Heterocyclic Letters
Vol. 15| No.4|807-823|August-October|2025
ISSN : (print) 2231–3087 / (online) 2230-9632
CODEN: HLEEAI
http://heteroletters.org

ANTIFUNGAL ACTIVITY OF PEPPERMINT AND ORIGANUM ESSENTIAL OIL PROPORTIONS AGAINST ORAL CANDIDA ALBICANS - IN VITRO STUDY

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Abstract

Background:

Candida-associated denture stomatitis (CADS) is a frequent inflammatory condition in denture wearers, primarily caused by Candida albicans. Treatment is complicated by rising antifungal resistance and the limitations of conventional drugs. There is increasing interest in natural alternatives, particularly essential oils with known antimicrobial properties.

Objective:

This study evaluates the antifungal activity of peppermint and origanum essential oils both individually and in combination, against oral Candida albicans, including standard and clinical isolates, to determine their potential as alternative antifungal agents.

Methods:

The chemical compositions of the essential oils were characterized using Gas Chromatography-Mass Spectrometry (GC-MS) and Fourier Transform Infrared Spectroscopy (FTIR), confirming carvacrol as the main component in origanum oil and menthol in peppermint oil.

Antifungal efficacy was assessed via the well diffusion method (measuring inhibition zones) and the broth dilution method (evaluating minimal inhibitory/fungicidal concentrations and colony counts) according to CLSI guidelines, using various oil ratios (including pure and mixed forms).

Results:

Both essential oils demonstrated significant antifungal activity against *C. albicans* isolates.

Origanum oil showed a superior inhibition zone compared to peppermint oil when used alone.

Synergistic effects were observed combinations of the oils, particularly at higher concentrations, produced larger inhibition zones than either oil alone and exceeded the efficacy of the standard antifungal agent fluconazole.

Complete inhibition of fungal growth was observed in several oil combinations, especially those containing at least 25% of both oils.

FTIR data supported the presence of major bioactive constituents responsible for the observed antifungal effect.

Conclusion:

The combination of peppermint and origanum essential oils exhibits potent, synergistic antifungal activity against oral *Candida albicans*, often outperforming fluconazole in vitro. These findings support the potential of essential oil formulations as natural, effective alternatives or adjuncts to conventional antifungal therapies for oral candidiasis.

Keywords: *Candida albicans,* Peppermint oil, Origanum oil, GC-MS and FTIR.

Introduction

Denture stomatitis is a common inflammatory condition among denture wearers, primarily caused by Candida infections [I, III]. Candida albicans is the predominant pathogen, though other species such as C. tropicalis, C. glabrata, C. parapsilosis, and C. krusei may also contribute [IV,V]. Treatment of Candida-associated denture stomatitis (CADS) remains challenging due to frequent relapses and increasing antifungal resistance [VI–VIII]. Hence, there is a growing need to explore new, effective, and safer antifungal agents.

Herbal medicine has long been utilized in dentistry, offering promising alternatives in managing microbial infections [IX, XI]. CADS affects approximately 65–70% of denture wearers, with C. albicans responsible for about 93% of cases [XII, XV]. Its multifactorial nature involves factors such as poor denture hygiene, continuous use, surface irregularities of denture

bases, and systemic conditions like diabetes and immunosuppression [XII]. Biofilm formation on denture surfaces further enhances fungal persistence and resistance to treatment.

Current management of CADS combines mechanical cleaning, denture adjustment, and antifungal therapy [XVI]. Common drugs, including polyenes (Amphotericin B, Nystatin) and azoles (Miconazole, Fluconazole), are effective but limited by side effects, toxicity, cost, and drug resistance [XVII]. Therefore, plant-based essential oils (EOs) have emerged as potential antifungal agents due to their safety, accessibility, and broad-spectrum antimicrobial activity [XVIII].

Among EOs, Origanum vulgare (oregano) and Mentha piperita (peppermint) have shown potent antifungal effects. Oregano oil, rich in carvacrol and thymol, disrupts fungal cell membranes, leading to cell death [XIX, XX]. Peppermint oil, containing menthol and menthone, exhibits antifungal, antibacterial, and antibiofilm activities and is widely used in oral hygiene formulations [XXI].Recent findings suggest that combining essential oils may produce synergistic effects, enhancing efficacy while reducing toxicity [XXII].

This study aims to evaluate the antifungal potential of Origanum and Mentha piperita essential oils in various ratios against Candida albicans (ATCC 90028) and clinical isolates from denture stomatitis patients, exploring their potential as natural, cost-effective alternatives to conventional antifungal therapy.

Materials and methods

Procurement of Essential Oils

Peppermint essential oil (Mentha piperita) was procured from LOBA CHEMIE Pvt. Ltd., Mumbai, India. The oil was stored at 4°C in a dark environment until further use to maintain its stability.

