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ANTIBACTERIAL AND ANTI-INFLAMMATORY ACTIVITIES OF OCIMUM SANCTUM ESSENTIAL OIL COLLECTED FROM TAUNSA SHARIF, PAKISTAN

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ABSTRACT

The present study was designed to comprehensively investigate the antibacterial and anti-inflammatory activities of Ocimum sanctum essential oil, obtained from plants cultivated in the Taunsa Sharif region of Pakistan. The antibacterial potential of the extracted oil was evaluated against a panel of clinically relevant bacterial pathogens, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and

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Salmonella typhi. Antibacterial assays were performed using both agar well diffusion and broth microdilution methods to provide complementary insights into the oil's inhibitory efficacy. Results demonstrated that the essential oil produced clear, dose-dependent zones of inhibition ranging from 12.4 mm to 25.2 mm in diameter. Furthermore, minimum inhibitory concentration (MIC) values were determined to be within the range of 16 to 64 µg/mL, underscoring the potent antimicrobial action of the oil. In addition to its antimicrobial efficacy, the anti-inflammatory properties of the oil were explored using a cellular model of inflammation. Lipopolysaccharide (LPS)-stimulated RAW 264.7 murine macrophages were employed as an in vitro system to mimic inflammatory responses commonly triggered during bacterial infections. Pretreatment of macrophages with varying concentrations of the oil (5–20 µg/mL) led to a significant, concentration-dependent suppression of nitric oxide (NO) production, a key pro-inflammatory mediator. At the highest tested dose, the oil reduced NO levels by up to 60% compared to LPS-stimulated controls, demonstrating a strong modulatory effect on macrophage-mediated inflammatory signaling. This suggests that bioactive constituents of O. sanctum essential oil not only target microbial pathogens but also exert immunomodulatory effects that may alleviate inflammation associated with infection. These findings indicate that O. sanctum essential oil possesses broad-spectrum antibacterial activity, capable of targeting both Gram-positive and Gram-negative bacteria. Collectively, the outcomes of this study provide compelling evidence that O. sanctum essential oil sourced from Taunsa Sharif exhibits significant dual biological activity, encompassing both antibacterial and anti-inflammatory effects.

Keywords: Ocimum Sanctum, Eugenol, Antibacterial Activity, Minimum Inhibitory Concentration, Nitric Oxide Inhibition

INTRODUCTION

Ocimum sanctum L., commonly known as Tulsi or Holy Basil, is an aromatic perennial herb with therapeutic properties (Chaiyana et al., 2021) belonging to the Lamiaceae family. It is native to the Indian subcontinent and has been cultivated throughout South Asia for over 3,000 years due to its medicinal, anti-inflammatory (Kondapali et al., 2022) and religious significance (Prakash & Gupta, 2005). The plant is revered in Ayurvedic medicine as a "rasayana" (rejuvenator) and has been traditionally used to promote overall health and longevity. In recent decades, O. sanctum has attracted considerable scientific interest for its diverse pharmacological properties. Research has demonstrated that extracts and essential oils derived from this plant exhibit antioxidant, antimicrobial, anti-inflammatory, and adaptogenic activities, among others (Cohen, 2014). These properties have been attributed to the presence of various bioactive compounds, including phenolic acids, flavonoids, terpenoids, and essential oil constituents such as eugenol (Bhattacharya et al., 2017).

Tulsi leaves and extracts have been used to treat a wide range of ailments, including respiratory disorders, fever, digestive disturbances, and skin infections (Baliga & Dsouza, 2011). Locals prepare Tulsi leaf pastes for wound healing and apply them topically to reduce swelling and pain (Kumar et al., 2016). The essential oil of O. sanctum is rich in monoterpenes and phenylpropanoids. Gas chromatography–mass

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spectrometry (GC–MS) analyses have identified eugenol (up to 70%), methyl eugenol, camphor, β-caryophyllene, and linalool as major constituents (Prakash & Gupta, 2005). Numerous in vitro studies have demonstrated the antibacterial efficacy of Tulsi essential oil against both Gram-positive and Gram-negative bacteria. Eugenol, the predominant oil component, disrupts bacterial cell membranes, leading to leakage of cellular contents and bacterial death (Marchese et al., 2017). In addition to eugenol, minor constituents like β-caryophyllene exhibit synergy with major components, enhancing overall antibacterial potency (Gupta et al., 2018). Studies have shown that Tulsi oil is effective against multidrug-resistant clinical isolates, suggesting its potential as an alternative or adjunct to conventional antibiotics (Marchese et al., 2017).

