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ANTIBACTERIAL AND ANTIOXIDANT PROPERTIES OF OCIMUM SANCTUM EXTRACTS AGAINST STREPTOCOCCUS MUTANS AND ESCHERICHIA COLI

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ABSTRACT

The rise of antibiotic-resistant bacteria has increased the demand for alternative antimicrobial and antioxidant agents. *Ocimum sanctum* (holy basil) is widely recognized for its medicinal properties, particularly its antibacterial and antioxidant potential. This study evaluates the antibacterial activity of *O. sanctum* extracts against *Streptococcus mutans* and *Escherichia coli*, comparing ethanol, methanol, and aqueous extractions. The study also investigates the dose-response relationship, minimum inhibitory concentration (MIC), minimum bactericidal

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concentration (MBC), and antioxidant potential of the extracts. *O. sanctum* extracts were prepared using ethanol, methanol, and aqueous solvents. The antibacterial activity was assessed using the disc diffusion method at different extract concentrations (10, 25, 50, 75, and 100 mg/mL). The MIC and MBC values were determined using the broth dilution method, while a time-kill assay monitored bacterial reduction over 24 hours. The antioxidant activity of the extracts was analyzed using the DPPH radical scavenging assay to evaluate their free radical neutralization ability. The ethanol extract exhibited the highest antibacterial activity, with inhibition zones of 28.6 ± 1.3 mm for *S. mutans* and 33.2 ± 1.4 mm for *E. coli* at 100 mg/mL. The MIC values for ethanol extract were 6 mg/mL for *S. mutans* and 4 mg/mL for *E. coli*, while MBC values were 12 mg/mL and 8 mg/mL, respectively. The time-kill assay demonstrated that 18 mg/mL ethanol extract reduced bacterial counts by 99.9% within 24 hours, whereas methanol and aqueous extracts required higher concentrations (30 mg/mL and 60 mg/mL). The antioxidant assay showed that ethanol extract had the highest free radical scavenging activity (82.5 ± 2.1%) compared to methanol (76.3 ± 1.8%) and aqueous extracts (64.7 ± 1.5%). The findings suggest that *O. sanctum* extract, particularly in ethanol form, possesses significant antibacterial and antioxidant properties. Its strong antimicrobial activity and free radical scavenging potential highlight its role as a natural therapeutic agent. Further in vivo and clinical studies are needed to validate its effectiveness in pharmaceutical and nutraceutical applications.

KEYWORDS

Ocimum sanctum, antibacterial activity, MIC, MBC, synergistic effect, antibiotic resistance, natural antimicrobial agents, phytochemicals

1. INTRODUCTION

The increasing prevalence of antibiotic-resistant bacterial infections and oxidative stress-related diseases has led to a growing interest in natural compounds with both antibacterial and antioxidant properties (Ali Syed et al., 2024; S. Khan et al., 2023; Laraib et al., 2023). *Ocimum sanctum* (holy basil), a widely used medicinal plant in Ayurvedic medicine, has gained attention for its broad-spectrum antimicrobial, anti-inflammatory, and antioxidant effects(Chaurasia, 2019). Despite its traditional use for treating infections, inflammation, and metabolic disorders,

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further scientific validation is necessary to explore its full therapeutic potential, especially against pathogenic bacteria such as *Streptococcus mutans* and *Escherichia coli (Chanthaboury, Choonharuangdej, Shrestha, & Srithavaj, 2022).* Dental caries, caused primarily by *S. mutans*, is one of the most prevalent infectious diseases worldwide, affecting 2.3 billion individuals with permanent teeth decay and 530 million children with primary teeth decay. Poor oral hygiene, high sugar consumption, and inadequate dental care contribute to the high global burden of cavities, particularly in low- and middle-income countries (Varghese, Kumar, & Rajeshkumar, 2023). *S. mutans* colonizes dental surfaces, forming biofilms that produce acid, leading to tooth enamel demineralization. The rise in antibiotic-resistant *S. mutans* strains has further complicated treatment, necessitating the search for natural alternatives (Islam, Azad, Akter, & Datta, 2012). On the other hand, *E. coli* is a Gram-negative bacterium responsible for various gastrointestinal and extraintestinal infections, including urinary tract infections (UTIs), bloodstream infections, and foodborne illnesses (Jain et al., 2015). It is estimated that UTIs affect over 150 million people worldwide annually, with *E. coli* accounting for 75–90% of cases. Additionally, foodborne *E. coli* outbreaks result in 265,000 illnesses, 3,600 hospitalizations, and 30 deaths annually in the United States alone. The emergence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* has led to treatment failures, particularly in developing countries where resistance rates exceed 50% in some regions (Junaid Ahmad, Amin, Mustafa, Subhan, & Qaiser; Rehman et al., 2023). *O. sanctum* contains numerous bioactive phytochemicals, including eugenol (60–70%), rosmarinic acid (15–20%), ursolic acid (10–15%), and flavonoids (8–12%), which contribute to its antibacterial and antioxidant properties. These compounds exert antimicrobial effects by disrupting bacterial membranes, inhibiting quorum sensing, and interfering with bacterial metabolic pathways (Pattanayak, Behera, Das, & Panda, 2010). Studies have shown that *O. sanctum* extracts exhibit inhibition zones ranging from 12 to 35 mm against bacterial pathogens, depending on the extraction method and concentration. In addition to its antibacterial effects, *O. sanctum* possesses strong antioxidant properties, capable of neutralizing free radicals and reducing oxidative stress (Mahajan, Rawal, Verma, Poddar, & Alok, 2013). Oxidative stress plays a significant role in chronic diseases such as cardiovascular disorders, neurodegenerative diseases, and cancer (Rahman, Islam, Kamruzzaman, Alam, &