Origanum essential oil (Origanum vulgare) was acquired from Suyash Herbs, New Delhi, India. The oil was also provided in light-protective packaging and stored under refrigeration to prevent oxidation and volatilization of active compounds such as carvacrol and thymol.

Ethical Clearance

Prior to the commencement of the study, ethical clearance was obtained from the Institutional Review Board (IRB) of the concerned institution. The study protocol, including sample collection, microbial analysis, and use of patient data, was reviewed and approved under ethical guidelines for research involving human subjects. The ethical approval reference number NU/CEC/2018/0202 from the central ethics committee NITTE Deemed to be University.

Organisms Used

Two strains of Candida albicans were used:

Candida albicans ATCC 90028: A standard reference strain from the American Type Culture Collection (ATCC), commonly used for reproducibility and antifungal susceptibility testing.

Candida albicans – Clinical Oral Isolate: Strains were collected from the oral mucosa of denture stomatitis patients at a dental outpatient clinic following aseptic procedures. Informed consent was obtained, and patient identities were anonymized. All isolates were confirmed as C. albicans by morphological, cultural, and biochemical methods.

Gas Chromatography-Mass Spectrometry (GC-MS)

The chemical composition of peppermint (Mentha piperita) and origanum (Origanum vulgare) essential oils was analyzed using a PerkinElmer Autosystem XL GC with TurboMass Gold MS (Software Version: TurboMass 6.1.0.1963). Separation was achieved on a BP20 capillary column (30 m × 0.25 mm × 0.25 μm). The oven program started at 40 °C (3 min), increased at 5 °C/min to 110 °C, then 3 °C/min to 150 °C, and finally 15 °C/min to 200 °C (held 5 min). Injector temperature: 250 °C, carrier gas: 1 mL/min, split ratio 1:20. Essential oils (1% in diethyl ether) were injected (1 μL). MS conditions: transfer line 200 °C, ion source 180 °C, scan range 40–400 m/z, solvent delay 3 min. This protocol allowed identification of major compounds, e.g., menthol, menthone, menthyl acetate (peppermint), and carvacrol, thymol, p-cymene (origanum) [XXIII, XXV].

Infrared (IR) Spectroscopy

FTIR with ATR was used to identify functional groups in peppermint and origanum oils. 1–2 drops of each oil were placed on the ATR crystal, and spectra were recorded from 4000–400 cm^{-1} with 4 cm^{-1} resolution and 16 scans. Background correction was applied using air [XXVI, XXVII].

Essential Oil Combinations

To evaluate synergistic or additive effects, peppermint (PE) and origanum (OE) oils were tested in varying ratios: 100% PE, 75% PE + 25% OE, 50% PE + 50% OE, 25% PE + 75% OE, and 100% OE (Table 1). Oils were diluted in DMSO, which dissolves hydrophobic compounds while showing minimal antimicrobial activity.

Fluconazole preparation: 0.1 g in 10 mL DMSO.

For testing, 50 μL of each essential oil at designated concentrations was combined to a final volume of 100 μL.

Table 1: Design of Peppermint and Origanum Essential Oil Concentrations

Oil Concentration		**Origanum essential oil**				
		0	25	50	75	100
Peppermint essential oil	0	P0 O0	P0 O25	P0 O50	P0 O75	P0 O100
	25	P25 O0	P25 O25	P25 O50	P25 O75	P25 O100
	50	P50 O0	P50 O25	P50 O50	P50 O75	P50 O100
	75	P75 O0	P75 O25	P75 O50	P75 O75	P75 O100
	100	P100 O0	P100 O25	P100 O50	P100 O75	P100 O100

Determination of Antifungal Activity

Broth Dilution Method:

MICs of peppermint (PE) and origanum (OE) essential oil combinations against Candida albicans (ATCC 90028 and clinical isolate) were determined using the broth dilution method per CLSI guidelines, in triplicate [XXVIII]. Inoculum was prepared by emulsifying 2–3 colonies from overnight SDA cultures in SDB and incubating for 4 h at 35 ± 2 °C. SDB was sterilized at 121 °C for 15–30 min. Fungal suspensions (50 μL) were added to tubes containing different EO ratios, with DMSO as negative and fluconazole as a positive control, and incubated at 37 °C for 24 h. MIC was the lowest concentration inhibiting visible growth [XXIX]. For MFC, MIC samples were plated on SDA and incubated at 37 °C for 24 h; the lowest concentration showing no colonies was recorded. Tests were repeated three times [XXX, XXXI].

