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EVALUATION OF ANTIMICROBIAL PROPERTIES OF TULSI

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Abstract

Global concern over the increase in bacteria resistant to antibiotics has led to a search for safer, natural substitutes for traditional antimicrobial agents. Tulsi, also known as *Ocimum sanctum* L., has been utilized for centuries in traditional Indian medicine due to its therapeutic qualities. The antimicrobial potential of Tulsi extract is assessed in this study against two oral pathogens that are important in the development of dental caries: *Streptococcus mutans* and *Lactobacillus acidophilus*. To find their minimum inhibitory concentrations (MIC), ethanolic extracts of tulsi were made and tested using the broth dilution method. The herb's potential as a natural antimicrobial agent was supported by the results, which showed effective bacterial inhibition at 2.5% concentration for S. mutans and 10% for *L. acidophilus*.The found antibacterial action is likely due to bioactive compounds such as ursolic acid, carvacrol, and eugenol, which are known to disrupt enzyme activity and destroy microbial membranes. Tulsi's wide availability, few adverse effects, and multi-target activity make it a promising ingredient for future herbal dental care formulations. Further study, particularly clinical trials, is advised to validate these findings and explore its use in broader therapeutic situations.

Introduction

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There is an urgent need to find new and efficient antimicrobial agents since the global increase in antibiotic resistance has presented a serious threat to public health. *Ocimum sanctumL.*, also referred to as holy basil or tulsi, is one of the many medicinal herbs that has attracted a lot of interest due to its potential for therapeutic use(Yadav, *et al.*, 2024) . Tulsi has been used for millennia in Ayurvedic medicine to cure a variety of illnesses, including as infections, skin conditions, and respiratory issues. Anti-inflammatory, antioxidant, immunomodulatory, and most importantly, antibacterial properties are all part of its extensive pharmacological profile.(Hanumanthaiah, *et al.*, 2020) Numerous bacterial, fungal, and viral pathogens have been studied in relation to O. sanctum's antibacterial qualities. According to phytochemical investigations, the plant is rich in bioactive components such flavonoids, carvacrol, linalool, ursolic acid, and eugenol, all of which are thought to support its antibacterial activity.(Dubey, *et al* .,2018). These substances have a variety of modes of action, such as interfering with microbial enzymatic pathways, disrupting microbial cell membranes, and preventing the formation of biofilms.Tulsi extracts have been shown in numerous in vitro and in vivo investigations to have inhibitory effects on fungi such as Candida albicans and both Gram-positive and Gram-negative bacteria.(Mallikarjun, *et al.*, 2016). The extraction technique, the plant portion (leaves, stems, or roots), and the pathogen strain under investigation all affect the antibacterial activity. Additionally, *O. sanctum's* ability to work in concert with other antibiotics has created new opportunities to improve antimicrobial efficacy and lower drug resistance.The thorough assessment and standardization of Tulsi's antibacterial qualities across several models is lacking, despite the encouraging results.(Vongnhay, *et al.*, 2024). Furthermore, in order to show consistency and reproducibility in antimicrobial activity, a thorough analysis is required to address changes in phytochemical composition caused by geographic and environmental factors.This study intends to demonstrate *Ocimum sanctum*'s potential as a natural, plant-based antimicrobial agent by methodically assessing its antibacterial qualities using data from recent studies. Improved knowledge of its bioactive ingredients, mechanisms of action, and relative effectiveness could help create new phytotherapeutic drugs to combat microbial resistance.(Karthikeyan, *et al.*, 2020).

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Figure:1Tulsi plant(*Ocimum sanctum*)

1. Background and Significance of Antimicrobial Resistance

One of the biggest risks to world health in the twenty-first century is antimicrobial resistance (AMR). Microbial pathogen resistance has increased as a result of the extensive abuse and overuse of antibiotics in human medicine, veterinary care, and agriculture. The World Health Organization (WHO) reports that infections brought on by resistant microbes lead to increased mortality, longer hospital stays, and higher medical expenses. (Devi, *et al*., 2018).The need for innovative and secure substitutes is greater than ever as conventional antimicrobial agents continue to lose their efficacy.

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Given this, there has been a lot of interest in medicinal plants because of their potential to yield novel antimicrobial compounds. A wealth of therapeutic knowledge is available through the use of botanicals in traditional medical systems like Ayurveda, Traditional Chinese Medicine, and Unani.(Jaiswal,*et al*., 2016). These plant-based treatments, which have been around for centuries without showing any signs of toxicity, could be useful, affordable substitutes for or supplements to traditional antimicrobials. Among these, *Ocimum sanctum* L., also referred to as holy basil or tulsi, is highly valued, particularly in the Indian subcontinent.