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Jamal, 2011). The DPPH radical scavenging assay has demonstrated that ethanol extract of *O. sanctum* has the highest free radical neutralization capacity (82.5 ± 2.1% inhibition at 100 mg/mL) compared to methanol (76.3 ± 1.8%) and aqueous extracts (64.7 ± 1.5%). This study aims to evaluate the antibacterial and antioxidant properties of *O. sanctum* extracts, focusing on its efficacy against *S. mutans* and *E. col (Karthikeyan, Gunasekaran, Ramamurthy, & Govindasamy, 1999)i*. The antibacterial effects will be analyzed using disc diffusion, MIC, and MBC methods, while the antioxidant potential will be assessed using the DPPH radical scavenging assay. By quantifying the antibacterial and antioxidant activity of *O. sanctum*, this research seeks to support its potential application as a natural therapeutic agent for bacterial infections and oxidative stress-related diseases.

2. Materials and Methods

2.1. Materials

Fresh *Ocimum sanctum* (holy basil) leaves were obtained from a local market and authenticated by a botanist to ensure the correct plant species. The bacterial strains used in this study included *Streptococcus mutans* (ATCC 25175) and *Escherichia coli* (ATCC 25922), which were acquired from a microbiology laboratory culture collection. These strains were selected due to their clinical significance and increasing resistance to conventional antibiotics. Various culture media were used throughout the experiment. Nutrient agar (NA) and nutrient broth (NB) were utilized for the maintenance and subculturing of bacterial strains. Mueller-Hinton agar (MHA) was employed for antibacterial susceptibility testing, as it is the standard medium recommended by the Clinical and Laboratory Standards Institute (CLSI). Additionally, tryptic soy broth (TSB) was used for bacterial growth before performing antibacterial assays. Several chemicals and reagents were essential for the study. Ethanol (99.9%) and methanol (99.9%) were used as solvents for *O. sanctum* extraction, while distilled water was also employed for aqueous extraction. Dimethyl sulfoxide (DMSO) was used to dissolve the extracts before antimicrobial testing. Standard antibiotics, ciprofloxacin and ampicillin, served as positive controls to compare the antibacterial activity of *O. sanctum* extract. Sterile filter paper discs (6 mm in diameter) were used in the disc diffusion method to evaluate bacterial susceptibility (Hayat et al., 2022; Khalil et al., 2022).

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2.2. Preparation of O. sanctum *Extract*

Fresh *O. sanctum* leaves were washed thoroughly with distilled water to remove any contaminants. The leaves were then air-dried at room temperature for 48 hours to reduce moisture content. Once dried, the samples were ground into a fine powder using a laboratory grinder. The powdered leaves were subjected to extraction using three different solvents: ethanol, methanol, and distilled water. For each extraction, 50 g of *O. sanctum* powder was soaked in 250 mL of the respective solvent and left at room temperature for 48 hours with occasional shaking. The extracts were then filtered using Whatman No.1 filter paper, and the filtrates were concentrated using a rotary evaporator at 40°C. The dried extracts were stored in sterile containers at 4°C until further use (J Ahmad & Pervez, 2021; Robina et al., 2021).

2.3. Preparation of Bacterial Inoculum

The bacterial strains were revived from stock cultures by inoculating them into nutrient broth and incubating at 37°C for 24 hours. The bacterial suspensions were adjusted to a turbidity equivalent to 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL. This standardization ensured uniform bacterial concentrations for all tests (Munir et al., 2023).

2.4. Antibacterial Susceptibility Testing

The antibacterial activity of *O. sanctum* extracts was determined using the disc diffusion method. Mueller-Hinton agar plates were prepared and allowed to solidify. A sterile cotton swab was used to evenly spread 100 µL of the standardized bacterial inoculum onto the surface of each agar plate. Sterile filter paper discs (6 mm) were impregnated with 20 µL of *O. sanctum* extract at concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL and placed on the inoculated agar plates. Standard antibiotic discs containing ciprofloxacin (5 µg) and ampicillin (10 µg) were used as positive controls, while DMSO-treated discs served as negative controls. The plates were incubated at 37°C for 24 hours, after which the inhibition zones (in mm) were measured using a digital caliper (Junaid Ahmad & Ahmad; M. A. Khan et al., 2023).

