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EVALUATION OF ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF OCIMUM SANCTUM LEAF EXTRACT

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Abstract

The antimicrobial and antifungal properties of Ocimum sanctum leaf extract were the primary motivation for this investigation. The aim of this review is to summarize current antimicrobial research and identify the potential treatments for specific parasitic and bacterial infections. The ethnomedicinal herb Ocimum sanctum was studied for its microbiological activity, particularly in the development of potential antimicrobials against therapeutically significant bacterial and parasitic strains. Methanol was used to extract the active components from the leaves. The antibacterial effects of the extracts were assessed using the agar well diffusion method. A variety of microorganisms were tested, including two Gram-positive bacteria (Staphylococcus aureus and Streptococcus pyogenes), two Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa), and three fungal strains (Aspergillus niger, Aspergillus clavatus, and Candida albicans). The results indicated a significant inhibition of microbial growth, suggesting strong antimicrobial activity. Phytochemical analysis of the plant revealed a variety of secondary metabolites in close proximity, which may have acted synergistically to enhance the antimicrobial effect of Ocimum sanctum. These findings suggest that Ocimum sanctum holds promise as a source of bioactive compounds for further pharmaceutical research and development.

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Introduction to Antimicrobial Activity and Bacteria

Medicinal plants have been used in traditional medicine since ancient times, playing a crucial role in global healthcare. According to the World Health Organization (WHO), around 80% of the world's population relies on plant-based medicine for primary healthcare needs. These remedies are especially valued for their affordability, broad therapeutic effects, and minimal side effects. The rise of drug-resistant bacteria and viruses has further driven interest in plant-derived antimicrobials, which are considered safer and less likely to cause resistance compared to synthetic drugs [1-2]. Medicinal plants are rich in secondary metabolites like tannins, alkaloids, terpenoids, and flavonoids-many of which have shown significant antibacterial properties [4]. Plants such as Artocarpus heterophyllus, Berberis aristata, Chromolaena odorata, and Vitex negundo have been studied for their effectiveness against microbial infections. These natural alternatives offer a promising solution in the fight against antimicrobial resistance [5].

Bacteria

Bacteria such as Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Klebsiella pneumoniae are among the most common clinical pathogens. E. coli, a member of the Enterobacteriaceae family, is a Gram-negative, motile bacillus typically found in the gut. It can cause infections ranging from gastrointestinal illnesses to urinary tract infections, meningitis, and septic wounds [6-8].

Colonies of E. coli on MacConkey agar appear rose-pink, while on CLED medium they vary based on lactose fermentation. Though once considered harmless, E. coli is now associated with serious diseases, especially in immunocompromised individuals. Despite the threat, many community-acquired E. coli strains remain susceptible to standard antibiotics.

Conversely, Staphylococcus aureus, a Gram-positive cocci, can form grape-like clusters and often requires resistance testing due to its frequent resistance to multiple antibiotics via R plasmids [9-12].

Overview of S. aureus, P. aeruginosa, and Antimicrobial Resistance

Staphylococcus aureus is a Gram-positive, non-motile coccus that appears in grape-like clusters. It grows best at 37 °C and pH 7.5. Colonies are buff-golden, smooth, and often show β-hemolysis. S. aureus commonly colonizes human skin and mucosa, and is responsible for a range of infections like pneumonia, bacteremia, osteomyelitis, and septic arthritis. Around 90% of strains produce β-lactamase, rendering them resistant to penicillin G, with many also resistant to methicillin (MRSA). MRSA strains are now common in both hospital and community settings [13].

Pseudomonas aeruginosa is a Gram-negative, rod-shaped, motile aerobe that produces pigments like pyocyanin (blue) and fluorescein (yellow-green). It thrives at 37–42 °C and inhabits moist environments, including hospital surfaces and medical devices. It causes respiratory, urinary tract, skin, and wound infections, especially in immunocompromised individuals. P. aeruginosa shows resistance to multiple antibiotics, complicating treatment.

Escherichia coli is another multidrug-resistant pathogen. It produces extended-spectrum β-lactamases (ESBLs) like CTX-M, which deactivate many antibiotics. The global rise of antimicrobial-resistant strains of S. aureus, P. aeruginosa, and E. coli poses a severe threat to public health, highlighting the urgent need for new, effective antimicrobial strategies [14].

