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ANTIMICROBIAL ACTIVITY OF TULSI (OCIMUM TENUIFLORUM)

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ABSTRACT

In recent years, researchers worldwide have recognized that the effective duration of any antimicrobial agent is limited due to the rising development of microbial resistance. As a result, numerous investigations have focused on identifying alternative antimicrobial sources, particularly from plant origins. The objectives of this study were to evaluate the antimicrobial potential of essential oils extracted from Australian-grown *Ocimum tenuiflorum* (Tulsi), to quantify the volatile constituents present in flower spikes, leaves, and essential oil, and to identify the compounds responsible for the observed activity.

The broth micro-dilution technique was employed to determine the minimum inhibitory concentration (MIC) of Tulsi essential oil against selected microbial strains. The essential oil, at concentrations of 4.5% and 2.25%, completely suppressed the growth of *Staphylococcus aureus* (including MRSA) and *Escherichia coli*, while exhibiting only partial inhibition against *Pseudomonas aeruginosa*. Out of 54 compounds detected in Tulsi leaves, flower spikes, and essential oil, three—camphor, eucalyptol, and eugenol—are suggested to be primarily responsible for the antimicrobial activity.

Since *S. aureus* (including MRSA), *P. aeruginosa*, and *E. coli* are significant pathogens responsible for skin and soft tissue infections, Tulsi essential oil may serve as a promising topical antimicrobial agent for managing such infections.

INTRODUCTION

The application of medicinal plants in traditional healthcare systems has been documented in literature spanning several millennia. Ayurvedic texts, composed during the Vedic era (3500–1600 B.C.), describe various therapeutic practices, including the use of herbal remedies, which laid the foundation for subsequent medical sciences in the Indian subcontinent. In modern complementary and alternative medicine, plants remain a primary source of

therapeutic agents, with every plant part—such as seeds, roots, stems, leaves, and fruits—potentially containing bioactive compounds.

These bioactive substances are largely composed of secondary metabolites, which contribute to the medicinal properties of plants. The use of medicinal plants offers several advantages, including affordability, widespread availability, and comparatively fewer adverse effects. However, due to variability in plant metabolism, herbal products must undergo proper standardization, rigorous quality assessment, and safety evaluation before being approved for primary healthcare use.

Among medicinal plants, aromatic herbs are considered rich sources of biologically active constituents valuable in both agriculture and medicine. One such plant, *Ocimum tenuiflorum* (also known as *Ocimum sanctum*, Tulsi, or Holy Basil), belongs to the Lamiaceae family and is often referred to as the "Queen of herbs" or "Mother medicine of nature" because of its extensive therapeutic potential. It has been widely utilized in traditional Indian medicine for centuries, with nearly all parts of the plant demonstrating medicinal properties.

Traditionally, Tulsi is consumed in various forms. Aqueous extracts of its leaves (either fresh or dried powder) are commonly prepared as herbal teas or combined with other herbs or honey to enhance their therapeutic efficacy. These preparations have been traditionally used to treat conditions such as poisoning, abdominal pain, common cold, headaches, malaria, inflammation, and cardiovascular disorders. Essential oils extracted from Tulsi leaves and inflorescences are reported to possess multiple pharmacological properties, including expectorant, analgesic, anti-emetic, antipyretic, anti-asthmatic, hypoglycemic, hepatoprotective, hypotensive, hypolipidemic, and immunomodulatory effects, as well as stress-relieving and anti-inflammatory activities.

Several studies have explored the pharmacological properties of Tulsi extracts obtained through different extraction techniques, including steam distillation and solvent extraction methods. Previous reviews have highlighted the therapeutic importance of Tulsi and its major bioactive component, eugenol. These findings provide a scientific basis for its medicinal applications, particularly concerning its effects on the central nervous, immune, cardiovascular, reproductive, gastric, and urinary systems.

Skin and soft tissue infections (SSTIs) contribute significantly to global morbidity and healthcare costs. The primary causative organisms include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. While many infections are mild to moderate, severe cases often require hospitalization and treatment with systemic antimicrobial agents.

The growing prevalence of multidrug-resistant pathogens has further complicated the management of SSTIs.

To limit the spread of antimicrobial resistance, it is crucial to distinguish between infections that require antibiotic therapy and those that do not. Studies indicate that antibiotics are frequently overprescribed for conditions that may not necessitate systemic treatment. In this context, essential oils and their active constituents may serve as effective alternatives for managing mild to moderate skin infections, thereby reducing unnecessary antibiotic use and slowing the development of resistance.