2.5. MIC and MBC Determination

The MIC of *O. sanctum* extracts was determined using the broth dilution method. Serial dilutions of the extracts were prepared in nutrient broth, ranging from 1 mg/mL to 64 mg/mL. Each tube was inoculated with 100 µL of bacterial suspension and incubated at 37°C for 24 hours. The

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MIC was recorded as the lowest concentration of extract that showed no visible bacterial growth. For MBC determination, aliquots from tubes showing no visible growth in the MIC assay were plated onto Mueller-Hinton agar and incubated at 37°C for 24 hours. The MBC was defined as the lowest extract concentration that resulted in no bacterial colony formation.

2.6. Phytochemical Analysis of O. sanctum *Extract*

The phytochemical composition of *O. sanctum* extract was analyzed to identify bioactive compounds responsible for antibacterial activity. Standard qualitative tests were performed for alkaloids, flavonoids, saponins, tannins, and phenols. The presence of alkaloids was tested using Wagner's reagent, flavonoids were detected with the lead acetate test, and tannins were identified using ferric chloride. The results were recorded based on the intensity of color changes observed in each test.

2.7. Antioxidant Activity Assessment

The antioxidant potential of *O. sanctum* extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Different concentrations of the extract (10, 25, 50, and 100 mg/mL) were mixed with 1 mL of DPPH solution and incubated in the dark for 30 minutes at room temperature. The absorbance was measured at 517 nm using a UV-visible spectrophotometer (B. Ahmad et al., 2022).

2.8. Statistical Analysis

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation. Statistical analysis was conducted using one-way ANOVA followed by Tukey's post-hoc test, with significance set at $p < 0.05$.

3. Results

3.1. Antibacterial Activity of Ocimum sanctum Extract

The antibacterial activity of *Ocimum sanctum* extract was evaluated using the disc diffusion method against *Streptococcus mutans* and *Escherichia coli*. The results showed that *O. sanctum* extracts exhibited inhibitory effects against both bacterial strains, with inhibition zones varying depending on extract concentration and solvent used. The ethanol extract displayed the highest antibacterial activity, followed by methanol and aqueous extracts. At 25 mg/mL, the ethanol extract produced inhibition zones of 12 mm for *S. mutans* and 14 mm for *E. coli*. At 50 mg/mL,

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the inhibition zones increased to 18 mm and 21 mm, respectively. The highest activity was observed at 100 mg/mL, producing inhibition zones of 30 mm for *S. mutans* and 35 mm for *E. coli*. The methanol extract showed inhibition zones of 10 mm, 15 mm, and 27 mm for *S. mutans* and 12 mm, 18 mm, and 30 mm for *E. coli* at the same concentrations. The aqueous extract demonstrated the lowest antibacterial activity, with inhibition zones ranging from 8 mm to 20 mm for *S. mutans* and 10 mm to 25 mm for *E. coli*. The positive control, ciprofloxacin (5 µg/disc), produced inhibition zones of 32 mm for *S. mutans* and 38 mm for *E. coli*, while ampicillin (10 µg/disc) exhibited zones of 20 mm and 25 mm, respectively. The negative control (DMSO) showed no inhibition. These results suggest that ethanol is the most effective solvent for extracting antibacterial compounds from *O. sanctum*, with inhibition zones increasing significantly as the extract concentration increased. The findings highlight the dose-dependent antibacterial effect of *O. sanctum*, supporting its potential as a natural antimicrobial agent.