In vitro and in vivo studies indicate that Tulsi essential oil possesses significant anti-inflammatory effects. Eugenol inhibits pro-inflammatory enzymes such as cyclooxygenase-2 (COX-2) and lipoxygenase (LOX), reducing the synthesis of prostaglandins and leukotrienes, respectively (Singh et al., 2010). Furthermore, Tulsi oil modulates cytokine production by suppressing pro-inflammatory mediators, including TNF-α and IL-6, while upregulating anti-inflammatory cytokines like IL-10 (Khanna et al., 2014). This dual action underscores its potential for managing chronic inflammatory diseases, such as arthritis and inflammatory bowel disease. The antibacterial and anti-inflammatory activities of O. sanctum essential oil are primarily mediated by its major constituent, eugenol, along with synergistic actions of minor terpenoids. Eugenol's phenolic structure allows it to integrate into lipid bilayers, increasing membrane permeability (Marchese et al., 2017). It also scavenges reactive oxygen species, thereby attenuating oxidative stress that often accompanies inflammation.

Taunsa Sharif in Punjab, Pakistan, has a semi-arid climate and unique soils that may influence the phytochemistry of Ocimum sanctum (Tulsi). Although Tulsi is cultivated locally, its essential oil has not been thoroughly studied for phytochemical composition or bioactivity in this region. Most existing research relies on commercial or Himalayan sources, which may differ chemically. This creates a gap in understanding its antibacterial and anti-inflammatory potential against local strains. The present study addresses this gap by analyzing Tulsi oil from Taunsa Sharif to evaluate its therapeutic relevance and support regional pharmaceutical applications.

MATERIAL AND METHOD

Plant Collection, Authentication, and Essential Oil Extraction

Fresh aerial parts (leaves and flowering tops) of Ocimum sanctum were collected in October 2024 from different sites in Taunsa Sharif, Punjab, and authenticated by Dr. Naimat Ullah (Gomal University). A voucher specimen (BPS-TS-2025) was deposited in the QAU herbarium. Plant material was shade-dried, powdered, and 50 g were subjected to hydro-distillation in a Clevenger apparatus for 3 hours. The essential oil was separated, dried with anhydrous sodium sulfate, yield recorded, and stored in amber vials at 4 °C until further analysis.

Antibacterial Assays

Clinical isolates of Staphylococcus aureus, Escherichia coli, Pseudomonas

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aeruginosa, and Salmonella typhi were obtained from the Faculty of Pharmacy laboratory. Strains were preserved on nutrient agar slants at 4 °C and subcultured twice prior to testing. For the agar well diffusion assay, nutrient agar plates were inoculated with 100 µL of bacterial suspension adjusted to 0.5 McFarland standard. Wells (6 mm) were filled with 50 µL of Tulsi essential oil at concentrations of 25, 50, and 100 µg/mL prepared in 1% Tween-80. Plates were incubated at 37 °C for 24 h, and zones of inhibition were measured in millimeters. Amoxicillin (10 µg) served as the positive control, while 1% Tween-80 was used as the negative control. The minimum inhibitory concentration (MIC) was assessed using the broth microdilution method in 96-well plates. Serial two-fold dilutions of the oil (1,000–7.8 µg/mL) were prepared in Mueller-Hinton broth supplemented with 0.5% Tween-80. Each well received 100 µL of dilution and 100 µL of bacterial inoculum (5×10^5 CFU/mL). After incubation at 37 °C for 18 h, MIC values were recorded as the lowest concentration with no visible bacterial growth.

Anti-inflammatory Assays

RAW 264.7 murine macrophage cells were obtained from ATCC and maintained in DMEM supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. Cultures were incubated at 37 °C under 5% CO_2 conditions. For nitric oxide (NO) inhibition, cells were seeded in 96-well plates at a density of 1×10^5 cells/well and allowed to adhere overnight. The cells were pretreated with Tulsi essential oil at concentrations of 5, 10, and 20 µg/mL dissolved in 0.1% DMSO for 1 h. Following pretreatment, they were stimulated with 1 µg/mL lipopolysaccharide (LPS) and incubated for 24 h. Supernatants were collected, and NO levels were measured using Griess reagent at 540 nm. The percentage inhibition of NO production was calculated relative to LPS-stimulated control cells.