Well Diffusion Method:
Antifungal activity was also assessed using the well diffusion method per CLSI guidelines [32]. C. albicans cultures were adjusted to 0.5 McFarland (~1.5 × 10^6 CFU/mL) and evenly streaked on SDA plates. Wells (8 mm) were filled with 100 µL EO combinations, fluconazole as a positive control, and plates incubated at 37 °C for 24 h. Zones of inhibition were measured in mm to evaluate efficacy [XXXIII, XXXIV].

RESULTS

GC-MS Analysis Report of Essential Oil (Peppermint and Origanum)

The major volatile constituents of peppermint (Mentha piperita) and origanum (Origanum vulgare) essential oils were identified using GC–MS.

Instrumentation and Method:
BP20 (polyethylene glycol) column, 30 m × 0.25 mm × 0.25 µm; carrier gas: helium (1 mL/min); injection: 1 µL (1% oil in diethyl ether); split ratio: 1:20. Oven: 40 °C (3 min) → 110 °C at 5 °C/min → 150 °C at 3 °C/min → 200 °C at 15 °C/min (5 min). MS: transfer line 200 °C, ion source 180 °C, scan range 40–400 m/z, solvent delay 3 min.

The GC–MS profile of Origanum oil showed carvacrol as the major constituent, with a prominent peak at 4.809 min and characteristic ions at m/z 93 (base peak), 121, 136, 91, and 77, confirming its identity. Thus, carvacrol was identified as the principal compound in the analyzed Origanum essential oil.

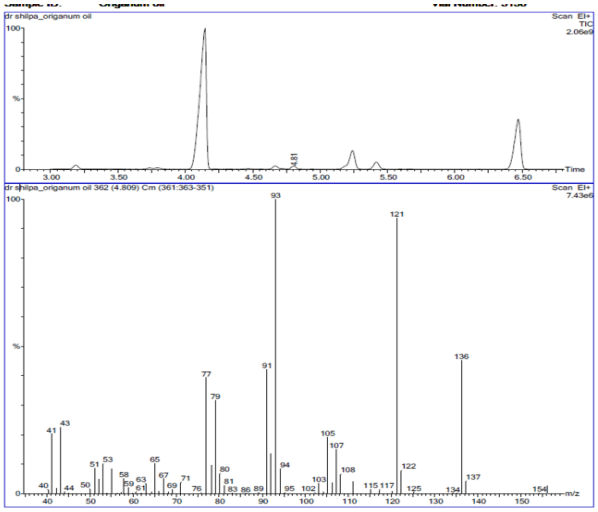

Figure 1: The GC-MS analysis of Origanum oil

The GC–MS analysis of peppermint oil identified menthol as the major component, showing a prominent peak at 5.419 min in the total ion chromatogram (Figure 2). The mass spectrum exhibited characteristic ions at m/z 43, 81, 84, 93, 108, and 154, consistent with menthol's known fragmentation (MW 156.27 g/mol). The m/z 81 ion results from cleavage near the hydroxyl group, while m/z 43 represents a common terpenoid fragment. The m/z 154 peak corresponds to the molecular ion minus water (M–18). Although menthone (MW 154.25) is another expected constituent, its characteristic m/z 138 peak was not dominant, confirming menthol as the principal compound, with minor contributions from menthone and other monoterpenes.

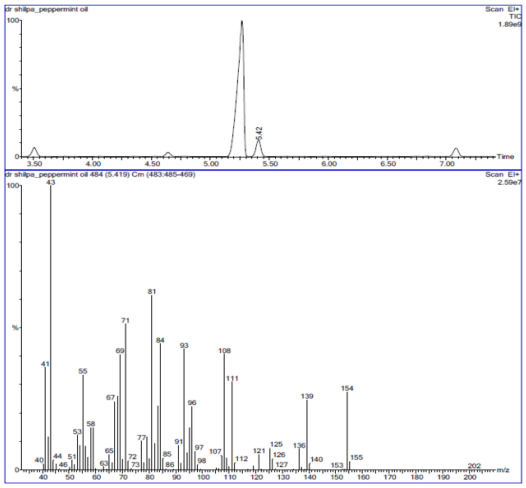

Figure 2: The GC-MS analysis of peppermint oil

FTIR Results

The FTIR spectrum of Origanum oil confirms the presence of functional groups characteristic of carvacrol. A broad band near 3300 cm^{-1} indicates O–H stretching of phenolic groups, while peaks at 2960–2850 cm^{-1} correspond to aliphatic C–H stretching and ~3020 cm^{-1} to aromatic C–H stretching. Strong absorptions at 1600 cm^{-1} and 1500 cm^{-1} denote aromatic C=C vibrations, and the 1260–1280 cm^{-1} band represents C–O stretching typical of phenols. Out-of-plane C–H bending at 800–900 cm^{-1} confirms aromatic substitution. These findings collectively verify carvacrol as the principal constituent of Origanum oil (Figure 3).