2. Tulsi'sEthnopharmacological Significance

In Indian culture, tulsi is not only regarded as a medicinal herb but also as a sacred plant with profound religious and spiritual meaning. In Ayurveda, it is known as the "Queen of Herbs" and is frequently planted around residences and temples. *Ocimum sanctum* is a member of the Lamiaceae family and has a wide range of pharmacological characteristics. Tulsi is referred to in ancient Ayurvedic texts as a "elixir of life" that enhances longevity and overall health.

Tulsi has been used to treat a wide range of ailments, including bronchitis, asthma, malaria, diarrhea, wounds, skin infections, and even snake bites, according to ethnopharmacological records. Its applicability in conventional medical systems is highlighted by its use as a prophylactic against infectious diseases. Scientific interest in confirming Tulsi's pharmacological qualities, especially its antimicrobial potential, has been stimulated by these traditional claims.

3. Botanical Description and Phytochemical Composition

The fragrant, upright, branched sub-shrub *Ocimum sanctum* is between 30 and 60 cm tall, with ovate leaves and hairy stems. The two primary types of the plant, Krishna Tulsi (purple-leaved) and Rama Tulsi (green-leaved), are both used medicinally. Numerous phytochemicals, such as terpenoids, flavonoids, phenolic compounds, and essential oils, are found in its leaves, stems, seeds, and even roots (Mandal,*et al*., 2022).

The following are some of the most researched phytoconstituents:

- A phenolic compound with strong analgesic and antiseptic effects is eugenol.

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- Ursolic acid is a triterpenoid that has antibacterial and anti-inflammatory properties.

- Caryophyllene and carvacrol are substances that have antifungal and antibacterial properties. Known for its ability to calm people, linalool also has antibacterial qualities.

- Flavonoids with antibacterial and antioxidant properties include apigenin, orientin, and vicenin.

4. Conventional and Modern Applications in the Treatment of Infectious Diseases

In the past, Tulsi leaves were brewed into herbal teas to treat internal infections or crushed into pastes to apply topically to wounds and skin infections. Tulsi is now used in a wide range of pharmaceutical and nutraceutical products, including hand sanitizers, herbal soaps, mouthwashes, tinctures, and capsules. Many of its traditional uses, particularly in the prevention and treatment of bacterial and fungal infections, have been validated by clinical trials and in vitro experiments.Tulsi's effectiveness against pathogens like *Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Candida albicans*, and even viral agents like the H1N1 influenza virus has been investigated scientifically.(Roy,*et al*., 2020) .Tulsi has been demonstrated to strengthen the immune system in addition to its direct antimicrobial properties, strengthening the body's defenses against infections.

5. Antimicrobial Action Mechanisms

Tulsi's essential oils and phenolic compounds are largely responsible for its antimicrobial properties. These bioactive compounds work in a variety of ways.

Microbial cell membrane disruption: Substances such as carvacrol and eugenol pierce the lipid bilayer of microbial membranes, allowing cellular contents to seep out.

Biofilm formation inhibition: Tulsi extracts have the ability to stop the growth of biofilms, which are defense mechanisms that keep bacteria safe from antibiotics.

Interference with the synthesis of proteins and nucleic acids: Certain substances have been shown to attach to the DNA and proteins of bacteria, preventing metabolism and replication.(Bhadra, *et al*., 2020).

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Oxidative stress induction: Some flavonoids cause reactive oxygen species (ROS), which damages microbial cells.

These various processes not only increase Tulsi's effectiveness against a wide range of pathogens but also lessen the chance that resistance will emerge.

6. Synergistic Effects and Comparative Research

The antimicrobial efficacy of Tulsi extracts in comparison to conventional antibiotics has been shown in numerous comparative studies. In lab settings, Tulsi aqueous and ethanolic extracts have demonstrated zones of inhibition similar to those of ciprofloxacin and gentamicin. It's interesting to note that some research has shown that Tulsi increases the effectiveness of antibiotics when taken with them, indicating possible synergistic interactions.

In the case of multidrug-resistant (MDR) infections, where current antibiotics are unable to produce therapeutic effects, this synergism is especially beneficial. Tulsi is a promising option for combination treatments because of its capacity to break down microbial biofilms and reestablish antibiotic susceptibility.