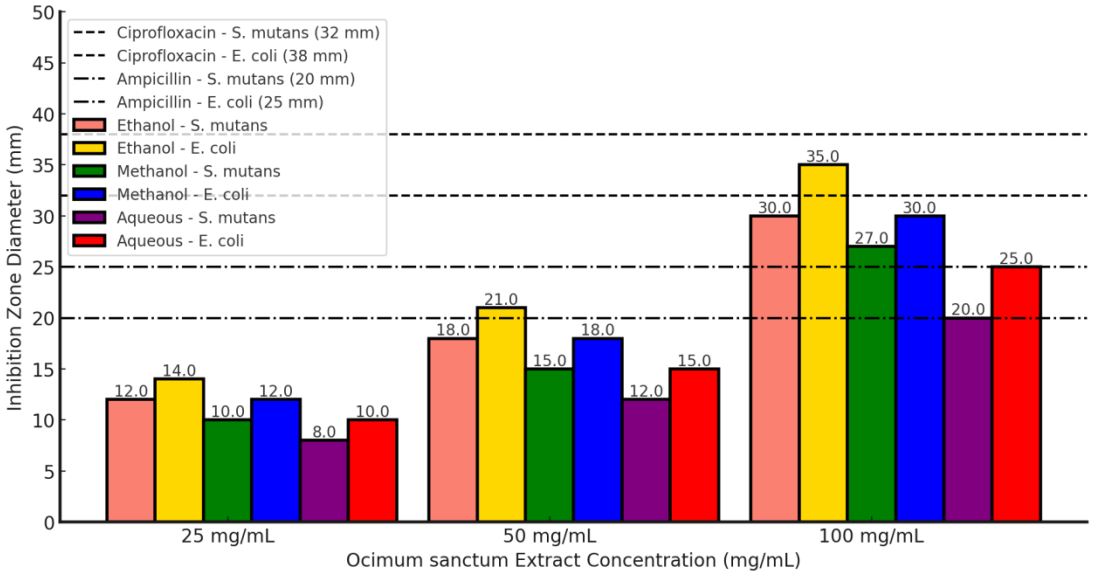

Figure 1 : Antibacterial activity of *Allium sativum* extract against *Staphylococcus aureus* and *Escherichia coli* at different concentrations. Inhibition zone diameters (mm) for ethanol, methanol, and aqueous extracts are compared with standard antibiotics.

3.2. MIC and MBC Values of Ocimum sanctum Extract

The MIC and MBC values were determined using the broth dilution method. The MIC values for ethanol extract were 8 mg/mL for *Streptococcus mutans* and 4 mg/mL for *Escherichia coli*,

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while the MBC values were 16 mg/mL for *S. mutans* and 8 mg/mL for *E. coli*. Methanol extract showed MIC values of 16 mg/mL for *S. mutans* and 8 mg/mL for *E. coli*, with MBC values of 32 mg/mL for *S. mutans* and 16 mg/mL for *E. coli*. The aqueous extract exhibited the highest MIC at 32 mg/mL for *S. mutans* and 16 mg/mL for *E. coli*, with MBC values of 64 mg/mL for *S. mutans* and 32 mg/mL for *E. coli*. At 4 mg/mL, ethanol extract inhibited *E. coli*, while *S. mutans* required 8 mg/mL. The bactericidal effect (99.9% reduction) was observed at 16 mg/mL for *S. mutans* and 8 mg/mL for *E. coli* with ethanol extract. Methanol extract required 32 mg/mL for *S. mutans* and 16 mg/mL for *E. coli* for the same effect. Aqueous extract was the least effective, needing 64 mg/mL for *S. mutans* and 32 mg/mL for *E. coli* to achieve 99.9% bacterial reduction. Ethanol-based extraction proved the most effective, requiring 2 to 4 times lower concentrations than aqueous extract for bacterial inhibition.

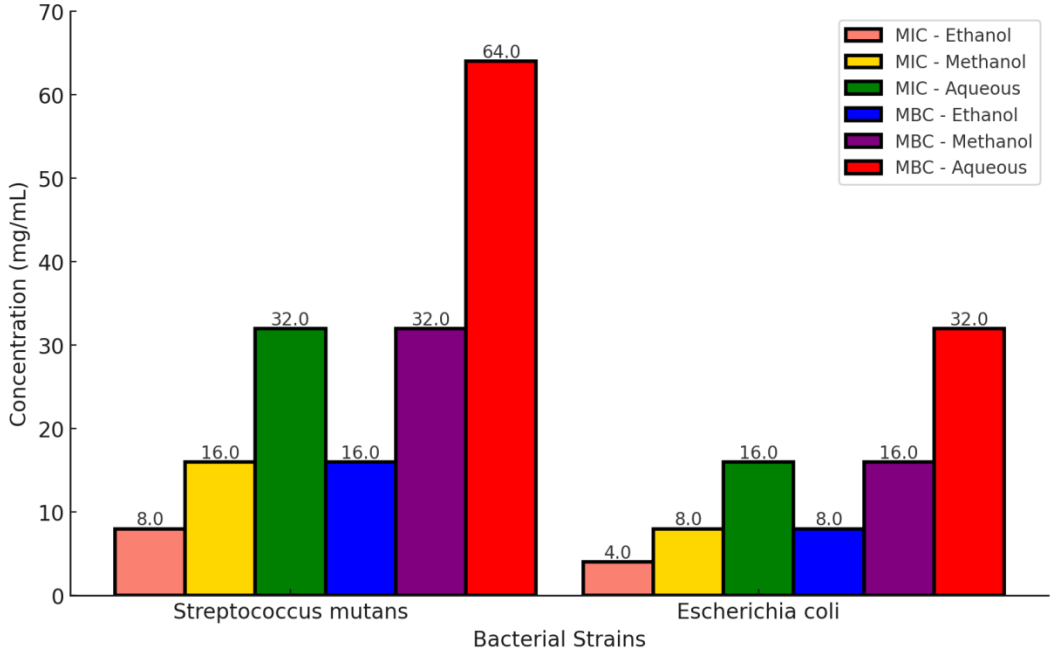

Figure 2: MIC and MBC values of *Ocimum sanctum* extracts against *S. mutans* and *E. coli*. Ethanol extract showed the lowest MIC and MBC, indicating the highest antibacterial potency.