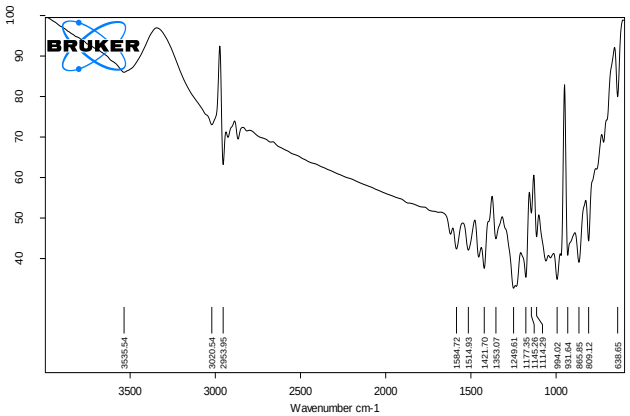

Figure 3: The FTIR spectrum of Origanum oil

The FTIR spectrum of peppermint oil confirms the presence of key functional groups associated with menthol and menthone. A broad band around 3300 cm^{-1} indicates O–H stretching from menthol's hydroxyl group, while peaks between 2960–2850 cm^{-1} correspond to C–H stretching in both compounds. A strong absorption near 1715 cm^{-1} signifies C=O stretching from menthone's ketone group. Additional bands at 1450–1370 cm^{-1} (C–H bending) and 1050–1150 cm^{-1} (C–O stretching) further support the presence of alcohols. Overall, the spectrum confirms the presence of both menthol and menthone in peppermint oil as sown in figure 4.

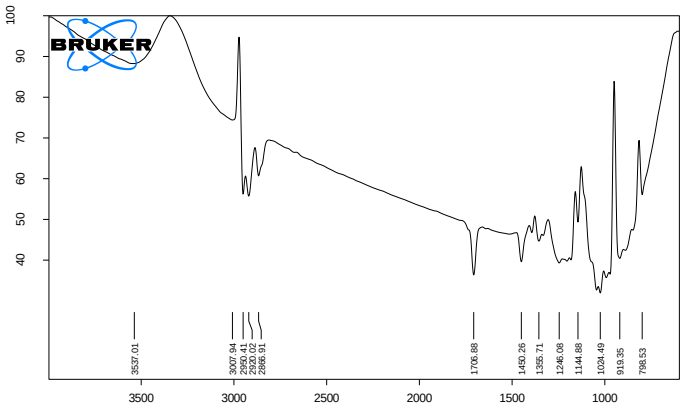

Figure 4: The FTIR spectrum of Origanum oil

Assessment of Antifungal Activity by Well Diffusion Method

Microbial Culture:
Candida albicans (clinical strain and ATCC 90028) was cultured on Sabouraud Dextrose Agar (SDA) and incubated for 18 h. The fungal suspension was prepared in Sabouraud Dextrose Broth (SDB) to match 0.5 McFarland standards (≈1.5 × 10^6 CFU/ml).

Antifungal Assay:
Antifungal activity of peppermint (P) and origanum (O) essential oils, individually and in combination, was evaluated by the well diffusion method. Lawn cultures were prepared on SDA plates, and 8 mm wells were filled with 100 µl of test samples at varying concentrations (25%, 50%, 75%, and 100%) prepared in DMSO. For combinations, 50 µl of each oil at the required concentration was mixed to yield 100 µl per well.
Controls:
Fluconazole (0.1 g/10 ml DMSO) served as the positive control, while DMSO alone acted as the negative control.
Measurement:
Plates were incubated at 37°C for 24 h, and zones of inhibition (mm) were measured against C. albicans (ATCC 90028 and clinical strain). The procedure was identical for all controls and test samples.

Table 2: Zone of inhibition was observed against Candida albicans (ATCC 90028), tabulated as follows (in mm)

	Origanum Essential Oil (%)					
Peppermint Essential Oil (%)	**Concentration**	**0**	**25**	**50**	**75**	**100**
	0	**0**	**36**	**42**	**45**	**50**
	25	**35**	**37**	**39**	**46**	**55**
	05	**30**	**47**	**47**	**48**	**49**
	75	**35**	**35**	**50**	**45**	**53**
	100	**45**	**48**	**51**	**58**	**60**

The antifungal activity of peppermint (P) and origanum (O) essential oils, individually and combined, was evaluated against Candida albicans (ATCC 90028) using the agar well diffusion

method. Results (Table 2) showed no inhibition at P0O0, confirming the need for active components. Increasing origanum concentration (25–100%) enhanced inhibition from 36 mm to 50 mm, while combinations exhibited synergistic effects—P25O100, P50O100, and P75O100 produced 55, 49, and 53 mm zones, respectively. The maximum inhibition (60 mm) was observed at P100O100, confirming strong synergy and concentration-dependent antifungal activity.

