaceae family and is a cornerstone of Hindu traditions, esteemed for its diverse therapeutic properties and its role in Ayurvedic and Unani medicine systems (Murkar et al., 2023).

Ocimum sanctum holds a revered place in Hindu culture, celebrated for its profound spiritual and health benefits. Known as the "elixir of life" in Ayurvedic

medicine, it is praised for enhancing longevity, balancing bodily functions, and promoting well-being (Nagbanshi & Mishra, 2024). Its adaptogenic properties help the body adapt to stress, boosting resilience against environmental and psychological stressors. Botanically, it is an aromatic, much-branched sub-shrub, typically 30-60 cm tall, and is widely cultivated across tropical regions, including Nepal, particularly around the Himalayan foothills and plains, due to its adaptability (Singh & Chaudhuri, 2018).

The therapeutic value of *Ocimum sanctum* is attributed to its rich composition of secondary metabolites, including polyphenolic compounds, flavonoids, alkaloids, saponins, and tannins. These metabolites are known for their antioxidant properties, which play a crucial role in neutralizing free radicals and mitigating oxidative stress (Devi et al., 2024). The concentration and efficacy of these compounds can vary depending on environmental stressors such as water availability, temperature fluctuations, and soil conditions. This variation in metabolite profiles influences *Ocimum sanctum's* medicinal effectiveness and presents challenges in standardizing its therapeutic use (Das et al., 2020).

It is rich in various chemical constituents that contribute to its medicinal properties. Key compounds include eugenol, known for its antiseptic and anti-inflammatory effects; ursolic acid, with antioxidant, anti-inflammatory, and anticancer activities; and rosmarinic acid, an antioxidant with antiviral properties (Dobhal et al., 2024). Additionally, it contains apigenin and luteolin, flavonoids with anti-inflammatory and antioxidant benefits; caryophyllene and camphor, which provide anti-inflammatory and antimicrobial effects; and linalool, known for its calming properties (Pooja & Kumar, 2023). Other important constituents are beta-sitosterol, which has cholesterol-lowering effects, and oleanolic acid, recognized for its hepatoprotective and anticancer properties (Hasan et al., 2023). These diverse compounds make *Ocimum sanctum* a potent adaptogen and therapeutic agent (Dobhal et al., 2024).

This study investigates the variation in secondary metabolite profiles of *Ocimum sanctum* grown in elevated regions of the Terai, focusing on analysing the phytochemical composition and antioxidant capacity of water and ethanol extracts. By examining how environmental factors at higher elevations influence the production of bioactive compounds such as flavonoids, phenols, terpenoids, and alkaloids, the research aims to uncover potential differences in metabolite concentration and activity. Antioxidant capacity will be assessed using DPPH and FRAP assays, providing a comparative evaluation of the efficacy of both extracts. The findings will offer valuable insights into the impact of altitude on metabolite synthesis, guiding optimal cultivation practices for enhancing therapeutic properties and supporting the development of natural antioxidants and antimicrobials. By using ethanol solvent condition of extract, the research seeks to identify potential constituents and biochemical activity of *Ocimum sanctum*-based

herbal preparations (R & P, 2018). The findings are expected to contribute to more consistent and clinically effective *Ocimum sanctum*-based treatments, thereby enhancing their therapeutic value and reliability (Jamshidi & Cohen, 2017).

By integrating traditional knowledge with modern scientific techniques, the research aims to bridge the gap between ethnobotanical knowledge and contemporary drug discovery. The goal is to enhance our understanding of *Ocimum sanctum*'s potential, support the development of new herbal medicines, and contribute to the sustainable cultivation and utilization of this valuable medicinal plant. This approach not only honours the traditional uses of *Ocimum sanctum* but also seeks to validate and expand its role in modern therapeutic practices, ensuring that its benefits can be consistently harnessed and applied in contemporary medicine.

