International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 22s, 2025
https://theaspd.com/index.php

Evaluation And Comparison Of Antimicrobial Efficacy Of Tea Tree Peppermint And Rosemary Essential Oils Against Streptococcus Mutans Staphylococus Aureus And Candida Albicans

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Abstract

INTRODUCTION-*Essential oils derived from medicinal plants are recognized for their natural antimicrobial properties and are considered viable alternatives to synthetic drugs in the treatment of bacterial and fungal infections. Despite their widespread use, there has been limited research to evaluate the antimicrobial efficacy of tea tree oil, peppermint oil, and rosemary oil against Streptococcus mutans, Staphylococcus aureus, and Candida albicans. This study was designed to investigate the antimicrobial potential of these essential oils against the above-mentioned pathogens and to assess their effectiveness as natural therapeutic agents.*

MATERIALS AND METHODOLOGY-*Fresh (24-hour) cultures of Staphylococcus aureus, Streptococcus mutans, and Candida albicans were inoculated onto nutrient agar (NAM) and Sabouraud dextrose agar (SDA) plates. Essential oils of tea tree, peppermint, and rosemary were prepared in DMSO at serial concentrations (100%, 75%, 50%, 25%, 12.5%, 6.25%, 3.12%, and 1.6%) and applied to sterile absorbent discs. Antimicrobial efficacy was determined by measuring the zones of inhibition surrounding each disc.*

RESULTS: *Fosfomycin and fluconazole demonstrated mean zones of inhibition of 25.00 ± 0.71 mm against Staphylococcus aureus, Streptococcus mutans, and Candida albicans, respectively. While all three essential oils—tea tree, peppermint, and rosemary—exhibited antimicrobial activity across tested concentrations, none achieved inhibition zones comparable to the control agents at any dilution level, including undiluted applications.*

CONCLUSION: *This study suggests that tea tree, peppermint, and rosemary essential oils possess supplementary antimicrobial potential against Staphylococcus aureus, Streptococcus mutans, and Candida albicans. However, the test organisms were standard laboratory strains rather than resistant clinical isolates. Therefore, the efficacy of these essential oils against resistant pathogens remains inconclusive and warrants further investigation.*

KEYWORDS: *Tea Tree Oil, Peppermint Oil, And Rosemary Oil,Fosfomycin,Streptococcus Mutans, Staphylococcus Aureuand Candida Albicans.*

INTRODUCTION-

The rise of antimicrobial resistance among clinical isolates has intensified the global demand for novel therapeutic agents. In this context, essential oils (EOs) extracted from medicinal plants have garnered attention as promising natural antimicrobial alternatives to synthetic drugs. EOs have been widely utilized to treat urinary tract infections, respiratory disorders, gastrointestinal disturbances, and dermal diseases [1].

Tea tree oil (TTO), extracted from *Melaleuca alternifolia* (family Myrtaceae), contains a complex mixture of bioactive constituents, including thymol, carvacrol, *p*-cymene, and γ-terpinene. In vitro studies have shown that

antimicrobial-resistant strains of *Staphylococcus aureus* and *Candida albicans* exhibit susceptibility to TTO. Although historical data on TTO's medicinal benefits is largely anecdotal, contemporary evidence highlights its antibacterial, antifungal, antiviral, and antiprotozoal properties [2]. However, many of these effects remain underexplored in laboratory and clinical settings.

Peppermint oil (PEO), extracted from *Mentha* × *piperita* L., is extensively cultivated and applied across various industries. Studies indicate that menthol and menthone—its primary constituents—possess significant antimicrobial activity. Tullio et al. reported fungicidal activity of PEO against yeasts and fungistatic effects on dermatophytes, including *Candida spp.*, *Cryptococcus neoformans*, and *Trichophyton mentagrophytes* . Despite these findings, the precise mechanisms of action remain unclear, necessitating further investigation [3].

Rosemary oil (REO), derived from *Rosmarinus officinalis* L. (family Lamiaceae), has a longstanding history of medicinal use across Europe, Asia, North Africa, and Australia. REO has demonstrated antimicrobial activity against both gram-positive and gram-negative bacteria, as well as *Candida albicans*[4].

Recent findings suggest TTO exhibits superior antibacterial activity against *Streptococcus mutans* compared to PEO . *S. mutans* is highly acidogenic, adheres to tooth surfaces, and contributes significantly to biofilm formation and dental caries [5]. While promising data exists, limited research has directly compared the efficacy of TTO, PEO, and REO against *S. mutans*.