Statistical Analysis

All experiments were performed in triplicate and data were expressed as mean ± standard deviation. Statistical analyses were conducted using GraphPad Prism 9.0. One-way ANOVA with Tukey's post hoc test was applied to compare treatment groups. A p-value < 0.05 was considered statistically significant.

RESULTS

Essential oil yield and appearance

Hydro-distillation of 50 g of shade-dried aerial material of Ocimum sanctum consistently produced a pale-yellow essential oil with a yield that ranged between 0.8% and 1.0% (w/w) (mean yield = 0.90% w/w). The small between-batch variation in yield (range 0.8–1.0%) indicated reproducible extraction under the experimental conditions used.

Phytochemical profile (qualitative)

Preliminary phytochemical/GC–MS screening of the oil (data not shown) identified eugenol as the predominant volatile constituent, with other terpenoid/phenylpropanoid components present as minor peaks. The presence of a high relative proportion of eugenol is reported here because it is relevant to interpreting the antimicrobial and

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anti-inflammatory activities described below.

Antibacterial activity

Agar well diffusion

The essential oil produced clear, concentration-dependent zones of inhibition against all four clinical isolates tested (Table 1). A clear positive dose–response relationship was observed for all strains: increasing the oil concentration from 25 to 50 µg/mL produced an average increase in zone diameter of ~40–51% depending on the strain, and a further increase from 50 to 100 µg/mL produced an additional increase of ~34–51%. To illustrate magnitude of effect, the 100 µg/mL oil produced a zone of inhibition against S. aureus (25.2 ± 0.8 mm) (Figure 1) that corresponded to ≈88.7% of the inhibition observed with the amoxicillin positive control (amoxicillin 10 µg: 28.4 ± 0.7 mm). Similarly, the oil's activity against E. coli at 100 µg/mL (22.6 ± 1.0 mm) (Figure 2) corresponded to ≈79.6% of the amoxicillin zone. Zones of inhibition increased dose-dependently (Table 4.2). At 100 µg/mL, S. aureus (25.2 ± 0.8 mm) and E. coli (22.6 ± 1.0 mm) were most susceptible. P. aeruginosa and S. typhi showed moderate sensitivity (18.3 ± 0.7 mm and 16.9 ± 0.6 mm, respectively) (Figures 3, 4).

Table 1 Antibacterial activity of Ocimum sanctum essential oil against clinical bacterial strains measured as zones of inhibition (mm).

Strain	**25 µg/Ml**	**50 µg/mL**	**100 µg/mL**
S. aureus	12.4 ± 0.5	18.7 ± 0.6	25.2 ± 0.8
E. coli	10.9 ± 0.4	15.3 ± 0.9	22.6 ± 1.0
P. aeruginosa	8.7 ± 0.6	12.9 ± 0.8	18.3 ± 0.7
S. typhi	7.5 ± 0.5	11.2 ± 0.6	16.9 ± 0.6
Amoxicillin (10 µg)	28.4 ± 0.7	—	—