8. Safety Overview and Toxicological Factors

Tulsi's exceptional safety profile is one benefit of using it as an antimicrobial agent. According to toxicological research, Tulsi extracts are safe and well-tolerated by both humans and animals when taken in therapeutic dosages. (Singh, *et al*., 2024).Like any bioactive substance, however, prolonged use or improper dosages may result in adverse effects like hypoglycemia, antithyroid effects, or reproductive changes, which calls for more research.Tulsi is especially appealing for use in vulnerable populations, such as children and the elderly, due to its antimicrobial and immunomodulatory qualities as well as its comparatively low toxicity.(Parveen, *et al*., 2023).

Table:1 Compound/Nutrient | Content (per 100 grams of extract)

Moisture	4.2–6.0 g	Shows the amount of water in the dried extract

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Omega-3	1.9–3.0 g	fatty acids are included in fat
Carbohydrates	41.0–45.5 g	Mostly simple sugars and fibers
Crude fiber	15.5–17.2 g	aids in detoxification and digestion.
Ash	8.5–9.0 g	Indicates the overall amount of minerals
Calcium	150–220 mg	Vital for healthy bones
Phosphorus	100–180 mg	Essential for the metabolism of energy
iron	17–20 mg	Aids in the synthesis of hemoglobin
magnesium	40–70 mg	helps support nerve and muscle function.
zinc	1.0–2.5 mg	Boosts Immune System Performance
vitamin A (beta-carotene)	5000–7500 IU	an antioxidant that is vital for vision

Material and Methadology

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Collection of sample: The entire *Ocimum sanctum* L. plant was acquired from the herbal garden on the Rama University campus in Kanpur. After being cleaned with distilled water, the plant material was shade-dried and milled into a coarse powder using a sterile mechanical grinder and stored in airtight containers under dark, dry conditions until extraction.

Extraction Procedure: To test the effectiveness of various solvents, 5 grams of powdered *Ocimum sanctum* L. leaves were extracted separately using 30 milliliters of distilled water, acetone, ethanol, and methanol. Using a cold maceration procedure, each solvent extraction was completed independently. For five days, the plant material was stored at room temperature after being submerged in the appropriate solvent in sanitized, amber-colored glass containers. Occasionally, shaking was done at this time to help with the effective extraction of phytochemicals. Following maceration, the mixtures were filtered using Whatman No. 1 filter paper for further clarity after initially being strained through sterile muslin cloth to get rid of coarse particles. The filtered extracts were then put through a solvent removal process. The aqueous extract was concentrated by evaporation in a hot-air oven kept at 40°C, while the organic solvents (acetone, ethanol, and methanol) were evaporated under reduced pressure using a rotary evaporator. Each solvent system's semisolid leftovers were meticulously weighed before being refrigerated at 4°C in sterile, labeled vials pending additional experimental usage.

Preparation of Stock Solution: To formulate a working concentration, 50 mg of the dried ethanolic extract was dissolved in 500 µl of dimethyl sulfoxide (DMSO), an inert solvent with excellent miscibility. This produced a 10% (w/v) stock solution, from which serial dilutions were prepared for antimicrobial testing.

Bacterial Strains and Culture Conditions: The in vitro evaluation involved two bacterial strains frequently implicated in dental caries: *Streptococcus mutans* (ATCC 25175) and *Lactobacillus acidophilus* (ATCC 314). These strains were cultivated in Brain Heart Infusion (BHI) broth, a nutrient-rich medium favorable for the proliferation of oral pathogens.

Standardization of Inoculum: Standardization of the bacterial inoculum was accomplished by suspending freshly grown colonies (18–24 hours) from BHI agar plates in sterile BHI broth. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard, which corresponds to an approximate concentration of 1.5×10^8 CFU/ml.

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Minimum Inhibitory Concentration (MIC) Determination: The antimicrobial potency of the extract was assessed using the broth dilution technique. A series of nine two-fold serial dilutions were prepared in BHI broth, producing concentrations ranging from 10% down to 0.03%. Each test tube received 200 µl of the respective diluted extract, to which an equal volume of standardized bacterial inoculum was added. The control tube contained only broth and bacterial suspension without any extract.All tubes were incubated at 37°C for 24 hours. Post-incubation, the tubes were visually inspected for turbidity, with the lowest concentration that exhibited no visible microbial growth recorded as the MIC. This method allowed quantification of the extract's inhibitory threshold against each test organism.