Studies also highlight that TTO has stronger antibacterial effects against *S. aureus* than PEO. *S. aureus*, including methicillin-resistant strains (MRSA), poses a major public health challenge due to its biofilm-forming ability and resistance profile, especially in healthcare settings. Despite its clinical relevance, the comparative efficacy of these EOs against *S. aureus* remains inadequately explored.

Similarly, TTO's antifungal activity against *Candida albicans* exceeds that of PEO. While *C. albicans* is a normal commensal organism, it can cause severe opportunistic infections in immunocompromised individuals. Its biofilm-forming capacity and resistance to conventional antifungals complicate treatment outcomes [6]. Given the limited research on EO efficacy against *C. albicans*, further studies are warranted.

Therefore, this study aims to assess and compare the antimicrobial activity of three essential oils—TTO, PEO, and REO—against *S. mutans*, *S. aureus*, and *C. albicans*, contributing to the expanding literature on plant-derived antimicrobials.

MATERIAL AND METHODOLOGY-

It is a Observational study conducted in TMU, Moradabad with ethical clearance obtained from Institutional Review Board . A total of 72 samples, determined using G*Power software (80% power, 5% alpha error), divided into three essential oil groups (n=24 per group). Test Organisms choosen was*Staphylococcus aureus* (ATCC9144), *Streptococcus mutans* (ATCC25175), and *Candida albicans* (ATCC90028) using Kirby–Bauer disk diffusion assay using Nutrient Agar (NAM) for bacterial strains and Sabouraud Dextrose Agar (SDA) for fungal strain. **Essential Oil Groups:**

- **Group 1 (Tea Tree Oil):** Eight concentrations (100%, 75%, 50%, 25%, 12.5%, 6.25%, 3.12%, 1.6%).
- **Group 2 (Peppermint Oil):** Same concentration gradient.
- **Group 3 (Rosemary Oil):** Same concentration gradient.
- **Controls:** Fosfomycin used as positive control for bacterial strains; fluconazole for fungal strain.

Aim-

To evaluate antimicrobial effectiveness of essential oils Melaleuca Alternifolia, Mentha Piperita, and Rosmarinus Officinalis L. against S. mutans, S. aureus, and C. albicans.

Objectives-

1. To evaluate antimicrobial properties of the antimicrobial properties of Melaleuca Alternifolia (Group I) and determination of minimum inhibition concentration via examination of zone of inhibition against S. mutans , S. aureus and C. albicans.
2. To evaluate antimicrobial properties of Mentha Piperita (Group II) and determination of minimum inhibition concentration via examination of zone of inhibition against S.mutans , S. aureus and C. albicans.
3. To evaluate antimicrobial properties of Rosmarinus Officinalis L. (Group III) and determination of minimum inhibition concentration via examination of zone of inhibition against S.mutans , S. aureus and C. albicans
4. To compare and correlate the finding between the different groups.
5. To compare and correlate finding with the controls (fosfomycin for bacteria , fluconazole for fungus).

Experimental Protocol Disc Preparation and Application: Sterile 6 mm diameter discs were immersed in tea tree oil (TTO), peppermint oil (PEO), and rosemary oil (REO) at 100% concentration, air-dried for 1 minute, and placed onto nutrient agar (NAM) plates inoculated with *Staphylococcus aureus* (ATCC9144) and *Streptococcus mutans* (ATCC25175), or Sabouraud Dextrose Agar (SDA) plates inoculated with *Candida albicans* (ATCC90028). This process was repeated for each oil at seven additional concentrations (75%, 50%, 25%, 12.5%, 6.25%, 3.12%, and 1.6%), with discs placed equidistantly—four per plate—to ensure uniform diffusion.
Controls: Fosfomycin (bacterial) and fluconazole (fungal) were applied using the same disc-diffusion method as positive controls.
Incubation & Measurement: Plates were labeled appropriately and incubated for 24 hours at 37°C. Zones of inhibition (ZOIs) surrounding each disc were measured in millimeters using a vernier caliper.
Statistical Analysis: Data were analyzed using SPSS v23.0. Intergroup comparisons were performed using the Kruskal–Wallis One-Way ANOVA for non-parametric data.

RESULTS AND OBSERVATION –

The antimicrobial efficacy of tea tree oil (TTO), peppermint oil (PEO), and rosemary oil (RO) against *Staphylococcus aureus* and *Streptococcus mutans* was assessed using zones of inhibition (ZOI) in millimeters. Fosfomycin, serving as the positive control, produced a consistent ZOI of 25.00 ± 0.71 mm (mean ± SD), confirming the susceptibility of both test organisms.