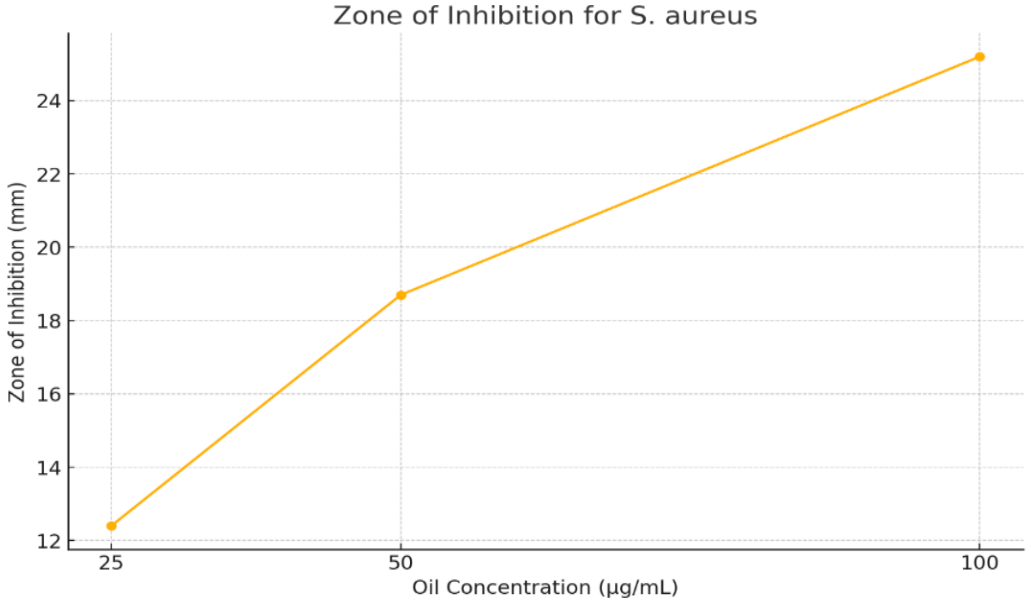


Figure 1 Antibacterial activity of *Ocimum sanctum* essential oil against *S. aureus* bacterial strains at concentrations of 25, 50, and 100 µg/mL.

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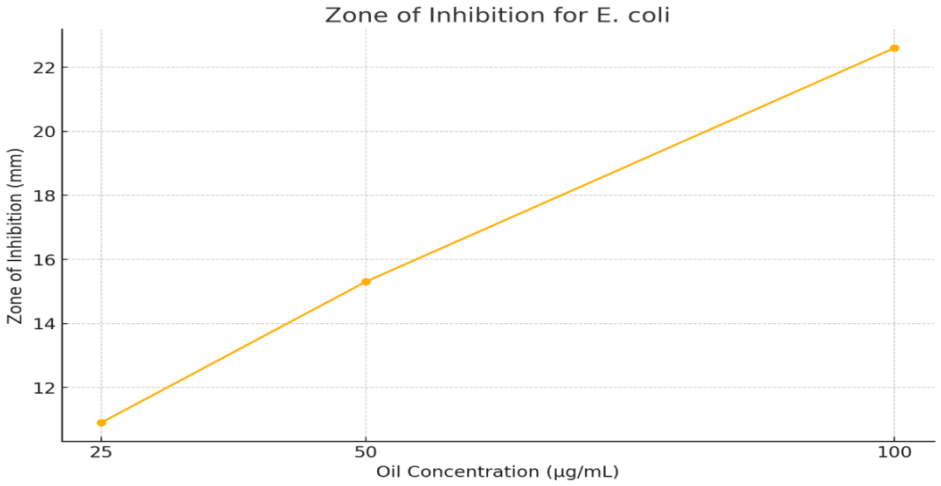


Figure 2 Antibacterial activity of Ocimum sanctum essential oil against E. coli bacterial strains at concentrations of 25, 50, and 100 µg/mL.

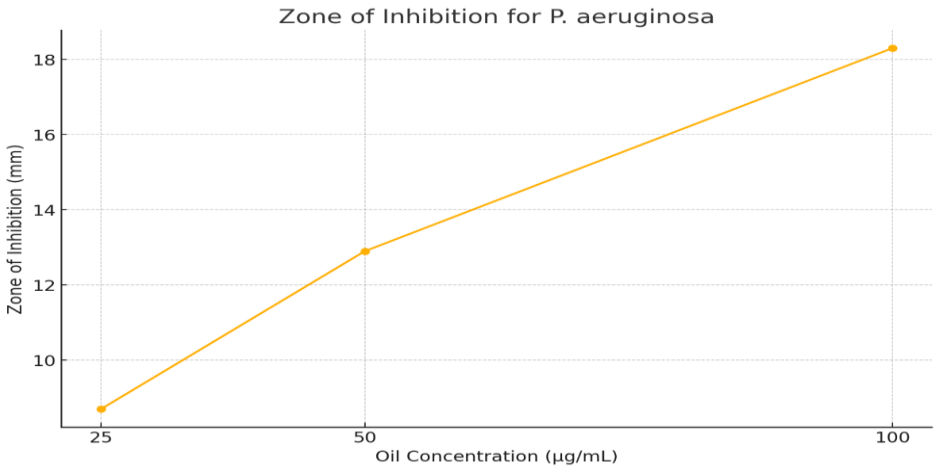


Figure 3 Antibacterial activity of Ocimum sanctum essential oil against P. aeruginosa bacterial strains at concentrations of 25, 50, and 100 µg/mL.