The present study aimed to (i) assess the antimicrobial efficacy of Tulsi essential oil, (ii) analyze the volatile composition of leaves, flower spikes, and extracted oil from Australian-grown Tulsi using headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC-MS), and (iii) identify the key volatile compounds responsible for its antimicrobial activity based on literature evidence. To the best of our knowledge, this is the first study to comprehensively analyze fresh Tulsi leaves, flower spikes, and essential oil derived from Australian-grown plants using HS-SPME-GC-MS.

MATERIALS AND METHODS

Source of Tulsi

Ocimum tenuiflorum (Tulsi), voucher specimen number PHARM-14-0028, was obtained from the Medicinal Plant Herbarium at Southern Cross University, NSW, Australia, and used for this investigation. Fresh leaves and inflorescences (350 g) were subjected to steam distillation for 6 hours using a modified Clevenger-type apparatus. The yield of essential oil, expressed as the percentage of oil weight relative to plant material weight, was 0.57% (v/w). The obtained yellow-colored volatile oil was stored in airtight containers at temperatures below 4°C in dark conditions until further use.

Assessment of Antimicrobial Activity of Tulsi Essential Oil

The bacterial strains selected for this study included *Staphylococcus aureus* ATCC 25923, a clinical isolate of methicillin-resistant *S. aureus* (MRSA) NCTC 6571 (Oxford strain), *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

The extracted essential oil was emulsified in Mueller–Hinton Broth (MHB) using dimethyl sulfoxide (DMSO) as a solvent. Specifically, 90 µL of essential oil was combined with 10 µL of DMSO in a sterile microcentrifuge tube and mixed thoroughly by vortexing. Subsequently,

900 µL of MHB was added incrementally in 30 µL portions with intermittent mixing to ensure uniform dispersion.

The broth microdilution method was employed to determine the minimum inhibitory concentration (MIC) of the essential oil. Serial two-fold dilutions were prepared starting from 9% concentration in a 96-well sterile microtiter plate. Each well contained 50 µL of diluted essential oil, followed by the addition of 50 µL of bacterial suspension to achieve a final concentration of 5×10^5 CFU per well. This resulted in an initial test concentration of 4.5% essential oil in the first well.

The microplates were incubated for 24 hours at 37°C under dark conditions on an orbital shaker set at 100 rpm to prevent cell aggregation. Following incubation, bacterial growth was quantified by measuring optical density at 620 nm using a spectrophotometer.

For determination of minimum bactericidal concentration (MBC), 100 µL aliquots from each well were plated onto agar media and incubated for 24 hours at 37°C, after which viable bacterial colonies were assessed.

Statistical evaluation was conducted using IBM SPSS (version 22). Data analysis included analysis of variance (ANOVA), followed by Tukey's post hoc test to compare differences between treatment groups. A significance level of $p < 0.05$ was considered statistically meaningful. All experiments were performed in duplicate, with each set conducted in triplicate.

Isolation and Identification of Volatile Compounds

Fresh leaves and flower spikes of *Ocimum tenuiflorum*, obtained from the same source as the essential oil, were collected from the Chinese medicinal garden at RMIT University (Bundoora Campus, Melbourne, Australia) during the summer season (22–35°C). Samples were transported on ice to preserve integrity.

Approximately 0.15 g of plant material (either ground leaf tissue or flower spikes) or essential oil was placed into a 4 mL sealed vial equipped with a PTFE/silicone septum and prepared immediately for analysis.

Extraction of Volatile Compounds Using HS-SPME

Volatile constituents were extracted using headspace solid-phase microextraction (HS-SPME), following a modified protocol. An 85 µm polyacrylate fiber was preconditioned in a gas chromatograph injection port at 250°C for 30 minutes prior to use.

After cooling, the fiber was exposed to the headspace of the sample vial, which was maintained at 40°C for 50 minutes to facilitate adsorption of volatile compounds. Desorption of analytes was then carried out by inserting the fiber into the GC injection port for 5 minutes.

The extraction temperature was optimized at 40°C to simulate environmental conditions and enhance volatile compound recovery, resulting in improved peak resolution and compound detection compared to lower temperatures.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

Identification of volatile compounds was performed using an Agilent 5973 GC–MS system equipped with a DB-5 MS capillary column. Helium (99.99% purity) served as the carrier gas at a flow rate of 1.5 mL/min, with a split ratio of 50:1.

The oven temperature program began at 40°C (held for 3 minutes), increased to 250°C at a rate of 6°C/min, and was maintained at the final temperature for 5 minutes. Injection port, transfer line, and ion source temperatures were maintained at 250°C, 280°C, and 230°C, respectively.