100% Essential Oil Concentration:

Pathogen	TTO (mm)	PEO (mm)	RO (mm)
S. aureus	17.00 ± 0.71	20.00 ± 0.71	21.00 ± 0.71
S. mutans	22.00 ± 0.71	20.00 ± 0.71	20.00 ± 0.71

- Kruskal–Wallis ANOVA: **p = 0.0010** for both species.
- Mann–Whitney U test: Significant differences between all EOs and fosfomycin (**p = 0.0122**), and among TTO, PEO, and RO except RO vs PEO (p = 0.0947).

Lower Concentrations (75%, 50%, 25%, ≤12.5%)

- Inhibitory effect declined sharply below 50%.
- At 25%, negligible activity against *S. aureus* and *S. mutans*, with ZOIs ≤ 0.01 mm.
- No inhibition observed at concentrations ≤12.5%.

Antifungal Activity
Control:

- *Fluconazole* yielded a ZOI of **29.00 ± 0.71 mm** against *C. albicans*.

100% Essential Oil Concentration:

Oil Type	ZOI (mm) Against *C. albicans*
TTO	19.00 ± 0.71
PEO	19.00 ± 0.71
RO	15.00 ± 0.71

- Kruskal–Wallis ANOVA: **p = 0.0010**
- Mann–Whitney U test: Significant differences among most pairwise groups (**p = 0.0122**), except TTO vs PEO (p = 0.9168).

At 75% and 50% Concentrations:

- TTO: 15.00 ± 0.71 mm → 9.00 ± 0.71 mm
- PEO: 16.00 ± 0.71 mm → 10.00 ± 0.71 mm
- RO: 10.00 ± 0.71 mm → 7.00 ± 0.71 mm

At 25% Concentration:

- Only PEO showed moderate activity (6.00 ± 0.71 mm); others were inactive (0.00 ± 0.00 mm).
- No antifungal effect observed below 25%.

DISSCUSSION-

Plant-based therapeutics have gained increasing attention due to their pharmacological benefits, low toxicity, and cost-effectiveness [7]. Interest in phytochemicals and essential oils derived from medicinal plants has intensified, given their potential role in combating microbial infections and oxidative stress.

Within dentistry, rising resistance to conventional antimicrobials like chlorhexidine (CHX) has triggered the search for alternative agents [8]. Although over 300,000 plant extracts are recorded, only a fraction has been studied for antimicrobial efficacy [9].

The strongest antibacterial activity against *Staphylococcus aureus* was observed with rosemary oil (RO), followed by peppermint oil (PEO) and tea tree oil (TTO). RO yielded ZOIs of 21.0, 16.0, 11.0, and 8.0 mm at concentrations of 100%, 75%, 50%, and 25%, respectively. PEO showed slightly reduced activity, while TTO demonstrated the lowest inhibition among the three.

In contrast, TTO showed the most potent activity against *Streptococcus mutans*, followed by RO and PEO. Notably, only TTO maintained activity at 25% concentration, producing an 8.0 ± 0.7 mm ZOI. Literature supports TTO's effectiveness: Patri et al. [10] found *S. mutans* susceptible to concentrations ≤1% , and Takarada et al. [11] established an MIC of 1% . Song et al. [12] Discrepancies may be due to strain variations or methodological differences.

Tea tree oil's efficacy is linked to constituents such as terpinen-4-ol, linalool, and α-terpineol. Terpinen-4-ol disrupts bacterial cell walls and induces cellular leakage, evidenced by loss of 260-nm nuclear material and morphological changes in *S. aureus* under electron microscopy [13].

Peppermint oil (PO) demonstrated antifungal efficacy comparable to tea tree oil (TTO), showing a zone of inhibition (ZOI) of 19.0 ± 0.7 mm against *Candida albicans* at 100% concentration. Notably, PO maintained antifungal activity at a lower dose (6.0 ± 0.7 mm at 25%), whereas TTO and rosemary oil (RO) showed no inhibition at this concentration. This identifies PO as the most effective antifungal agent against *C. albicans* in this study.

These findings are consistent with prior research by Saharkhiz MJ, El Gendy ZKHA, and Hitokoto H, which reported similar antifungal effects of PO against diverse pathogenic fungi [14]. The antifungal potency of PO is largely attributed to its active constituents—menthol (43.9%) and menthone (23.1%)—whose high concentrations may underlie its pronounced anticandidal activity.