Cefotaxime binds more strongly to proteins than other beta-lactams like ceftriaxone or cefepime. ESBL-producing E. coli contributes to serious infections, especially UTIs. Medicinal plants have ancient roots in Ayurveda, Unani, and other systems, valued for low cost, accessibility, and minimal side effects. Herbal medicine is often used in chronic conditions and complements modern drugs. Pain types include nociceptive and neuropathic, with treatments ranging from NSAIDs to opioids. Inflammation, a defense response, involves vascular and cellular changes. Diagnosis uses imaging and biomarkers. Fever is often infection-related and treated with antipyretics. R.I.C.E., NSAIDs, and herbal remedies reduce inflammation and promote healing [15-19].

Aim and Objectives

The purpose of this research is to evaluate the antimicrobial and antifungal properties of Ocimum sanctum (commonly known as Tulsi).

Objectives:

- To collect and prepare the whole Ocimum sanctum herb.
- To extract phytochemicals from Ocimum sanctum using suitable solvents (e.g., methanol).
- To investigate the extracted phytochemicals for their antibacterial and antifungal activities.

Plan of Work

The work plan involves the following sequential steps:

Step 1: Selection of Ocimum sanctum for phytochemical study using methanolic extracts.

Step 2: Collection and preparation of plant material (drying, grinding, and storage).

Step 3: Extraction of phytochemicals using methanol.

Step 4: Phytochemical screening of the extract to identify active compounds.

Step 5: Evaluation of antimicrobial properties of the extract against selected bacterial strains.

Step 6: Evaluation of antifungal activity using standard fungal strains.

Step 7: Analysis and interpretation of results.

Plant Profile

Table 01: Plant Profile

Field	Information
Botanical Name	*Ocimum sanctum*
Kingdom	Plantae
Order	Lamiales
Family	Lamiaceae
Genus	*Ocimum*
Species	*O. tenuiflorum*
Common Name	Krishna Tulsi
Hindi	Tulsi
English	Holy Basil
Marathi	Krishna Tulsi

Figure 01: Ocimumsanctum

Chemical Constituents

Tulsi (Ocimum sanctum) contains key phytochemicals like eugenol (70%), β-caryophyllene, and rosmarinic acid. Traditionally used in Ayurveda for purification, it shows pain-relieving, antioxidant, and antimicrobial properties. Studies confirm its antibacterial, antiviral, and anticancer effects against various organisms.

Methodology

Ocimum sanctum plants were identified, collected, and transported to the lab. Leaves were shade-dried and powdered. About 25 g of powder was defatted with petroleum ether, then extracted with 900 ml methanol using a Soxhlet extractor for 72 hours. Extracts were dried, concentrated under vacuum, purified, and stored in sterile vials.

Phytochemical analyses tested for carbohydrates, glycosides, flavonoids, tannins, proteins, amino acids, sterols, and triterpenes using standard chemical tests like Molisch, Bernstein, and Libermann-Buchard.

For antimicrobial testing, eight pathogens including bacteria and fungi were cultured on specific media. Methanolic extracts were sterilized and tested against microbes using the agar disk diffusion method at concentrations of 25-250 µg/ml. Standard antibiotics and antifungals were used as controls. Inhibition zones were measured after incubation; zones under 8 mm were considered inactive.

Results and Discussion

Table 02: Qualitative Phytochemical Evaluation of Ocimum sanctum

Parameters	Value
1. Alkaloid	+
2. Carbohydrates	+
3. Glycosides	-
4. Flavonoids	++
5. Tannins & Phenolic compounds	+
6. Proteins & Amino acids	+
7. Saponins	+
8. Sterols or Triterpenes	+

++:high content,+: moderate, -:Negative,

Based on an individual's subjectivephytochemical evaluation of an Ocimum sanctum methanolic extract. It contains a tall whole of flavonoids, alkaloids, tannins, phenolic compounds, sterols, saponins, amino acids, and proteins.