Method and Methodology

Sample Collection

The plant material of *Ocimum sanctum* was collected in January 2022 from the local area of Lahan, Siraha at some elevated land, Nepal. The selected plant parts were carefully removed and washed under running tap water to eliminate any dirt.

Sample Preparation

After collection, the plant material was dried in the shade for one week. Once dried, the plant parts were ground into a fine powder using an electric grinder. For extraction, 150 g of the powdered sample was soaked in 1000 to 1500 ml of methanol and distilled water in a conical flask separately, shaken occasionally to ensure proper mixing, and macerated for 72 hours at room temperature. This maceration process helps to soften and break the plant's cell walls, facilitating the release of soluble phytoconstituents. After filtration samples are named as *Ocimum sanctum* in ethanol (TW) for water extract and TE for ethanol extract. To get solid sample solvent was evaporated stored in 2ml vial (Abubakar & Haque, 2020).

The sample preparation and the phytochemical analysis were conducted at the Chemistry Laboratory, J. S. M. M. Campus, Lahan. A Phoenix electronic analytical balance was used for accurately weighing the powdered sample, followed by shaking in a shaker with different solvents for solution preparation. Various test-tube tests were then performed using different chemicals to carry out the phytochemical analysis. Additionally, the antimicrobial and antioxidant tests were performed at the Central Department of Chemistry, Tribhuvan University.

Phytochemical Screening Tests

Phytochemical screening of the extract was conducted using standard procedures and chemical reagents to identify various phytoconstituents. Alkaloids were detected with Meyer's test, showing a white creamy precipitate. Amino acids

were tested with the Xanthoprotein test, where an orange colour appeared upon adding concentrated nitric acid and sodium hydroxide, indicating aromatic amino acids. Carbohydrates were identified using Molish's test, which produced a reddish ring. Phenols were tested with $FeCl_3$, resulting in a dark green or reddish-brown colour. Flavonoids were screened using the alkaline test, where yellow fluorescence signalled their presence. Tannins were detected with $FeCl_3$, indicated by a green colour. Saponins were identified through the foam test, showing stable foam formation. Terpenoids were tested with Salkowski's test, which produced a yellow or reddish-brown colour. Quinine was detected with the hydrochloric test, showing a yellow colour, while coumarins were identified using the sodium hydroxide test, resulting in a yellow colour. Glycosides were screened with the Kellar–Kilianis test, where reddish-brown or blue-green colour indicated their presence. Finally, anthocyanins were tested with sulfuric acid, showing a yellowish-orangecolour (Palaramb et al., 2023; Shridhar & Kumar, 2023).

Determination of Antioxidant Activity

The antioxidant activity was measured using a modified colorimetric method in a 96-well plate. A 0.1 mM DPPH solution was prepared by dissolving 3.9 mg of DPPH in 100 mL methanol, protected from light. Quercetin stock solution (1.0 mg/mL) was diluted to final concentrations of 170, 190, 60, 20, and10 µg/mL, and also the plant extracts were prepared in 50% DMSO with same concentrations as Quercetin. In the assay, 100 µL of each solution or 50% DMSO (negative control) was added to the wells, followed by 100 µL of DPPH solution. After 30 minutes of incubation in the dark, absorbance was measured at 517 nm, and the DPPH radical scavenging activity was calculated as the percentage inhibition (Chaves et al., 2020).

$$\text{Inhibition (\%)} = \frac{A\ control - A\ sample}{A\ control} \text{X100}$$

Where $A_{control}$ is absorbance of DPPH solution without the sample and A_{sample} is absorbance of the DPPH with the sample.

Antimicrobial Assay

The antimicrobial activity of TW and TE extracts against *Staphylococcus aureus* (SA), *Klebsiella pneumoniae* (KP), *Salmonella sonai* (SS), and *Escherichia coli* (E. coli) was evaluated using the agar well diffusion method. Bacterial cultures were spread on nutrient agar plates, and wells were filled with 50mg/mL concentrations of each extract dissolved in 50% DMSO solution. After incubation at 37°C for 24 hours, the zones of inhibition were measured in millimetre(mm) to assess antimicrobial effectiveness (Bubonja-Šonje et al., 2020).