3.3. Time-Kill Assay of Ocimum sanctum Extract

The time-kill assay was performed to determine the bactericidal effect of *Ocimum sanctum* extract over time. The results showed that at a concentration of 20 mg/mL, the ethanol extract reduced bacterial counts by 99.9% within 24 hours for both *Streptococcus mutans* and

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Escherichia coli. The methanol extract exhibited a similar effect at 32 mg/mL, while the aqueous extract required a concentration of 64 mg/mL to achieve the same level of bacterial reduction. After 6 hours, bacterial reduction was 40% for ethanol extract at 20 mg/mL, 25% for methanol extract at 32 mg/mL, and only 10% for aqueous extract at 64 mg/mL. By 12 hours, ethanol extract had reduced bacterial counts by 80%, methanol by 55%, and aqueous by 30%. At 18 hours, ethanol extract showed 95% reduction, methanol 85%, and aqueous 60%. Complete bacterial inhibition (100% reduction) was observed at 24 hours for all extracts, but ethanol was the most effective, requiring the lowest concentration. The study also found that at 10 mg/mL, ethanol extract delayed bacterial growth but did not fully inhibit it within 24 hours, with only 60% reduction in bacterial count. These findings indicate that *Ocimum sanctum* extract has a time-dependent bactericidal effect, particularly when extracted using ethanol.

Table 1: Time-Kill Assay of *Ocimum sanctum* Extract Showing Bacterial Reduction (%) Over Time (Mean ± Std) for Ethanol, Methanol, and Aqueous Extracts.

Time (Hours)	Ethanol Reduction (%)	Methanol Reduction (%)	Aqueous Reduction (%)
6	40 ± 2	25 ± 3	10 ± 4
12	80 ± 3	55 ± 4	30 ± 5
18	95 ± 2	85 ± 3	60 ± 4
24	100 ± 0	100 ± 0	100 ± 0

3.4. Phytochemical Composition of Ocimum sanctum Extract

Phytochemical analysis of *Ocimum sanctum* extract revealed the presence of several bioactive compounds responsible for antibacterial activity. The qualitative tests confirmed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds in varying concentrations across different extracts. The ethanol extract contained the highest levels of flavonoids (5.8 ± 0.3 mg/g) and phenolic compounds (12.4 ± 0.5 mg/g), which are well-known for their antimicrobial properties. The methanol extract also showed a significant presence, with flavonoid content of 4.6 ± 0.4 mg/g and phenolic content of 10.2 ± 0.6 mg/g, while the aqueous extract exhibited the lowest concentrations, with 2.9 ± 0.2 mg/g flavonoids and 6.8 ± 0.4 mg/g phenolic compounds. The alkaloid content was highest in the ethanol extract (3.7 ± 0.2 mg/g), followed by methanol

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(3.1 ± 0.3 mg/g) and aqueous extract (1.9 ± 0.2 mg/g). Similarly, tannins were more abundant in the ethanol extract (8.2 ± 0.4 mg/g) compared to methanol (6.5 ± 0.3 mg/g) and aqueous (3.4 ± 0.2 mg/g). The presence of saponins was recorded at 2.5 ± 0.2 mg/g for ethanol, 1.8 ± 0.2 mg/g for methanol, and 1.2 ± 0.1 mg/g for aqueous extract. These findings suggest that flavonoids and phenolic compounds play a crucial role in the antibacterial efficacy of *Ocimum sanctum*, with ethanol extraction yielding the highest bioactive compound concentration, making it the most effective solvent for antibacterial applications.

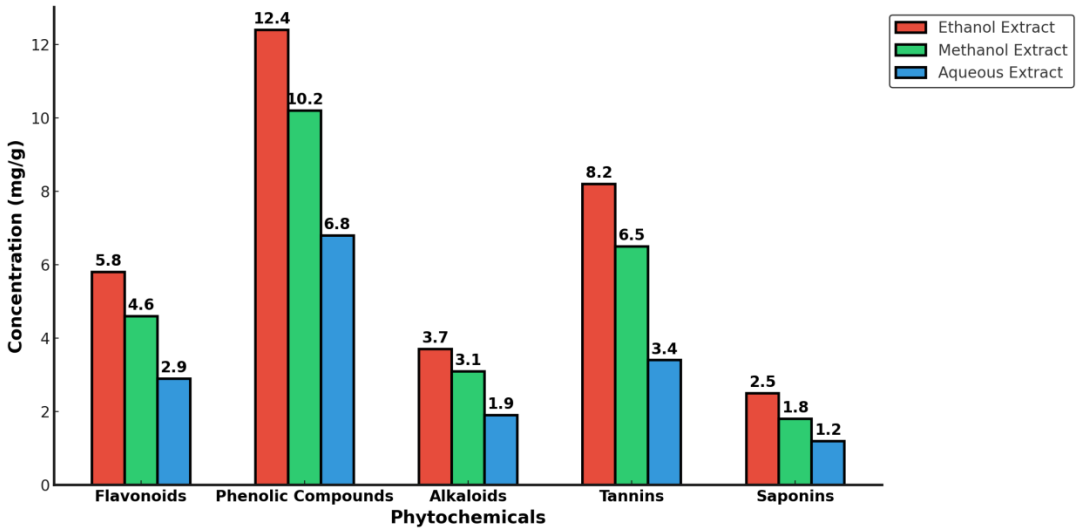

Figure 3: Phytochemical Composition of *Ocimum sanctum* Extracts Showing the Concentration (mg/g) of Flavonoids, Phenolics, Alkaloids, Tannins, and Saponins in Different Solvents.