Table 3: Zones of inhibition of peppermint oil and Origanum oil as per their concentration

OriganumEssential Oil (%)					
Peppermint Essential Oil (%)	**0 mm**	**25 mm**	**50 mm**	**75 mm**	**100 mm**
0	**0mm**	**47 mm**	**50 mm**	**53 mm**	**53 mm**
25	**47 mm**	**51 mm**	**51 mm**	**52 mm**	**58 mm**
50	**42 mm**	**51 mm**	**51 mm**	**54 mm**	**58 mm**
75	**39 mm**	**42 mm**	**50 mm**	**49 mm**	**58 mm**
100	**45 mm**	**46 mm**	**46 mm**	**55 mm**	**60 mm**

Control groups:-DMSO, pure culture (ATCC 90028 and clinical strains), and standard Fluconazole:

The antifungal efficacy of peppermint (PEO) and origanum (OEO) essential oils, individually and in combination, was evaluated against Candida albicans (ATCC 90028) using the agar well diffusion method. The inhibition zones (mm) are shown in Table 3 and Figure 6. The negative control (DMSO) showed minimal inhibition (8 mm), confirming no intrinsic antifungal activity, while fluconazole (positive control) produced a 35 mm zone.

In contrast, essential oil formulations exhibited significantly higher activity. Even at lower concentrations, P0O25 and P25O0 showed 47 mm zones, surpassing fluconazole. A clear dose-dependent increase was observed, with maximum inhibition (60 mm) at P100O100. Combinations such as P25O100, P50O100, and P75O100 also showed strong synergistic effects (≥58 mm), confirming enhanced efficacy when both oils were combined.

The superior antifungal action is attributed to bioactive constituents—carvacrol and thymol (in OEO) and menthol and menthone (in PEO)—which disrupt fungal membranes and inhibit ergosterol biosynthesis.

Determination of Antifungal Activity by Broth Dilution Method:

Table 4: Concentration of Origanum and Peppermint Essential oil

Origanum Peppermint Essential Oil (%)					
Essential Oil (%)	**0**	**25**	**50**	**75**	**100**
0	P0 O0	P0 O25	P0 O50	P0 O75	P0 O100
25	P25 O0	P25 O25	P25 O50	P25 O75	P25 O100
50	P50 O0	P50 O25	P50 O50	P50 O75	P50 O100

75	P75 O0	P75 O25	P75 O50	P75 O75	P75 O100
100	P100 O0	P100 O25	P100 O50	P100 O75	P100 O100

After incubation, 50 µL and 100 µL of Candida albicans inocula (ATCC and clinical strains) were added to tubes containing different essential oil concentrations (Table 4). All tubes, including controls (DMSO, fluconazole, and untreated), were incubated at 37 °C for 24 h. Fungal growth was assessed visually based on turbidity. Contents from each tube were then plated onto SDA using the lawn culture method and incubated for another 24 h. Colony formation was recorded to determine antifungal efficacy. Results were denoted as "+" (growth), "−" (no visible growth), and "NG" (no colony growth).

Table 5: Turbidity observed in concentration of peppermint and Origanum oil in Inoculum concentration 100 µl.

	Origanum Essential Oil (%)				
Peppermint Essential Oil (%)	**0**	**25**	**50**	**75**	**100**
0	**P0 O0** + +	**P0 O25** + +	**P0 O50** + +	**P0 O75** + +	**P0 O100** + +
25	**P25 O0** +	**P25 O25** +	**P25 O50** +	**P25 O75** +	**P25 O100** +
50	**P50 O0** +	**P50 O25** +	**P50 O50** +	**P50 O75** +	**P50 O100** +
75	**P75 O0** +	**P75 O25** +	**P75 O50** +	**P75 O75** +	**P75 O100** +
100	**P100 O0** +	**P100 O25** +	**P100 O50** +	**P100 O75** +	**P100 O100** +

DMSO ++, Fluconazole +

The antifungal activity of peppermint and origanum essential oil combinations was evaluated by the broth dilution method against Candida albicans (ATCC 90028 and clinical strains) at 100 µL inoculum, with turbidity assessed after 24 h at 37 °C (Table 5). All essential oil combinations showed visible turbidity ("+"), indicating fungal growth, even at 100% concentrations, suggesting partial inhibition. DMSO control was turbid, while fluconazole showed no turbidity, confirming its antifungal efficacy. These results indicate that the tested essential oil concentrations partially inhibited C. albicans but did not achieve complete growth suppression.