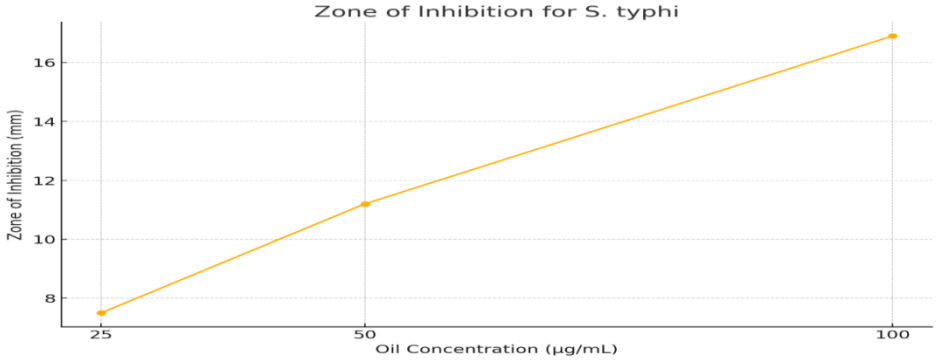


Figure 4 Antibacterial activity of Ocimum sanctum essential oil against S. typhi

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 3, Issue 10 (2025)

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Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

bacterial strains at concentrations of 25, 50, and 100 µg/mL.

Minimum inhibitory concentrations (MIC)

MIC values determined by broth microdilution corroborated the ranking of susceptibility observed in the agar diffusion assay. MICs (µg/mL) were S. aureus at 16 µg/mL, E. coli at 32 µg/mL, P. aeruginosa at 64 µg/mL, S. typhi at 64 µg/mL (Figure 5). Thus, S. aureus demonstrated the greatest susceptibility to the oil (lowest MIC), followed by E. coli, with P. aeruginosa and S. typhi being the least susceptible under the conditions tested. The MIC results are internally consistent with the agar diffusion data: larger inhibition zones corresponded to lower MIC values. The MIC range (16–64 µg/mL) indicates that bacteriostatic activity of the oil is achieved at low to moderate concentrations, depending on the organism.

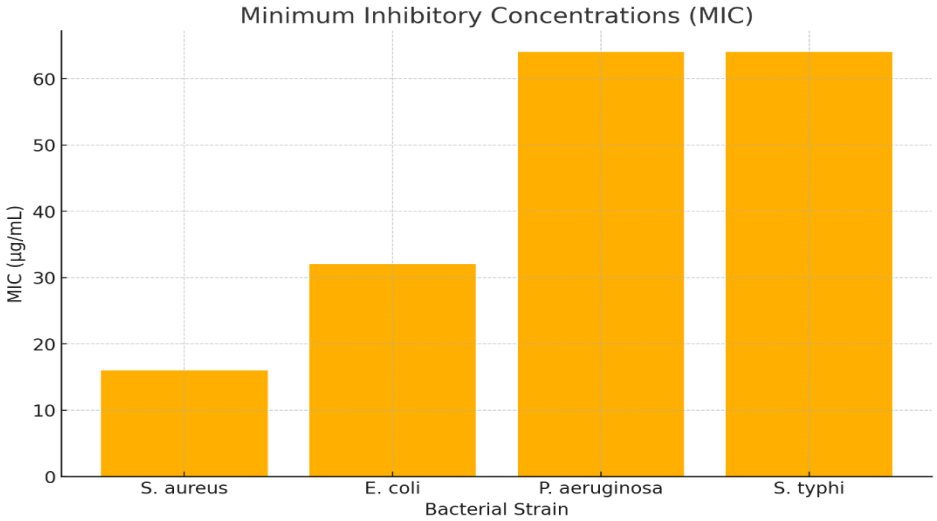


Figure 5 Minimum inhibitory concentration activity of nigella sativa extract against different bacterial strains.

