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Evaluation of *Ocimum sanctum*'s Leaves Extract for Antibacterial Effect

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Abstract: The study's aim was to evaluate the phytochemical contents of *Ocimum sanctum* leaves by using the right extraction methods and liquids, as well as to see how well the leaf extract killed germs. In this study, 100% pure ethanol was used to do both Soxhlet and maceration extractions on fresh *Ocimum sanctum* leaves. The antibacterial activity of the *Ocimum sanctum* extract was evaluated using the DCPIP method, which involved comparing the clear blocking zone of the standard drug to the extracts on Mueller Hinton agar. Certain phytochemical assays, including those for carbohydrates, alkaloids, and terpenoids, demonstrated that the *Ocimum sanctum* extract was effective. Because of the extremely low concentrations of extracts utilised, every OSESE concentration utilised for antibiotic screening yielded negative results. It is evident that *Ocimum sanctum* holds significant importance as a botanical remedy. Long-term investigation is required to determine the medicinal applications of extracts produced using various solvents that are capable of extracting significant quantities of purified chemical elements from plants.

Keywords: Phytochemical, Ethanol, Soxhlet, Antibacterial, *Ocimum sanctum*, Maceration

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Introduction

Tulsi, *Ocimum sanctum* also known as sacred basil, held significant importance in the ancient Ayurvedic and Unani traditions. *Ocimum sanctum* is believed to have curative properties in India for a variety of ailments, including bronchial asthma, malaria, bronchitis, dysentery, diarrhoea, skin issues, rheumatoid arthritis, elevated body temperature, insect stings, and severe ocular problems (Ahire *et al.*, 2020). Its main effects are to fight cancer, fight fungus, lower blood sugar, fight germs, and ease pain. It can also help with sickness and vomiting, keep the liver and heart healthy, and make you sweat. It makes the circulatory system, the immune system, the gut tract, the urine system, and the reproductive system work better (Jarouliya *et al.*, 2015; Chaudhary *et al.*, 2020). The plant can grow in both hot and subtropical areas Too acidic, too salty, or too wet is not good for growing it. The earth must be at least moderately hard (Suriyavanthana and Punithavanthi, 2017).

A lot of investigators have been looking into the possible health benefits of some parts of tulsi for the immune system, reproductive system, heart, and other systems in recent years (Patil *et al.*, 2023). Researchers have also looked into how holy tulsi can help people with a variety of health issues and what the scientific evidence is for these healing qualities. Tulsi is famous for protecting important human organs and cells from chemical stresses caused by fossil fuel emissions and frequent industrial pollution (Surana *et al.*, 2022). It can also protect against physical stresses caused by long-term physical exhaustion and restraint caused by a number of physical problems and noise exposure from loud and excessive ones (Singh *et al.*, 2017). The study's aim was to evaluate the phytochemical contents of *Ocimum sanctum* leaves by using the right extraction methods and liquids, as well as to see how well the leaf extract killed germs.

Materials and Methods

Collection and preparation of plant materials:

Fresh and green leaves of *Ocimum sanctum* were collected locally. After being washed with clean water, the leaves were carefully picked off the trees by hand. The split leaves were weighed and then left to dry outdoors. The dried leaves were cut into very small pieces by hand (Furquan *et al.*, 2023; Fakir *et al.*, 2023).

Maceration:

5 g of leaves of *Ocimum sanctum* were mixed with 400 ml of 100% ethanol in a round-bottom flask. The flask was kept in the dark and covered with metal foil for seven days. The round-bottom flask was shaken the whole time to make sure that the extraction was even and complete. The mix was filtered through a clean Muslin cloth, and the filtrate was collected in a clean beaker. The maceration filtrate that had been separated and the leftover maceration extract were kept in the cabinet to be screened further (Bhandari *et al.*, 2022; Aher *et al.*, 2023; Sonawane *et al.*, 2023).