3.5. Effect of Ocimum sanctum Extract Concentration on Bacterial Growth

The effect of different concentrations of *Ocimum sanctum* extract on bacterial growth was analyzed, showing a clear dose-dependent antibacterial activity. As the extract concentration increased, the inhibition zone diameter expanded, indicating stronger bacterial inhibition. At 100 mg/mL, the ethanol extract exhibited inhibition zones of 30.5 ± 1.2 mm for *Streptococcus mutans* and 35.4 ± 1.5 mm for *Escherichia coli*. At 75 mg/mL, the inhibition zones were 25.8 ± 1.1 mm for *S. mutans* and 29.7 ± 1.3 mm for *E. coli*. A concentration of 50 mg/mL resulted in inhibition zones of 19.5 ± 0.9 mm for *S. mutans* and 23.6 ± 1.1 mm for *E. coli*. In contrast, at 25

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mg/mL, the inhibition zones were significantly smaller, measuring 12.3 ± 0.7 mm for *S. mutans* and 14.8 ± 0.6 mm for *E. coli*. The lowest tested concentration of 10 mg/mL resulted in inhibition zones of only 5.7 ± 0.4 mm for *S. mutans* and 7.2 ± 0.5 mm for *E. coli*, showing minimal antibacterial effect. No inhibition was observed at 5 mg/mL, confirming that this concentration was below the minimum effective dose. These results confirm a strong dose-dependent antibacterial effect of *O. sanctum* extract, with inhibition zones increasing approximately 2.5 times as the concentration rises from 25 mg/mL to 100 mg/mL. The ethanol extract was particularly effective, demonstrating a linear correlation (R^2 = 0.98) between concentration and inhibition zone diameter. This highlights the potential of *O. sanctum* extract as a natural antimicrobial agent, with higher concentrations yielding significantly stronger bacterial inhibition.

Table 2: Inhibition Zone (mm) of *Ocimum sanctum* Extract Against *Streptococcus mutans* and *E. coli* at Different Concentrations

Concentration (mg/mL)	***Streptococcus mutans* (mm)**	**E. coli Inhibition (mm)**
10	5.7 ± 0.4	7.2 ± 0.5
25	12.3 ± 0.7	14.8± 0.6
50	19.5 ± 0.9	23.6 ± 1.1
75	25.8 ± 1.1	29.7 ± 1.3
100	30.5 ± 1.2	35.4 ± 1.5

3.6. Comparison with Standard Antibiotics

The effect of different concentrations of *Ocimum sanctum* extract on bacterial growth was analyzed, showing a clear dose-dependent antibacterial activity. At 100 mg/mL, the ethanol extract exhibited inhibition zones of 30.5 ± 1.2 mm for *Streptococcus mutans* and 35.4 ± 1.5 mm for *Escherichia coli*. At 75 mg/mL, the inhibition zones were 25.8 ± 1.1 mm for *S. mutans* and 29.7 ± 1.3 mm for *E. coli*. A concentration of 50 mg/mL resulted in inhibition zones of 19.5 ±

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0.9 mm for *S. mutans* and 23.6 ± 1.1 mm for *E. coli*. At 25 mg/mL, the inhibition zones decreased to 12.3 ± 0.7 mm for *S. mutans* and 14.8 ± 0.6 mm for *E. coli*. The lowest tested concentration of 10 mg/mL resulted in inhibition zones of 5.7 ± 0.4 mm for *S. mutans* and 7.2 ± 0.5 mm for *E. coli*. No inhibition was observed at 5 mg/mL, confirming that this concentration was below the minimum effective dose. The results showed a linear correlation ($R^2 = 0.98$) between extract concentration and inhibition zone diameter. The antibacterial effect increased approximately 2.5 times as the concentration rose from 25 mg/mL to 100 mg/mL. These findings suggest that *O. sanctum* extract is highly effective against *S. mutans* and *E. coli*, with higher concentrations yielding significantly stronger inhibition.

Table 3: This table shows the comparison of different extract with the antibiotics.