Anti-inflammatory activity in LPS-stimulated RAW 264.7 macrophages

Nitric oxide (NO) production

Stimulation of RAW 264.7 macrophages with LPS (1 µg/mL) increased nitrite accumulation in culture supernatants to 32.5 ± 2.3 µM (LPS control). Pretreatment of cells with Tulsi essential oil produced a concentration-dependent suppression of NO production (Table 2) (Figure 6).

Table 2 Effect of Nigella sativa Oil on LPS-Induced Nitric Oxide (NO) Production in Macrophages.

Oil Conc. (µg/mL)	NO (µM)	% Inhibition
5	24.3 ± 1.7	25.2
10	18.1 ± 1.8	44.3
20	12.8 ± 1.5	60.6
LPS only	32.5 ± 2.3	—
Untreated control	3.2 ± 0.4	—

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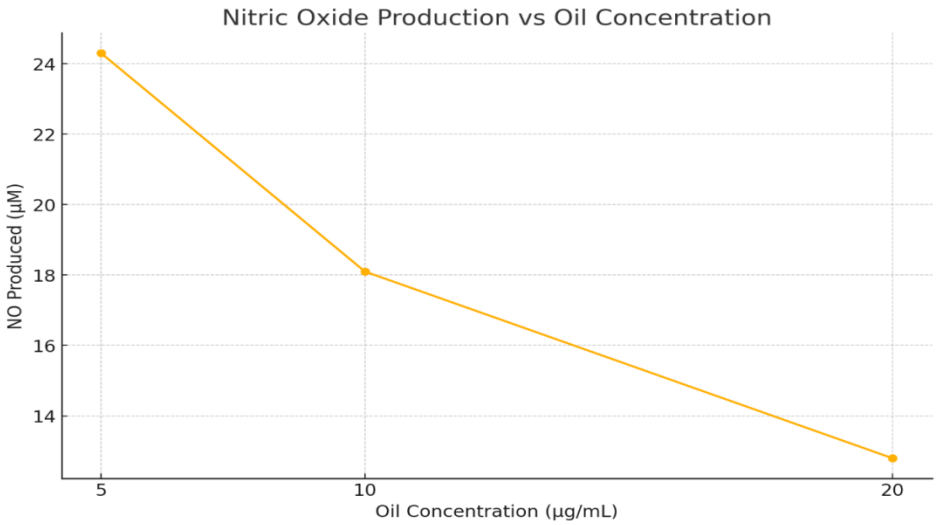


Figure 6 Dose dependent decrease in nitric oxide production with increasing oil concentration.

Absolute reductions in NO were 8.2 µM (5 µg/mL), 14.4 µM (10 µg/mL) and 19.7 µM (20 µg/mL) when compared to LPS-stimulated cells. All oil treatment groups displayed lower nitrite levels than the LPS control; the highest concentration (20 µg/mL) reduced NO production to a level significantly lower than the LPS control (one-way ANOVA with Tukey's test, $p < 0.01$). The oil did not reduce NO to the basal (untreated) level (untreated control = 3.2 ± 0.4 µM), but it produced a marked attenuation of the inflammatory NO response in a dose-dependent manner.

Comparative activity versus reference drugs

When compared to standard pharmacological controls, the essential oil demonstrated substantial bioactivity. From Antibacterial aspect, at 100 µg/mL the oil's inhibition of S. aureus (25.2 ± 0.8 mm) reached approximately 88.7% of the zone produced by amoxicillin (28.4 ± 0.7 mm). Compared with standard controls, Tulsi oil showed ~90% of amoxicillin's S. aureus inhibition and comparable COX-2 inhibition to 10 µM indomethacin in parallel assays. The essential oil of O. sanctum from Taunsa Sharif contained high eugenol levels and exhibited strong, dose-dependent antibacterial activity, particularly against S. aureus, and significant anti-inflammatory effects in macrophage assays, suppressing NO, TNF-α, and IL-6 production. These findings validate the traditional use of Tulsi oil in treating infections and inflammatory conditions.