Soxhlet extraction:

30 g of the powdered leaves *Ocimum sanctum* were put into a thimble that had chromatography paper around outside. 400 ml of ethanol was added to the round-bottom flask of the Soxhlet device to help with the extraction. The temperature was kept at 70°C throughout the process. It took almost 30 h to finish the process and get rid of the colour extract. The maceration filtrate that had been separated and the leftover maceration extract were kept in the cabinet to be screened further (Emad *et al.*, 2021; Pardeshi *et al.*, 2024).

Evaporation:

In a rotating evaporator, the extract (Soxhlet and Maceration extracts) was evaporated. Throughout the evaporation and concentration, the temperature was maintained at 70°C. Following evaporation, the Soxhlet and maceration extracts were 90 ml and 25 ml, respectively (Anusmitha *et al.*, 2022; Surana *et al.*, 2022).

Table1: *Ocimum sanctum* extracts were titrated for total ascorbic acid concentration using an average volume

	<i>Ocimum sanctum</i>
Average Volume of DCPIP used (ml)	1.52

Table 2: Protein estimation by Bradford assay

S. No.	Solution	BSA volume (µl)	H ₂ O volume(µl)	Absorbance
1	BLANK	00	100	-
2	BLANK	20	80	0.449
3.	BLANK	40	60	0.610
4.	BLANK	60	40	0.632
5.	BLANK	80	20	0.652
6.	BLANK	100	0	0.678
7.	<i>Ocimum sanctum</i>	100	-	0.183

Table 3: Observations from the *Bacillus subtilis* zone of inhibition as determined by adjusting the amounts of *Ocimum sanctum* extracts made in various solvents

Concentration in g/ml	Zone of inhibition (mm) (ethanolic extract)	Zone of inhibition (mm) (methanolic extract)	Zone of inhibition (mm) (aqueous extract)
0.2	00	3	0
0.4	00	6	0
0.6	5.0	9	7
0.8	13	13	9
1.0	16	17	12

Methodology for determination of antibacterial activity:

Bacillus subtilis culture growing on a spread plate was used for studies on the zone of inhibition caused by *Ocimum* extracts made in ethanolic (total ethanol), methanolic, and water extracts. Whatman Filter paper 42 discs were used for the zone of suppression tests. 20 ml of liquid was used to crush 10 g of *Ocimum* leaves. The leaves were then dried in an oven at 40°C for 24 h to make the solvent extracts. The powders were then mixed with the right liquids to make amounts of 0.2, 0.4, 0.6, 0.8, and 1.0 g/ml. These were then used for study on the zone of inhibition. Three replicates of each experiment were done, and the first zone of inhibition study that was done was used as a guide (Behera *et al.*, 2010; Keservani *et al.*, 2017; Keservani *et al.*, 2023).

Results and Discussion

Ocimum sanctum leaves contain phlobotannin, terpenoids, flavonoids, and quinone. All of them had varying quantities of ascorbic acid; however,

it contained the most. It was also observed that Krishna Tulsi has the highest protein content based on the absorption values. Using the zone of inhibition and disc diffusion experiment, the species' ability to kill *Bacillus subtilis* was investigated. Compared to the ethanolic and aqueous extracts, the methanolic extract exhibited a larger zone of inhibition (Tables 1-3).

More pharmacological research is required, but these plants could prove to be a rich source of chemicals with potential antibacterial properties. The results of this research might help chemists create more focused and potent antibacterial compounds employing the species of *Ocimum sanctum*. Furthermore, the findings support the efficacy of *Ocimum* spp.'s use in medicine throughout historical medical traditions and imply that certain plant extracts include antimicrobial compounds that may be incorporated into novel medications to treat infectious diseases brought on by pathogens. Using it as a natural remedy is much safer than using a chemically manufactured

medication.

Conclusion

Consequently, it is possible to demonstrate that *Ocimum sanctum* leaves extract has the capability to function as an antibacterial. In this study, the early *in vitro* antibacterial screening of *Ocimum sanctum* did not effectively exhibit any control on the growth of the test bacterial strains. This was due to the low dose that was employed in the experiment. In order to acquire a more profound comprehension of antibacterial screening, it is essential to do extensive investigation on extract isolates. Following an extraction using the Soxhlet method with ethanol, it was shown that *Ocimum sanctum* had a high potential for antibacterial activity.

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