Antimibrobial Activity

Ocimum sanctum extracts were tested for their antimicrobial activity at five, twenty-five, one hundred, and two hundred and fifty milligram concentrations against four harmful bacterial strains: two Gram-positive (Streptococcus pyogenes MTCC 442) and two Gram-negative (Escherichia coli MTCC 443, Pseudomonas aeruginosa MTCC 424).The selection of these strains was driven by the launch of its application, which enumerated development factors.In order to determine the extricates' antibacterial capability, the zone of restriction for bacterial development was used.The results of the antibacterial tests are shown in tables.The extricates' antibacterial properties were significantly amplified as the concentration increased to Î14g/ml. Bacterial activity inside the extricates was more difficult to detect in S. pyogenes and S. aureus compared to E. coli and P. aeruginosa when using conventional antibiotics.For each of the fragile microscopic organisms, the measured improvement prevention zone ranged from eleven to twenty millimeters.The results reveal that, out of all the living things tested, the Ocimum sanctum extracts were the most effective.

Table 03: Antibacterial properties of methanolic extracts of Ocimum sanctum leaves were tested against various bacterial organisms.

Antibacterial activity (Zone of inhibition)					
Microorganism	*Ocimum sanctum*- Zone of inhibition in mm				
	Concentration in µg/ml				
	Methanolic extracts (µg/ml)				
	5	25	50	100	250
E. coli	-	13	16	18	22
P. aeruginosa	-	10	14	17	20
S. pyogenes	-	9	12	16	19
S. aureus	-	12	16	18	19

Values are mean ± SD of three parallel measurements - = No zone of inhibition

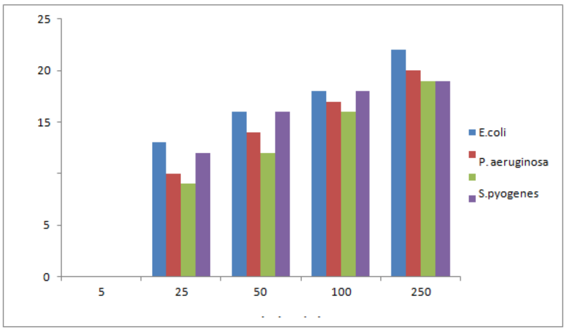

Figure 02: depicts the methanolic antibacterial activity of Ocimum sanctum leaf extracts against the tested bacterial organisms.

Table 04: Illustrates the antibacterial activity of standard medications against the bacterial organisms used for assessment.

Antibacterial activity (Zone of inhibition)					
Drug	Concentration (µg/ml)	Zone of inhibition in mm			
		E. coli	P. aeruginosa	S. pyogenes	S. aureus
Ampicillin	5	16	16	10	11
	25	18	19	14	13
	50	20	21	18	17
	100	22	24	19	19
	250	24	25	20	20
Chloramphenicol	5	15	15	11	12
	25	19	18	13	15
	50	20	20	17	18
	100	23	22	18	19
	250	23	23	19	20
Ciprofloxacin	5	17	19	15	16
	25	20	21	18	19
	50	23	24	19	21
	100	25	25	21	22
	250	26	27	22	23
Norfloxacin	5	20	18	17	16
	25	22	22	21	19
	50	24	24	23	20
	100	26	26	25	23
	250	28	28	27	25

Table 05: The results of the antibacterial activity of methanolic extracts of Ocimum sanctum leaves against the fungal test organisms are presented.

Antifungal activity (Zone of inhibition)					
Microorganism	*Ocimum sanctum*- Zone of inhibition in mm				
	Concentration in µg/ml				
	Methanolic extracts (µg/ml)				
	5	25	50	100	250
A. niger	-	16	17	18	21
A. clavatus	-	18	19	20	21
C. albicans	-	20	20	21	21

Values are mean ± SD of three parallel measurements; "–" = No zone of inhibition.

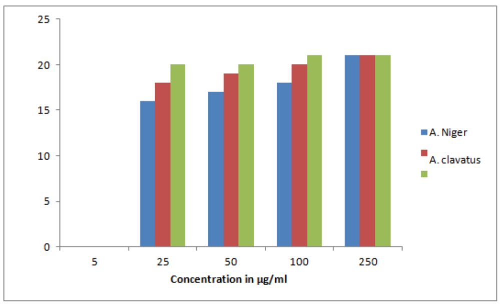

Figure 03: The antibacterial action of methanolic extracts of Ocimum sanctum against infectious test organisms is illustrated in