Data analysis

Data analysis was done by using MS excel.

Results

Phytochemical analysis

The phytochemical screening of *Ocimum sanctum* ethanol extract (TE) revealed the presence of various bioactive compounds, confirmed through distinct qualitative tests.

Table 1 Phytochemical analysis

Phytochemicals	Methanolic extract
Alkaloids	+
Amino acids	+
Carbohydrate	+
Phenol	+
Flavonoids	+
Tannin	-
Saponin	-
Terpenoid	+
Quinones	-
Coumarine	+
Glycoside	+
Anthocyanin	-

Key: + = present and - = absent

Alkaloids were identified via Mayer's test, indicated by the formation of a white creamy precipitate. Aromatic amino acids were detected using the Xanthoprotein test, which produced an orange color. Carbohydrates were confirmed through Molisch's test, showing a characteristic reddish ring. Phenols were detected using the $FeCl_3$ test, which resulted in a dark green or reddish-brown color, while flavonoids were confirmed by the alkaline test, producing yellow fluorescence. Tannins were also detected with the $FeCl_3$ test, indicated by a green color. Saponins were identified using the foam test, where stable foam formation confirmed their presence. Terpenoids were detected by Salkowski's test, indicated by a yellow or reddish-brown color. Quinine was confirmed through the hydrochloric acid test, where a yellow color indicated its presence. Coumarins were identified using the sodium hydroxide test, producing a yellow color, and glycosides were detected using the Keller-Kiliani test, indicated by a reddish-brown or blue-green color. Finally, anthocyanins were confirmed by sulfuric acid treatment, resulting in a yellowish-orange color. These findings highlight the diverse phytochemical profile of *Ocimum sanctum*, with each compound verified through specific extraction and detection methods.

Antioxidant Activity

The antioxidant potential of *Ocimum sanctum* extract prepared in ethanol (TE) was evaluated using the DPPH radical scavenging assay. In this assay, different concentrations of the ethanol extract were tested to determine its ability to

neutralize free radicals, measured by the percentage inhibition of the DPPH radical. The assay involved adding a methanol-based DPPH solution to varying concentrations of the TE extract. After 30 minutes of incubation in the dark, the absorbance was measured at 517 nm and % scavenging as shown in Table 2 and graphically in Figure 1 and 2, and the percentage inhibition and IC50 of radical scavenging activity was calculated which was found 195.243µg/mL for Quercetin and 341.315 µg/mL for *Ocimum sanctum.*

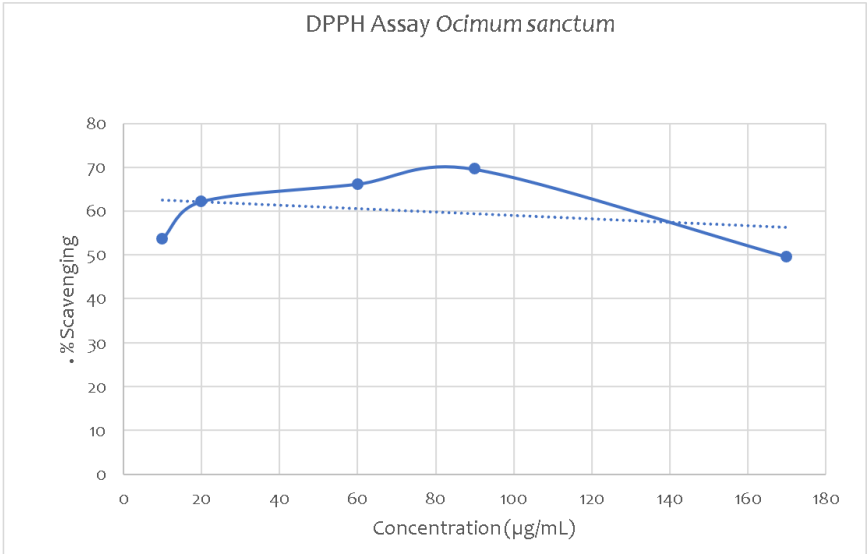

Figure 1 Graphical Representation of %Scavenging of different concentration of *Ocimum sanctum*