Mass spectra were recorded over a range of 41–415 m/z. Compound identification was achieved by comparison with standard spectral libraries (Adams, Wiley, and NIST), using a similarity threshold of 80%. Relative concentrations were calculated based on peak area integration, and results were averaged across three replicates. Kovats retention indices were determined using standard n-alkane mixtures.

Cytotoxicity Evaluation

The cytotoxic effect of Tulsi extract was assessed using HepG2 human liver cells and the PrestoBlue® cell viability assay. Cells were seeded in 96-well plates at a density of 4×10^4 cells per well and incubated for 24 hours at 37°C.

After washing with phosphate-buffered saline (PBS), cells were exposed to diluted samples in treatment medium for 1 hour. Following treatment, cells were washed again and incubated with PrestoBlue reagent for 1–2 hours.

Cell viability was determined by measuring absorbance at 570 nm using a spectrophotometer.

RESULTS

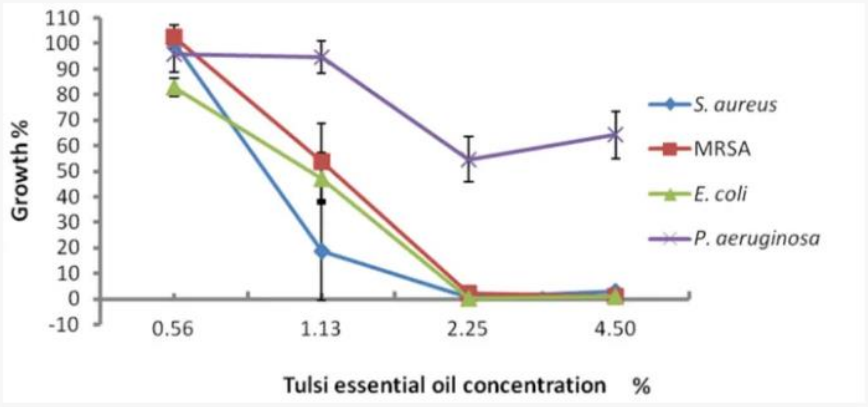
Antimicrobial Activity of Tulsi Essential Oil

Tulsi essential oil at concentrations of 4.5% and 2.25% completely suppressed the growth of *Staphylococcus aureus* (including MRSA) and *Escherichia coli*. In contrast, these concentrations exhibited only partial inhibitory effects against *Pseudomonas aeruginosa*.

Statistical analysis of optical density measurements revealed that both essential oil concentration and bacterial species significantly influenced bacterial growth ($p < 0.05$). Lower bacterial growth percentages were observed at higher oil concentrations—17.29% at 4.5% oil and 15.07% at 2.25%—compared to 56.62% at 1.13%.

Among the tested organisms, *S. aureus* (13.03%), MRSA (18.58%), and *E. coli* (17.99%) exhibited greater susceptibility to the essential oil, whereas *P. aeruginosa* (68.78%) showed markedly higher resistance.

No statistically significant differences were observed in growth inhibition among *S. aureus*, MRSA, and *E. coli* ($p > 0.05$), indicating comparable sensitivity to the Tulsi essential oil across these species. (Figure No. 1)



The antimicrobial effect of different concentrations of Tulsi essential oil on the growth rate of four bacterial strains.

Table 1

Bacterial growth (%) (Dependent variable)	Tests of Between-Subjects Effects	
Independent variable	*F*	Significance
Concentration	28.300	0.000
Bacteria	29.502	0.000
Replicate	2.423	0.126

Analysis of variance (ANOVA) main effect of independent variables: tests of between-subjects effects.

Tulsi Volatile Composition

The analysis of Tulsi leaves, inflorescence, and essential oil using HS-SPME coupled with GC–MS led to the identification of a total of 54 volatile constituents (Table 2). The predominant classes of compounds were monoterpenes and sesquiterpenes. Among the monoterpenes, camphor, cineole, estragole, and eugenol were the most abundant, while the प्रमुख sesquiterpenes included germacrene, caryophyllene, and bisabolene.

A total of nineteen main compounds were consistently detected across all plant parts, although their relative concentrations varied. In general, no significant differences were observed in the presence of the major volatile constituents among the leaves, inflorescence, and essential oil. However, several minor compounds were selectively identified in specific sample types.