Table 06: Antifungal Activity of Standard Drugs Against Parasitic Test Organisms

Antifungal activity (Zone of inhibition)				
Drug	Concentration in (µg/ml)	Zone of inhibition in mm		
		A. niger	A. clavatus	C. albicans
Griseofulvin	5	18	17	19
	25	22	20	21
	50	24	22	23
	100	26	24	25
	250	29	27	27
Nystatin	5	17	16	17
	25	20	19	18
	50	23	24	23
	100	27	25	24
	250	28	26	27

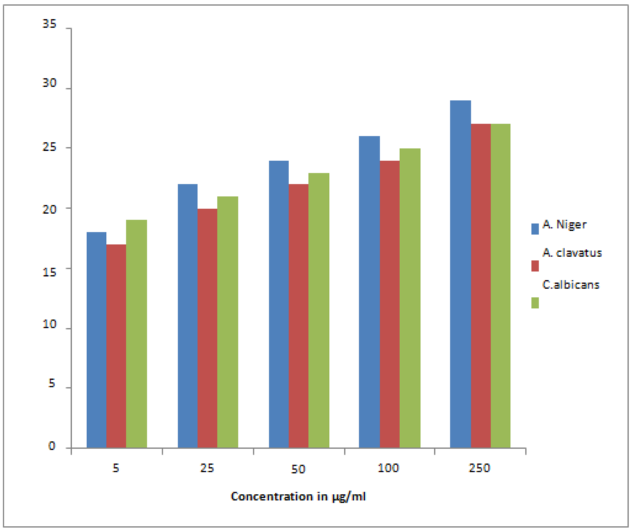

Figure 04: Activity of Griseofulvin as an antifungal agent.

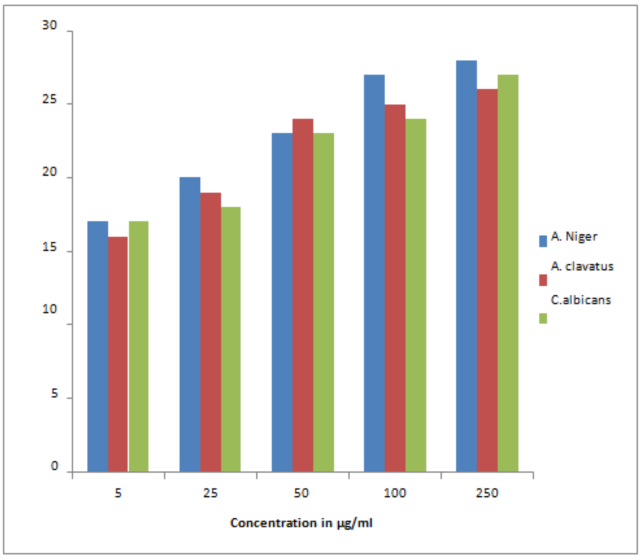

Figure 05: The antifungal action of Nystatin

Methanolic extracts of Ocimum sanctum are reported to exhibit significant antibacterial and antifungal effects, as stated in previous studies. Additionally, this article provides an overview of the potential therapeutic value of a combination of phytochemicals commonly found in the plant. The study identified various phytochemical components, including saponins, triterpenoids, steroids, glycosides, anthraquinones, flavonoids, proteins, and amino acids. The findings also suggest that plants rich in tannins and phenolics possess notable antimicrobial properties. These compounds have been shown to be effective against a variety of bacterial species [20-23].

Conclusion

The current experiment was conducted using an 80:20 methanolic extract, chosen based on its effectiveness. Since methanolic extracts are more suitable for clinical applications, further studies involving Minimum Inhibitory Concentration (MIC) and other characterization methods are recommended. The extract provided a detailed profile of the chemical constituents expected in this investigation. The novelty of this work lies in the promising results obtained with this specific methanol ratio. Compared to conventional treatments, the methanolic extracts of Ocimum sanctum demonstrated activity against most clinically relevant bacteria and fungi. The findings support the traditional use of Ocimum sanctum in treating various infectious diseases. However, further research is necessary to better evaluate the potential of crude extracts as antibacterial agents. The selection of plant species for isolating bioactive compounds will depend on ongoing research. The development of antimicrobial agents has begun, with researchers focusing on isolating and elucidating their structures.

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Conflicts of Interest

The authors declare no conflicts of interest.

Author Contribution

Both are contributed equally

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None

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