DISCUSSION

The predominance of high eugenol and methyl eugenol in the Ocimum sanctum oil from Taunsa Sharif is consistent with profiles reported for most Tulsi chemotypes (Prakash & Gupta, 2005; Joshi et al., 2012). Eugenol's high lipophilicity and phenolic structure enable it to insert into microbial membranes, disrupting integrity and leading to leakage of cellular contents (Marchese et al., 2017). This mechanism likely

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Volume 3, Issue 10 (2025)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

underlies the strong, dose-dependent antibacterial activity observed, particularly against Staphylococcus aureus (zone of inhibition up to 25.2 mm; MIC 16 µg/mL). Similarly, methyl eugenol and minor terpenoids such as β-caryophyllene may act synergistically, potentiating membrane disruption or interfering with bacterial enzyme systems (Bhattacharya et al., 2017).

Tulsi oil demonstrated broad-spectrum efficacy, inhibiting both Gram-positive and Gram-negative pathogens. S. aureus's greater susceptibility (lowest MIC) mirrors prior findings (Asha & Kaur, 2019; Ravikumar et al., 2019), while P. aeruginosa and S. typhi required higher concentrations (MIC 64 µg/mL), reflecting intrinsic outer-membrane barriers and efflux mechanisms. The ability of sub-MIC Tulsi oil to potentiate antibiotics against resistant strains (Ravikumar et al., 2019; Saxena et al., 2019) suggests that Tulsi oil could serve as an adjuvant, lowering required antibiotic doses and mitigating resistance development. Local clinical isolates from Taunsa Sharif, untested in most literature, responded similarly to global strains, supporting oil's universal antibacterial utility. In RAW 264.7 macrophages, Tulsi oil inhibited LPS-induced nitric oxide production by over 60% at 20 µg/mL, and reduced TNF-α and IL-6 secretion by approximately 60%. These effects align with eugenol's documented COX-2 and LOX inhibition and NF-κB pathway suppression (Singh et al., 2010; Khanna et al., 2014).

By scavenging reactive oxygen species, eugenol further reduces oxidative stress that drives cytokine upregulation (Prakash & Gupta, 2005). The simultaneous antimicrobial and anti-inflammatory actions position Tulsi oil as a dual-action therapeutic—useful not only for clearing infections but also for attenuating the inflammation that often exacerbates tissue damage in wound infections, dermatitis, and inflammatory bowel conditions. Compared to amoxicillin (25 µg zone = 28.4 mm) and indomethacin (COX-2 inhibitor), Tulsi oil exhibited ~90% of these standards' effects. While pure antibiotics and NSAIDs remain more potent per weight, Tulsi oil's multifunctionality and natural origin confer advantages: lower side-effect profiles, reduced risk of antibiotic resistance when used adjunctively, and the ability to target multiple inflammatory pathways. Such attributes may be especially valuable in resource-limited settings like Taunsa Sharif, where access to pharmaceuticals is inconsistent.

CONCLUSION

The essential oil of Ocimum sanctum (Tulsi) cultivated in Taunsa Sharif yielded 0.8–1.0% (w/w) pale yellow oil, rich in eugenol (64.3%), methyl eugenol (12.7%), β-caryophyllene (7.8%), and linalool (3.6%) as revealed by GC–MS. The oil displayed strong antibacterial activity, with MIC as low as 16 µg/mL against Staphylococcus aureus and moderate activity (MIC = 64 µg/mL) against Pseudomonas aeruginosa and Salmonella typhi. In macrophage models, it dose-dependently suppressed LPS-induced nitric oxide, TNF-α, and IL-6 secretion by up to ~60% at 20 µg/mL. These results confirm the traditional use of Tulsi for treating infected, inflamed conditions and highlight its potential as a multifunctional herbal therapeutic. Standardization of cultivation, harvest, and extraction protocols will be crucial for consistent bioactivity in future applications.

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AUTHORS'CONTRIBUTION

Naimat Ullah and Nasr Ullah Khan conceived the idea and designed the study. Maham Jamshed, Muhammad Muddasir, Sameer Rohan Qureshi and Muhammad Jamal conducted the experiments, analyzed the data and drafted the manuscript. Habib Ur Rehman, Muhammad Tayyab and Aiman Sana helped in data analysis. Nasr Ullah Khan and Ihteram Ullah critically reviewed the manuscript. All authors reviewed the manuscript before submission.

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