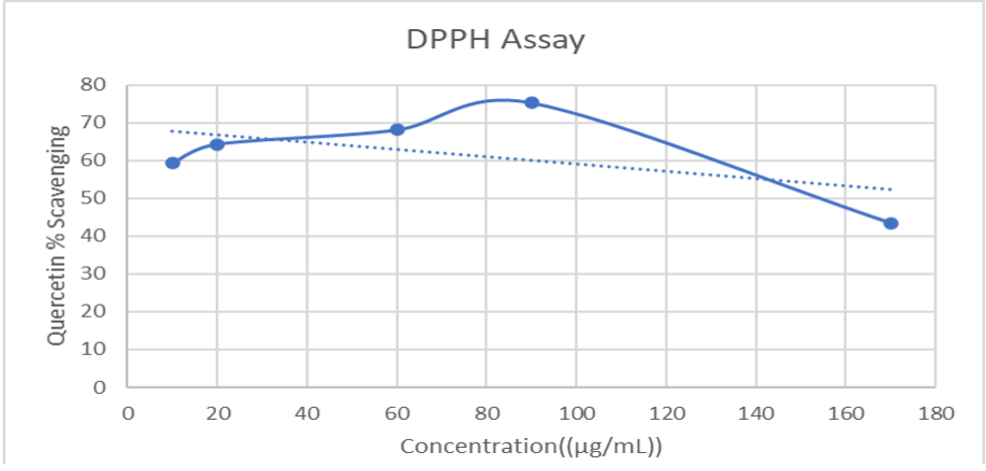

Figure 2. Graphical representation % Scavenging of Quercetin

The results showed that the TE extract exhibited concentration-dependent antioxidant activity, with higher concentrations generally showing greater inhibition percentages. This indicates that the ethanol extract has a significant capacity to neutralize free radicals, contributing to its potential as a natural antioxidant.

Table 2. %Scavenging of Ethanol extract and Quercitrin

Concentration (µg/mL)	Plant Extract % Scavenging	Quercetin % Scavenging
(Control)	0.00	0.00
10	53.65	59.32
20	62.23	64.34
60	66.23	68.12
90	69.63	75.34
170	49.57	43.45

Antimicrobial Assay

After incubation at 37°C for 24 hours, the zones of inhibition around the wells containing the TE extract were measured which are shown in **Table 3.**

Table 3. Zone of inhibition of *Ocimum sanctum* extract

Bacteria	ZOI of TE (mm)	ZOI of Neomycin(mm)
Staphylococcus aureus	14	25
Klebsiella pneumoniae	12	23
Salmonella sona	12	24
Escherichia coli	10	19

Discussion

The phytochemical screening of Ocimum sanctum extract prepared in ethanol (TE) revealed a diverse array of bioactive compounds, suggesting the plant's significant therapeutic potential. The presence of alkaloids, amino acids, carbohydrates, phenols, flavonoids, terpenoids, coumarins, and glycosides in the TE extract indicates a complex chemical profile that likely contributes to its biological activities. The detection of these compounds aligns with previous studies that have highlighted the medicinal properties of *Ocimum sanctum*, commonly known as Tulsi in traditional medicine systems. Interestingly, tannins, saponins, quinones, and anthocyanins were absent in the extract, which might be attributed to the solvent used or the specific extraction method (Pooja & Kumar, 2023). The absence of these compounds does not necessarily diminish the extract's efficacy but rather highlights the selectivity of the extraction process in isolating certain phytochemicals over others.