Camphor was the most dominant compound, occurring at slightly varying levels in the essential oil (31.5%), leaves (24.2%), and inflorescence (22.6%). This was followed by eucalyptol, which was present in higher concentrations in the essential oil (18.9%) and leaves (13.47%) compared to the inflorescence (1.2%). The third most abundant compound was eugenol, contributing 23.7% of the total volatile content in the leaves, 13.8% in the essential oil, and 7.5% in the inflorescence.

Comparative Volatile Composition

Consistent with the findings of Medina-Holguín et al. (2007), the present study also demonstrated that volatile compounds obtained from fresh plant material differ in composition from those extracted as essential oils from the same source. Consequently, comparing the relative distribution of volatile constituents across different plant parts provides valuable insights.

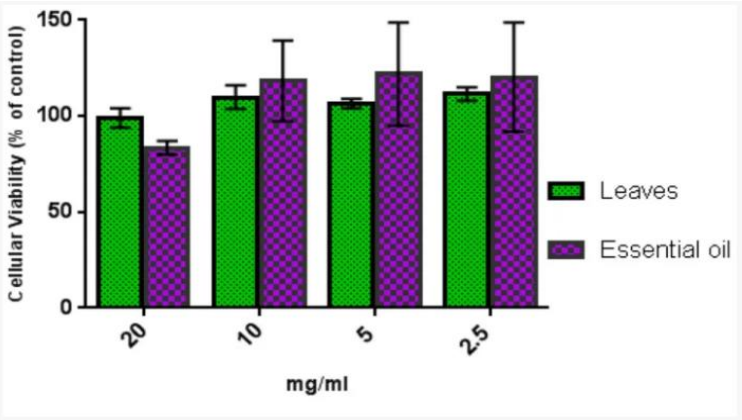
Monoterpenes, particularly eugenol and estragole, were found in the highest concentrations in the leaves (23.7% and 9.6%, respectively). In contrast, sesquiterpenes such as ocimene (9.30%), β-caryophyllene (4.9%), bergamotene (2.8%), germacrene (11.3%), β-bisabolene (10.7%), and α-bisabolene (16.7%) were more abundant in the inflorescence.

Additionally, certain volatile compounds were not detected in the essential oil but were present in trace quantities (<1%) in either the leaves or the inflorescence, indicating variability in compound distribution depending on plant part and extraction method.

Cytotoxicity Test

The cytotoxic effects of the extracts decreased proportionally with decreasing concentration. At a concentration of 20 mg/mL, cell viability was reduced by less than 20%, indicating minimal cytotoxicity. Both the essential oil and the concentrated leaf extract at this concentration were considered non-toxic, as the reduction in cell viability remained within acceptable limits.

Furthermore, concentrations below 20 mg/mL showed no significant impact on cell viability, confirming the relative safety of the extracts under the tested conditions (Figure 2).



Cell viability assay results of Tulsi essential oil and leaves extract.

DISCUSSION

Antimicrobial Activity of Tulsi Essential Oil

Two principal methodologies are commonly employed to evaluate the antimicrobial properties of essential oils: the agar diffusion method (including well and disk diffusion techniques) and dilution-based approaches (broth or agar dilution). Historically, agar diffusion techniques were widely utilized due to their simplicity and minimal requirement for sample volume. However, this approach has notable limitations when applied to essential oils.

Firstly, essential oils consist largely of volatile constituents that may evaporate during incubation, potentially leading to underestimation of antimicrobial activity. Secondly, their limited solubility in agar restricts the diffusion of active compounds, thereby affecting the accuracy and reproducibility of results.

In contrast, the broth microdilution method using 96-well microtiter plates has emerged as the preferred technique for assessing antimicrobial efficacy of essential oils. This method allows precise quantification of activity through determination of minimum inhibitory concentration

(MIC) and minimum bactericidal concentration (MBC). It has been recommended that MIC and MBC values be reported to enable reliable comparison across different studies.

The findings of the present study demonstrate that Tulsi essential oil exhibits bacteriostatic activity at concentrations ranging from 2.25 to 2.5 µg/mL against *Staphylococcus aureus* (including MRSA) and *Escherichia coli*, while showing comparatively reduced effectiveness against *Pseudomonas aeruginosa*. These observations are generally consistent with previous reports that employed disk diffusion or optical density-based assays, although variations exist in the degree of activity observed against Gram-positive and Gram-negative bacteria.

Earlier investigations have shown that Tulsi oil exerts stronger inhibitory effects against Gram-positive bacteria such as *S. aureus* compared to Gram-negative species. Some studies have reported substantial inhibition of both bacterial groups, though variability in susceptibility—particularly in *P. aeruginosa*—has been noted. Differences in antimicrobial performance may be attributed to variations in the chemical composition of essential oils, which are influenced by factors such as geographic origin, environmental conditions, and plant subspecies.