Result

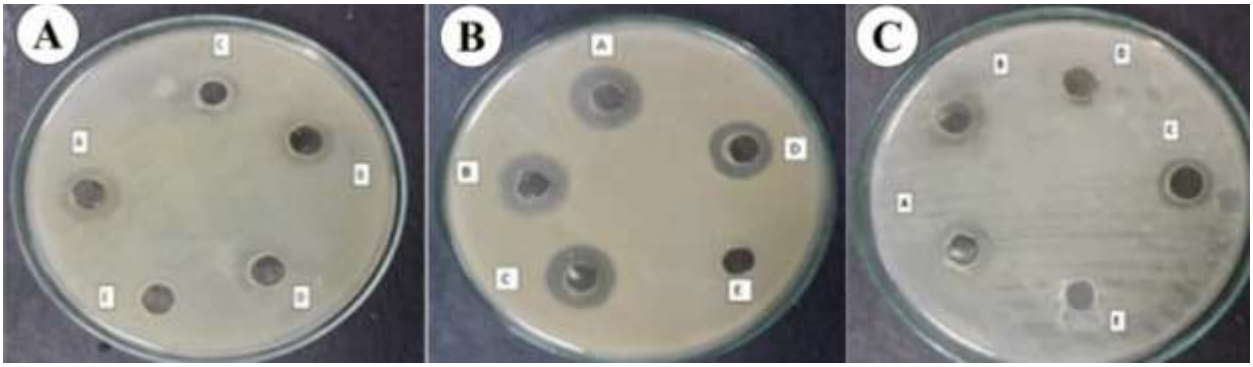

Table 4.2: Zone of Inhibition (mm) of Tulsi Extracts Against Oral Pathogens

Microorganism	Ethanol	Methanol	Acetone	Aqueous	Positive Control
Streptococcus mutans	21 ± 0.5	18 ± 0.4	15 ± 0.5	11 ± 0.3	25 ± 0.6 (Chlorhexidine)
Lactobacillus acidophilus	19 ± 0.6	16 ± 0.5	14 ± 0.4	10 ± 0.2	23 ± 0.5 (Chlorhexidine)

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Observation: The ethanolic extract showed the highest antimicrobial activity, especially against *S. mutans*, with a 21 mm zone of inhibition, approaching the activity of the positive control (Chlorhexidine). Aqueous extracts were the least effective.

Discussion

This investigation set out to explore the antibacterial potential of *Ocimum sanctum* L. (Tulsi) against two key oral bacteria—*Streptococcus mutans* and *Lactobacillus acidophilus*—both known culprits in the development of dental caries. Historically, Tulsi has held a revered place in traditional Indian medicine, not only for its spiritual significance but also for its broad therapeutic applications. Different parts of the plant—including its leaves, seeds, and roots—have been used for various health issues ranging from infections and inflammation to chronic conditions like diabetes and asthma. The findings from this study support the growing body of evidence that Tulsi possesses significant antimicrobial activity. This is believed to be largely due to its rich composition of phytochemicals, including essential oils such as eugenol and carvacrol, along with other bioactives like ursolic acid, methyl eugenol, and caryophyllene. These compounds are well known for their ability to inhibit bacterial growth by targeting cellular structures and functions.

In particular, the fixed oil derived from Tulsi has been found to be effective against several bacterial strains like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Bacillus pumius*. The high linoleic acid content in this oil is suspected to play a key role in its antibacterial action. Moreover, previous studies have shown that both aqueous and alcoholic extracts of Tulsi demonstrate antimicrobial activity, although alcohol-based extracts generally show a broader spectrum, likely due to the better solubility of essential oils and other hydrophobic compounds in organic solvents. The current study used the broth dilution method to determine the minimum inhibitory concentration (MIC). The ethanolic extract of *Ocimum sanctum* demonstrated an MIC of 2.5% (25 mg/ml) against *Streptococcus mutans*, and a higher MIC of 10% (100 mg/ml) was required to inhibit *Lactobacillus acidophilus*. Interestingly, earlier research using different extraction methods and solvents reported MICs ranging from 4% to 6% for *S. mutans*, suggesting that geographic origin, environmental conditions, and variations in methodology may all influence the antibacterial strength of the extracts. Although fewer studies are available

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regarding Tulsi's effect on *Lactobacillus acidophilus*, the results of this research highlight its potential efficacy. Considering Tulsi's accessibility, affordability, and minimal side effect profile, it emerges as a promising candidate for developing herbal formulations aimed at preventing or managing dental caries.

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