Table 6: Plate count after 24 hrs

	Origanum Essential Oil (%)				
Peppermint Essential Oil (%)	**0**	**25**	**50**	**75**	**100**
0	P0 O0 ≥300	P0 O25 NG	P0 O50 NG	P0 O75 NG	P0 O100 NG
25	P25 O0 NG	P25 O25 NG	P25 O50 NG	P25 O75 NG	P25 O100 NG
50	P50 O0	P50 O25	P50 O50	P50 O75	P50 O100

	40	NG	NG	NG	NG
75	P75 O0 36	P75 O25 NG	P75 O50 NG	P75 O75 NG	P75 O100 NG
100	P100 O0 17	P100 O25 NG	P100 O50 NG	P100 O75 NG	P100 O100 NG

The antifungal activity of peppermint and Origanum essential oils against Candida albicans (ATCC 90028 and clinical strain) was assessed by broth dilution with 100 µL inoculum. After 24 h at 37 °C, lower concentrations (especially P0 and O0–50%) were turbid, indicating growth, while higher concentrations (≥50% Origanum) showed clear broth, demonstrating antifungal activity. DMSO control was turbid (++), and fluconazole showed mild turbidity (+) (Table 6). Plating on SDA confirmed that combinations with Origanum ≥25% and Peppermint ≥25% completely inhibited growth (NG), with P100+O25 to P100+O100 showing strong synergistic antifungal effects.

***Table* 7: *Candida albicans*ATCC 90028 –Inoculum concentration: 50 µl**

Origanum Essential Oil (%)					
Peppermint Essential Oil (%)	**0**	**25**	**50**	**75**	**100**
0	P0 O0 +	P0 O25 +	P0 O50 +	P0 O75 +	P0 O100 +
25	P25 O0 +	P25 O25 +	P25 O50 +	P25 O75 -	P25 O100 -
50	P50 O0 +	P50 O25 +	P50 O50 +	P50 O75 -	P50 O100 -
75	P75 O0 +	P75 O25 +	P75 O50 -	P75 O75 -	P75 O100 -
100	P100 O0 -	P100 O25 -	P100 O50 -	P100 O75 -	P100 O100 -
DMSO		++	**Fluconazole**		+

Antifungal activity of peppermint and Origanum oil combinations against Candida albicans ATCC 90028 was assessed at 50 µL inoculum (Table 7). At 0% peppermint, turbidity remained (+) across all Origanum levels. Combinations of 25–50% peppermint with ≥75% Origanum showed clear broth (–), while 75–100% peppermint consistently achieved complete inhibition (–). DMSO was (++), and fluconazole (+).

Table 8: Plate Count of *Candida albicans* ATCC 90028 after 24 Hours Exposure to Peppermint and Origanum Essential Oil Combinations.

Origanum Essential Oil (%)					
Peppermint Essential Oil (%)	**0**	**25**	**50**	**75**	**100**
0	P0 O0 ≥300	P0 O25 NG	P0 O50 NG	P0 O75 NG	P0 O100 NG
25	P25 O0 NG	P25 O25 NG	P25 O50 NG	P25 O75 NG	P25 O100 NG
50	P50 O0	P50 O25	P50 O50	P50 O75	P50 O100

	NG	NG	NG	NG	NG
75	P75 O0 NG	P75 O25 NG	P75 O50 NG	P75 O75 NG	P75 O100 NG
100	P100 O0 NG	P100 O25 NG	P100 O50 NG	P100 O75 NG	P100 O100 NG

The antifungal activity of combined peppermint and Origanum essential oils against Candida albicans (ATCC 90028) was assessed based on colony formation on SDA plates (Table 8).
Control plates (P0 O0) showed heavy growth (≥300 CFU). Origanum oil alone (25–100%) completely inhibited growth, confirming its antifungal potential. Similarly, peppermint oil alone (25–100%) caused total inhibition. All combinations of peppermint and Origanum oils showed no growth (NG), indicating a strong synergistic antifungal effect.