The antioxidant activity of the TE extract, as evaluated through the DPPH radical scavenging assay, demonstrated a concentration-dependent inhibition of

free radicals. The observed IC_{50} value of 341.315 µg/mL for the TE extract suggests that it possesses a moderate ability to neutralize free radicals, though less potent than Quercetin, a known antioxidant, which exhibited an IC_{50} value of 195.243 µg/mL. The lower IC_{50} value of Quercetin compared to the TE extract indicates its higher effectiveness in scavenging DPPH radicals (http://dx.doi.org/10.5897/JMPR2019. 6880). However, the substantial antioxidant activity of the TE extract underscores its potential as a natural source of antioxidants, which could be further explored for therapeutic applications. The concentration-dependent increase in scavenging activity observed in the TE extract suggests the presence of bioactive compounds that interact synergistically to enhance the overall antioxidant effect. This is consistent with the presence of phenolic compounds such as flavonoids and phenols, which are known for their strong antioxidant properties.

The antimicrobial assay results highlight the ethanol extract's potential as an antimicrobial agent against various bacterial strains. The TE extract demonstrated inhibitory effects on all four tested bacteria, with zones of inhibition (ZOI) ranging from 10 to 14 mm. Although the zones of inhibition were smaller than those produced by Neomycin, a standard antibiotic, the results are significant in demonstrating the plant's natural antimicrobial properties. The highest ZOI of 14 mm was observed against Staphylococcus aureus, a common gram-positive pathogen, indicating the extract's effectiveness against this bacterium(Kumar et al., 2018). This suggests that the TE extract could be particularly useful in treating infections caused by gram-positive bacteria. The lower ZOI observed against Escherichia coli (10 mm) indicates a relatively weaker activity against gram-negative bacteria, which is often the case with plant-based antimicrobials due to the more complex cell wall structure of these organisms(Kumar et al., 2018).

Overall, the findings from this study support the traditional use of *Ocimum sanctum* from the elevated regions of the Terai in herbal medicine, particularly for treating oxidative stress-related conditions and microbial infections. The presence of multiple bioactive compounds in the ethanol extract (TE), combined with its significant antioxidant and antimicrobial activities, suggests that *Ocimum sanctum* from this specific locality could be a valuable source of natural therapeutics. Further research is warranted to isolate specific compounds responsible for these activities and to explore their mechanisms of action. Additionally, investigating the extract's efficacy in vivo models could provide deeper insights into its potential clinical applications and validate its traditional use in the Terai region.

Conclusion

This study highlights the phytochemical richness and bioactivity of *Ocimum sanctum* ethanol extract (TE), confirming its potential as a natural source of therapeutic agents. The phytochemical screening revealed the presence of several bioactive compounds, including alkaloids, amino acids, carbohydrates, phenols, flavonoids, terpenoids, coumarins, and glycosides, which are likely contributors to

its observed biological activities. The TE extract exhibited significant antioxidant activity, as demonstrated by its ability to scavenge DPPH radicals in a concentration-dependent manner. Although Quercetin showed greater potency in comparison, the antioxidant capacity of the TE extract underscores its potential for use in managing oxidative stress-related conditions.

Additionally, the TE extract demonstrated antimicrobial activity against multiple bacterial strains, particularly Staphylococcus aureus, suggesting its utility in combating certain infections. While the observed antimicrobial effects were less potent than those of standard antibiotics, the findings support the traditional use of *Ocimum sanctum* in herbal medicine for treating infections.

Ocimum sanctum ethanol extract possesses both antioxidant and antimicrobial properties, making it a promising candidate for further research and development as a natural therapeutic agent. Future studies should focus on isolating and characterizing the specific compounds responsible for these activities, as well as evaluating their efficacy in clinical settings.

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