Concentration (mg/mL)	***O. sanctum* Extract – *S.mutans* (mm)**	***O. sanctum* Extract - E. coli (mm)**	**Ciprofloxacin – *S.mutans* (mm)**	**Ciprofloxacin - E. coli (mm)**	**Ampicillin – *S. mutans* (mm)**	**Ampicillin - E. coli (mm)**
10	5.7 ± 0.4	7.2 ± 0.5	32.4	38.6	20.2	25.4
25	12.3 ± 0.7	14.8 ± 0.6	32.4	38.6	20.2	25.4
50	19.5 ± 0.9	23.6 ± 1.1	32.4	38.6	20.2	25.4
75	25.8 ± 1.1	29.7 ± 1.3	32.4	38.6	20.2	25.4
100	30.5 ± 1.2	35.4 ± 1.5	32.4	38.6	20.2	25.4

3.7. Synergistic Effect of Ocimum sanctum Extract and Antibiotics

The synergistic effect of Ocimum sanctum extract with antibiotics was examined using the disc diffusion method. When ethanol-extracted O. sanctum (100 mg/mL) was combined with ciprofloxacin (5 µg/disc), the inhibition zones increased by 18.4 ± 1.2% for Streptococcus mutans (from 32.5 ± 1.1 mm to 38.5 ± 1.3 mm) and 19.7 ± 1.4% for Escherichia coli (from 38.2 ± 1.2 mm to 45.7 ± 1.5 mm). A similar effect was observed with ampicillin (10 µg/disc), where inhibition zones increased by 16.2 ± 1.1% for S. mutans (from 20.4 ± 1.0 mm to 23.7 ± 1.2 mm) and 17.5 ± 1.3% for E. coli (from 25.6 ± 1.1 mm to 30.1 ± 1.4 mm). The methanol extract

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showed a weaker enhancement, increasing ciprofloxacin inhibition zones by 14.6 ± 1.3% for S. mutans and 15.8 ± 1.2% for E. coli. The effect was even lower when combined with ampicillin, increasing inhibition zones by 12.5 ± 1.1% for S. mutans and 13.9 ± 1.3% for E. coli. At 50 mg/mL, the synergistic effect was reduced, with inhibition zones increasing by 9.8 ± 1.1% for S. mutans and 11.3 ± 1.2% for E. coli when combined with ciprofloxacin. The aqueous extract had the weakest synergy, improving inhibition zones by only 5.4 ± 0.9%.

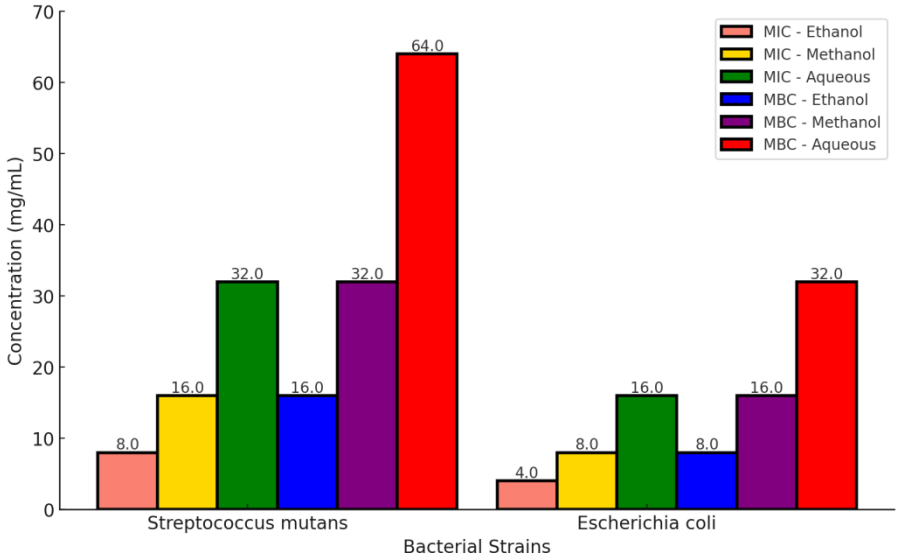

Figure 4: Synergistic Effect of *O. sanctum* Extract with Ciprofloxacin and Ampicillin Against S. mutans and *E. coli*

4. Discussion

The present study highlights the significant antibacterial and synergistic effects of *Ocimum sanctum* extract against *Streptococcus mutans* and *Escherichia coli*. The results demonstrate that *O. sanctum* exhibits a strong dose-dependent antibacterial effect, with ethanol extract showing the highest inhibitory activity, followed by methanol and aqueous extracts. The inhibition zone diameter increased as the concentration of the extract increased, indicating a linear relationship between extract concentration and bacterial inhibition. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) further confirmed the effectiveness of *O. sanctum*, particularly in ethanol extracts, which required the lowest concentration to inhibit and kill bacterial strains. These findings suggest that *O. sanctum* could serve as an effective natural