Table 9: Turbidity Observed in Candida albicans (Clinical strain) Inoculum concentration 100 µl:

OriganumEssential Oil (%)					
Peppermint Essential Oil (%)	**0**	**25**	**50**	**75**	**100**
0	**P0 O0 + +**	**P0 O25 +**	**P0 O50 +**	**P0 O75 +**	**P0 O100 +**
25	**P25 O0 +**	**P25 O25 +**	**P25 O50 +**	**P25 O75 +**	**P25 O100 +**
50	**P50 O0 +**	**P50 O25 +**	**P50 O50 +**	**P50 O75 +**	**P50 O100 +**
75	**P75 O0 +**	**P75 O25 +**	**P75 O50 +**	**P75 O75 +**	**P75 O100 +**
100	**P100 O0 +**	**P100 O25 -**	**P100 O50 -**	**P100 O75 -**	**P100 O100 -**
DMSO		++	**Fluconazole**		+

The antifungal activity of peppermint and Origanum essential oils against Candida albicans (clinical strain) was assessed based on turbidity after 24 h (Table 9).
Control (P0 O0) and DMSO groups showed intense turbidity (++), indicating high fungal growth, while fluconazole showed partial inhibition (+).
At low concentrations (0–75%), both oils showed limited effect (+). Complete inhibition (–) occurred only at 100% peppermint combined with ≥25% Origanum (P100 O25–O100), indicating that high peppermint levels with Origanum are essential for full antifungal activity. Results confirm a dose-dependent inhibition with maximum effect at higher peppermint concentrations.

Table 10: Plate count after 24 hours of Origanum and Peppermint essential oils

Origanum Essential Oil (%)					
Peppermint Essential Oil (%)	**0**	**25**	**50**	**75**	**100**
0	P0 O0 ≥300	P0 O25 NG	P0 O50 NG	P0 O75 NG	P0 O100 NG
25	P25 O0	P25 O25	P25 O50	P25 O75	P25 O100

	≥300	NG	NG	NG	NG
50	P50 O0 ≥300	P50 O25 NG	P50 O50 NG	P50 O75 NG	P50 O100 NG
75	P75 O0 ≥300	P75 O25 NG	P75 O50 NG	P75 O75 NG	P75 O100 NG
100	P100 O0 NG	P100 O25 NG	P100 O50 NG	P100 O75 NG	P100 O100 NG
DMSO		≥300	**Fluconazole**		178

Table 10 presents the CFU counts after 24 h of incubation to evaluate the antifungal activity of Origanum and Peppermint oil combinations against Candida albicans (clinical strain, 100 µL inoculum).

Control (P0 O0) and DMSO showed heavy growth (≥300 CFUs), while Fluconazole showed partial inhibition (178 CFUs).

From P0 O25 onward, complete inhibition (NG) was observed, except for P25 O0, P50 O0, and P75 O0, which showed ≥300 CFUs, indicating Peppermint alone at low levels was ineffective.

Combinations containing ≥25% of both oils (P25 O25 and above) achieved total inhibition, confirming a strong synergistic antifungal effect at higher concentrations.

Table 11: Turbidity Observation in *Candida albicans* (Clinical Strain) After 24 Hours – Inoculum Concentration: 50 µL

Origanum Essential Oil (%)					
Peppermint Essential Oil (%)	**0**	**25**	**50**	**75**	**100**
0	P0 O0 + +	P0 O25 +	P0 O50 +	P0 O75 +	P0 O100 +
25	P25 O0 +	P25 O25 +	P25 O50 +	P25 O75 +	P25 O100 +
50	P50 O0 +	P50 O25 +	P50 O50 -	P50 O75 -	P50 O100 -
75	P75 O0 +	P75 O25 _	P75 O50 -	P75 O75 -	P75 O100 -
100	P100 O0 +	P100 O25 -	P100 O50 -	P100 O75 -	P100 O100 -
DMSO		++	**Fluconazole**		+

The turbidity assay revealed a dose-dependent antifungal activity of the essential oil combinations. At lower concentrations of Peppermint and Origanum oils (0–25%), significant turbidity was observed, indicating active Candida albicans growth. However, increasing the concentration of both oils (especially above 50%) led to a reduction in turbidity. Notably:

The combination P50 O75 and higher showed marked reduction in turbidity.

At P100 O50 and beyond, complete growth inhibition was observed (–).

Compared to controls, the essential oil combinations performed comparably to fluconazole, with superior inhibition at higher concentrations.

DMSO control exhibited heavy turbidity (++) and confirmed that the vehicle had no antifungal effect.

These findings suggest that higher concentrations of combined peppermint and origanum essential oils exhibit synergistic antifungal efficacy against Candida albicans as shown in Table 11.