This study demonstrates that TTO, RO, and PO possess antimicrobial efficacy against *Streptococcus mutans*, *Staphylococcus aureus*, and *Candida albicans*. Their antibacterial and antifungal effects are primarily mediated by membrane disruption, increased permeability, and efflux of critical intracellular components [15]. However, several limitations are evident: clinical isolates and resistant strains were not addressed, and modern techniques for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination were not utilized.

CONCLUSION-

While they possess antimicrobial efficacy and have recognized as potent agents against various pathogens, inconsistencies in chemical composition and lack of standardization hinder reproducibility and result accuracy. Thus, future research should focus on the molecular characterization of essential oil constituents and their precise mechanisms of action, particularly through proteomic and genomic approaches.

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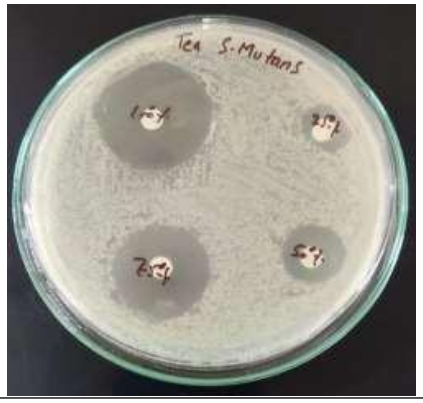
LEGENDS
PHOTOGRAPH

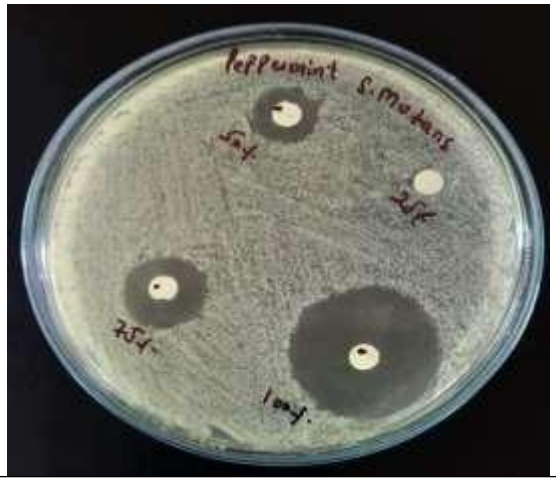
Photograph1depicting zone of inhibition of tea tree for *S.aureus* at concentration 100 % , 75% , 50%, 25%,

Photograph 2 depicting zone of inhibition of peppermint for *S.aureus* at concentration 100% , 75% , 50%, 25%

Photograph 3 depicting zone of inhibition of rosemary oil for *S.aureus* at concentration 100% , 75% , 50%, 25%



Photograph 4 depicting zone of inhibition of tea tree for *S.mutans* at concentration 100% , 75% , 50%, 25%,

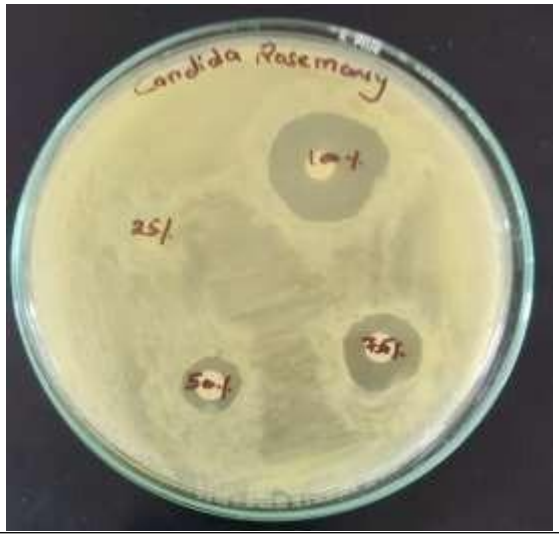


Photograph5 depicting zone of inhibition of peppermint for *S.mutans* at concentration 100% , 75% , 50%, 25%,

Photograph6 depicting zone of inhibition of rosemary for *S.mutans* at concentration 100% , 75% , 50%, 25%

Photograph7 depicting zone of inhibition of tea tree for *C.albicans* at concentration 100 % , 75% , 50%, 25%

Photograph8 depicting zone of inhibition of peppermint for *C.albicans* at concentration 100% , 75% , 50%, 25%



Photograph9 depicting zone of inhibition of rosemary for C.albicans at concentration 100% , 75% , 50%, 25%