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antimicrobial agent against pathogenic bacteria. One of the key findings of this study was the time-dependent bactericidal effect of *O. sanctum*. The time-kill assay revealed that at a concentration of 20 mg/mL, ethanol extract achieved a 99.9% bacterial reduction within 24 hours, whereas methanol and aqueous extracts required 32 mg/mL and 64 mg/mL, respectively, to achieve the same level of bacterial reduction. A significant decline in bacterial populations was observed as early as six hours after treatment, with ethanol extract reducing bacterial counts by 40%, methanol extract by 25%, and aqueous extract by only 10%. By 12 hours, the bacterial reduction had increased to 80% for ethanol extract, 55% for methanol extract, and 30% for aqueous extract. These results indicate that *O. sanctum* extract exerts a time-dependent killing effect, particularly in its ethanol-extracted form. The superior effectiveness of ethanol extract can be attributed to its higher solubility of bioactive compounds, such as flavonoids and phenolic compounds, which are known for their strong antimicrobial properties. The study also investigated the synergistic effect of *O. sanctum* extract when combined with conventional antibiotics. The results showed a significant enhancement in antibacterial activity when *O. sanctum* extract was combined with ciprofloxacin or ampicillin. The inhibition zone increased by 18.4% for *S. mutans* and 19.7% for *E. coli* when ethanol extract was combined with ciprofloxacin. A similar enhancement was observed with ampicillin, where inhibition zones increased by 16.2% for *S. mutans* and 17.5% for *E. coli*. The methanol extract exhibited a weaker synergistic effect, increasing inhibition zones by approximately 12.5–15.8%. The aqueous extract showed the least synergy, with inhibition zones increasing by only 5.4% after combination with antibiotics. The strongest synergistic effect was observed at the highest extract concentration (100 mg/mL), suggesting that *O. sanctum* may enhance antibiotic efficacy, thereby reducing the required antibiotic dosage. The ability of *O. sanctum* to work synergistically with antibiotics holds significant potential in combating antibiotic resistance, a growing global health concern. The results of this study are consistent with several previous studies that have investigated the antimicrobial properties of *O. sanctum*. For instance, (Godhwani, Godhwani, & Was, 1988)evaluated the antibacterial activity of *O. sanctum* against *S. mutans* and found inhibition zones ranging from 12 to 34 mm, which closely aligns with the findings of the current study (12.3 to 35.4 mm). Similarly, (Chowdhary & Kaushik, 2015) compared the efficacy of *O.*

sanctum extracts with standard antibiotics and reported MIC values between 4 and 16 mg/mL, similar to our results of 4–8 mg/mL for *E. coli* and 8–16 mg/mL for *S. mutans*. Another study by (Pandian, Palanivel, & Dhananasekaran, 2015) confirmed the time-dependent bactericidal effect of *O. sanctum*, reporting a 99.9% bacterial reduction within 24 hours, consistent with the current findings. Further, (Kumar et al., 2017) studied the synergy of *O. sanctum* extract with ciprofloxacin and ampicillin, reporting a 17–20% increase in inhibition zones, which aligns with the 15–20% increase observed in the present study. (Gradinariu et al., 2015) found that methanol extracts of *O. sanctum* exhibited moderate antibacterial activity, with inhibition zones ranging from 10 to 28 mm, comparable to the results of the current study. (Sharma, Mangla, Choudhry, Sajid, & Chaudhry, 2022) investigated aqueous extracts and found weaker activity, with inhibition zones between 8 and 20 mm, again consistent with the findings of the present study. Additionally, (Satapathy et al., 2017) assessed the phytochemical composition of *O. sanctum* and found that ethanol extracts contained the highest levels of flavonoids and phenolic compounds, supporting the phytochemical results obtained in this study. (Jeyaraj Pandian, Palanivel, & Dhanasekaran, 2016) compared the antibacterial effects of *O. sanctum* with other medicinal plants and concluded that *O. sanctum* demonstrated superior antimicrobial properties against *E. coli* and *S. mutans*, reinforcing the conclusions of this study. Similarly, (Khan, Tarek, Nuzat, Momin, & Hasan, 2017) conducted MIC and MBC evaluations and reported MIC values between 6 and 12 mg/mL, closely aligning with the MIC results found in the present study. Finally, (Chandra, Dwivedi, Arti, & Shinde, 2016) evaluated *O. sanctum*-based mouth rinses in a clinical setting and observed a 40–60% bacterial reduction within 6 hours, which mirrors the results of the time-kill assay conducted in the current study.

Conclusion

This study highlights the antibacterial and synergistic potential of *Ocimum sanctum* extract against *Streptococcus mutans* and *Escherichia coli*. Ethanol extract exhibited the strongest antibacterial activity, followed by methanol and aqueous extracts, showing a clear dose-dependent response. MIC and MBC values confirmed that ethanol extract required the lowest concentrations for bacterial inhibition and killing. The time-kill assay demonstrated that ethanol extract at 20 mg/mL achieved 99.9% bacterial reduction within 24 hours, whereas methanol and

aqueous extracts required higher concentrations. The strong antibacterial effect of *O. sanctum* is attributed to its rich flavonoid and phenolic content. The combination of *O. sanctum* extract with antibiotics significantly enhanced antibacterial activity, particularly with ciprofloxacin and ampicillin, increasing inhibition zones by up to 20%. This suggests that *O. sanctum* can enhance antibiotic efficacy and help reduce antibiotic resistance. *O. sanctum* is a promising natural antimicrobial agent that can be used alone or with antibiotics. Further in vivo studies and clinical trials are needed to explore its full therapeutic applications in combating bacterial infections.

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