Table 12: Plate count after 24 hours of Origanum and Peppermint essential oils

OriganumEssential Oil (%)					
Peppermint Essential Oil (%)	**0**	**25**	**50**	**75**	**100**
0	P0 O0 ≥300	P0 O25 60	P0 O50 NG	P0 O75 NG	P0 O100 NG
25	P25 O0 ≥300	P25 O25 NG	P25 O50 NG	P25 O75 NG	P25 O100 NG
50	P50 O0 ≥300	P50 O25 NG	P50 O50 NG	P50 O75 NG	P50 O100 NG
75	P75 O0 NG	P75 O25 NG	P75 O50 NG	P75 O75 NG	P75 O100 NG
100	P100 O0 NG	P100 O25 NG	P100 O50 NG	P100 O75 NG	P100 O100 NG

DMSO ≥300, Fluconazole 125 CFU

The plate count method demonstrated strong antifungal activity of Origanum and Peppermint essential oils, both individually and in combination as sown in table 12 and figure 14
At 0% essential oils (P0 O0) and DMSO control, heavy fungal growth was observed (≥300 CFU), confirming no inhibition.
Fluconazole, used as a standard antifungal control, showed moderate inhibition with 125 CFU.
At P0 O25, the count dropped to 60 CFU, showing partial inhibition.
Starting from P0 O50 and across all higher combinations (including P25 O25 and above), no growth (NG) was recorded, indicating complete inhibition.
The synergistic effect between peppermint and origanum oils was evident, especially at ≥50% of either oil, resulting in total suppression of fungal colonies.
These findings support the use of these essential oil combinations as potent natural antifungal agents against Candida albicans.

Discussion

Gas Chromatography–Mass Spectrometry (GC-MS) is a sensitive and reliable technique for identifying volatile compounds in essential oils [XXXV]. In this study, GC-MS analysis revealed carvacrol as the major component of Origanum vulgare oil (RT 4.809 min; m/z 93, 121, 136, 91, 77), consistent with literature reports [XXXVI]. Carvacrol, a phenolic monoterpene, is well known for its strong antimicrobial and antioxidant activities.
Similarly, Mentha piperita oil showed menthol as the predominant compound (RT 5.419 min; m/z 43, 81, 84, 93, 108, 154), confirming previous reports [XXXVII]. Menthol's fragmentation pattern indicated hydroxyl group cleavage, with no dominant menthone peak (m/z 138). These findings validate the purity and authenticity of the oils analyzed [XXXVIII, XXXIX].

FTIR spectroscopy further confirmed the presence of key functional groups in both oils. Origanum oil displayed O–H stretching at 3300 cm^{-1}, C–H stretches (2960–2850 cm^{-1}), aromatic C=C bands (1600–1500 cm^{-1}), and C–O stretch (1260–1280 cm^{-1}), confirming the phenolic structure of carvacrol [39]. Peppermint oil exhibited O–H stretching (3300 cm^{-1}),

C=O stretching (1715 cm^{-1}), and C–O bands (1050–1150 cm^{-1}), corresponding to menthol and menthone [XL]. These spectral features supported GC-MS findings.

The antifungal activity, assessed by the well diffusion assay, showed both oils to be highly effective against Candida albicans (ATCC 90028 and clinical strain). The control (DMSO) produced minimal inhibition (8 mm), while fluconazole showed 35 mm. Several oil combinations exceeded fluconazole, such as P25O0 (47 mm) and P100O100 (60 mm), indicating strong antifungal potential[XLI].
Origanum oil's efficacy is attributed to carvacrol and thymol, which disrupt fungal membranes [XLII], whereas peppermint oil's activity results from menthol's interference with ergosterol biosynthesis [XLIII, XLV]. The synergistic inhibition seen in combinations (e.g., P50O50 = 53 mm; P100O100 = 60 mm) reflects complementary mechanisms of action. Increased oil concentration enhanced inhibition, confirming a dose-dependent effect. These findings align with reports supporting essential oils as alternatives to antifungal drugs, particularly against fluconazole-resistant Candida strains[XLVI].

Overall, the results confirm that peppermint and origanum oils, especially in combination, possess strong, synergistic antifungal potential, making them promising natural agents for pharmaceutical or cosmeceutical applications.

Conclusion

Peppermint and origanum essential oils exhibited potent, dose-dependent, and synergistic antifungal effects against Candida albicans, surpassing fluconazole in activity. GC-MS and FTIR confirmed carvacrol, menthol, and menthone as key bioactive constituents responsible for their efficacy. These findings highlight the oils' potential as natural, safe alternatives or adjuncts to synthetic antifungals. Future work should focus on mechanistic studies, toxicity evaluation, and formulation development for clinical applications.

Acknowledgement: The authors thank Nitte (Deemed to be University), AB Shetty Memorial Institute of Dental Sciences (ABSMIDS), Department of Prosthodontics, Crown and Bridge, Deralakatte, Mangalore 575018 India, for providing facilities to carry out the research

Funding Sources: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The author(s) do not have any conflict of interest.

Data Availability Statement: This statement does not apply to this article.

Ethics Statement : This statement does not apply to this article.

Informed Consent Statement: This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration. This research does not involve any clinical trials

Author contributions: The sole author was responsible for the conceptualization, methodology, data collection, analysis, writing, and final approval of the manuscript.

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Received on August 4, 2025.