Additionally, discrepancies among studies may arise from methodological differences in antimicrobial testing procedures. It is also important to highlight that *Pseudomonas aeruginosa* is inherently resistant to many antimicrobial agents due to its intrinsic and acquired defense mechanisms, which may explain its reduced susceptibility in the present study.

Similar variability has been reported for other essential oils, where antibacterial efficacy depends on both the oil composition and the analytical technique employed. In general, Gram-positive bacteria are considered more susceptible to essential oils than Gram-negative bacteria, likely due to differences in cell wall structure. For instance, *S. aureus* has been shown to be effectively inhibited through synergistic interactions of compounds such as carvacrol and 1,8-cineole found in other essential oils.

Cytotoxicity Test

The impact of Tulsi extract and essential oil on cell viability was evaluated using the PrestoBlue assay. Cells were exposed to varying concentrations of the extract, ranging from 2.5 to 20 mg/mL. The findings indicated that cytotoxic effects decreased proportionally with decreasing concentration.

At the highest tested concentration (20 mg/mL), cell viability was reduced by less than 20%, suggesting minimal cytotoxicity. Both the essential oil and concentrated leaf extract at this

level were considered non-toxic, as the reduction in viability remained within acceptable limits. Furthermore, concentrations below 20 mg/mL did not produce any noticeable effect on cell viability, confirming the relative safety of the extracts under the experimental conditions.

Comparison of Tulsi Volatile Compounds from Different Geographical Regions

Although the main categories of volatile compounds identified in this study—monoterpenes and sesquiterpenes—are consistent with findings from previous research, notable quantitative variations exist in their distribution among plants cultivated in different geographical regions. Earlier reviews have reported that Tulsi essential oil typically contains monoterpenes such as linalool, estragole, and eugenol, along with smaller amounts of methyl cinnamate, cineole, tannins, camphor, and related compounds. However, both the concentration and relative abundance of major and minor constituents have shown considerable variation across different studies.

Supporting the present findings, previous investigations have demonstrated that extracts from fresh Tulsi leaves and stems contain a wide range of mono- and sesquiterpenes, including α-elemene, bornyl acetate, α- and β-pinene, campesterol, and camphene. Nevertheless, the composition and relative proportions of these compounds differ significantly from those observed in this study.

Such variations can be attributed to several factors, including geographical origin, climatic conditions, soil composition, and other environmental influences, all of which play a critical role in determining the chemical profile of plant-derived essential oils. Additionally, differences in extraction techniques and analytical methodologies may further contribute to discrepancies in reported compositions.

Comparative data from different regions (as presented in Table 4) clearly indicate that environmental conditions strongly influence the chemical makeup of Tulsi essential oil. Therefore, it is highly recommended to characterize the volatile composition of essential oils prior to evaluating their biological or antimicrobial properties, ensuring more accurate interpretation and reproducibility of results.

CONCLUSION

In conclusion, the essential oil derived from *Ocimum tenuiflorum* exhibited notable antimicrobial activity against *Staphylococcus aureus* (including MRSA) and *Escherichia coli*, while demonstrating comparatively lower efficacy against *Pseudomonas aeruginosa*. The reduced susceptibility of *P. aeruginosa* may be attributed to its well-documented resistance

mechanisms, including interactions with resistance–nodulation–division (RND) efflux pumps. Previous studies have also reported variable responses of this organism to essential oils, with some indicating higher resistance compared to other Gram-negative bacteria, while others have observed enhanced sensitivity or no significant difference.

Comprehensive profiling of volatile constituents from the essential oil, leaves, and inflorescence identified a total of 54 compounds, with variations in both composition and concentration across sample types. Based on literature evidence, the main bioactive compounds contributing to the antimicrobial activity of Tulsi oil are likely camphor, eucalyptol, and eugenol, with β-caryophyllene potentially playing a supplementary role due to its lower abundance.

Given that *S. aureus* (including MRSA), *P. aeruginosa*, and *E. coli* are main causative agents of skin and soft tissue infections (SSTIs), Tulsi essential oil shows promise as a topical antimicrobial agent for the treatment and prevention of such infections. Its application, either as a therapeutic agent or as part of wound care formulations, may help prevent infection progression, reduce dependence on systemic antibiotics, and ultimately contribute to mitigating the development of antimicrobial resistance.

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