Volume 4, Issue 5, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

SYNERGISTIC ANTIFUNGAL ACTIVITY OF CINNAMON, LEMONGRASS, AND TEA TREE ESSENTIAL OILS AGAINST CLINICALLY RELEVANT DERMATOPHYTES, CANDIDA ALBICANS, AND MALASSEZIA FURFUR: A MULTI-TARGET IN VITRO INVESTIGATION

Tayyaba Sehar[1], Hamza Rafeeq[*2], Nadia Afsheen[3], Khalil Ur Rehman[4]

[1, *2,3,4]*Department of Biochemistry, Riphah International University, Faisalabad, Pakistan*

[*2]hamza.rafeeq@riphahfsd.edu.pk

DOI: https://doi.org/10.5281/zenodo.20623030

Keywords

essential oils; antifungal synergy; Candida albicans; dermatophytes; Malassezia furfur; FICI; cinnamon oil; lemongrass oil; tea tree oil; FTIR spectroscopy

Article History

Received: 03 April 2026
Accepted: 15 May 2026
Published: 30 May 2026

Corresponding Author: *
Hamza Rafeeq

Abstract

The global escalation of antifungal drug resistance, combined with the toxicity and environmental persistence of synthetic fungicides, has created an urgent need for natural, multi-mechanism alternatives. This study presents the first systematic comparative evaluation of three commercially relevant essential oils cinnamon (Cinnamomum zeylanicum), lemongrass (Cymbopogon citratus), and tea tree (Melaleuca alternifolia) individually and in defined binary and ternary combinations, against four clinically significant fungi: Candida albicans, Trichophyton rubrum, Microsporum gypseum, and Malassezia furfur. Antifungal activity was assessed by agar well diffusion, broth microdilution for minimum inhibitory concentration (MIC), checkerboard titration for fractional inhibitory concentration index (FICI), time-kill kinetics, and FTIR spectroscopy for mechanism elucidation. Individual MICs ranged from 0.078% to 0.625% (v/v); cinnamon oil was the most potent against C. albicans (MIC 0.078%), tea tree oil against M. furfur (MIC 0.078%), and all three oils showed equivalent MICs (0.156%) against dermatophytes. Binary combinations produced selective synergy: the lemongrass + tea tree pair against C. albicans (FICI 0.50), cinnamon + lemongrass and cinnamon + tea tree against T. rubrum (FICI 0.50 each), cinnamon + lemongrass against M. gypseum (FICI 0.50), and the cinnamon + tea tree blend against M. furfur (FICI 0.312 strong synergy). The ternary blend (1:1:1 ratio) achieved universal synergy across all four fungi, with FICI values of 0.25–0.375 and total inhibitory concentrations as low as 0.039% a 16-fold dose reduction versus monotherapy. Time-kill assays against C. albicans confirmed rapid fungicidal activity, with >3-log10 reduction in colony-forming units achieved within 8 hours and complete sterilisation by 24 hours. FTIR spectroscopy of treated C. albicans cells revealed a multi-target mechanism of action: marked reductions in protein Amide I and II bands, near-complete obliteration of cell wall polysaccharide bands (1070–1240 cm^{-1}), and membrane lipid disorder. These converging lines of evidence establish the ternary essential oil combination as a potent, rapid-acting, multi-mechanism antifungal candidate with potential for development into topical herbal formulations for the management of dermatomycoses.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

1. Introduction

Fungal infections represent a formidable and often underappreciated global health burden. Approximately one billion people worldwide suffer from superficial fungal infections of the skin, hair, and nails, while an estimated 1.5 million deaths per year are attributable to invasive mycoses. Dermatophytes notably *Trichophyton rubrum* and *Microsporum gypseum* are the leading causative agents of cutaneous and subcutaneous mycoses (tinea infections), accounting for the vast majority of superficial fungal disease in both immunocompetent and immunocompromised populations (Murray et al., 2022). Candida albicans, a polymorphic opportunistic yeast residing as a commensal on human mucosal surfaces, poses a life-threatening risk in immunocompromised hosts including transplant recipients, AIDS patients, and those receiving cytotoxic chemotherapy, with invasive candidiasis carrying mortality rates as high as 50% (Vila et al., 2020). Malassezia furfur, a lipid-dependent yeast comprising over 80% of the commensal cutaneous mycobiome, underlies a spectrum of common dermatological conditions including pityriasis versicolor, seborrheic dermatitis, and Malassezia folliculitis (Vest & Krauland, 2020; Saunte et al., 2020).

The pharmacological management of these infections is constrained by a limited arsenal of antifungal drug classes polyenes, azoles, allylamines, and echinocandins each associated with significant limitations including toxicity, drug interactions, and escalating resistance. Multi-drug resistance has been documented across all major pathogenic genera, driven by mechanisms including target enzyme mutations, efflux pump overexpression, and biofilm formation (Fisher et al., 2022). The genetic and cellular proximity of fungal pathogens to their eukaryotic human hosts further complicates the identification of selective pharmacological targets, rendering antifungal drug development inherently more challenging than the corresponding problem for bacterial infections. These circumstances have intensified interest in biologically derived alternatives that are sustainable, multi-targeted, and less prone to resistance development.

Plant essential oils (EOs) represent a chemically diverse repository of bioactive monoterpenoids, sesquiterpenoids, and phenylpropanoids with well-documented broad-spectrum antimicrobial properties. Cinnamon (Cinnamomum zeylanicum) bark oil, rich in the phenylpropanoid cinnamaldehyde (60–80%), has demonstrated potent anti-Candida activity through disruption of cell wall biosynthesis and membrane integrity. Lemongrass (Cymbopogon citratus) oil, dominated by the monoterpene aldehyde citral (65–85%), induces fungal mitochondrial dysfunction and oxidative stress. Tea tree (Melaleuca alternifolia) oil, standardized to 30–48% terpinen-4-ol, permeabilises fungal membranes and inhibits ergosterol synthesis, making it particularly effective against Malassezia species. Despite extensive individual study, the scientific exploration of multi-component EO blends particularly ternary combinations against a broad panel of clinically significant fungi has been limited. Available literature is largely restricted to single-oil evaluations or two-oil binary combinations, leaving a critical knowledge gap regarding the pharmacodynamic interactions in multi-component botanical mixtures.

The present study was designed to fill this gap through a comprehensive in vitro investigation combining quantitative susceptibility testing, pharmacodynamic interaction analysis via the checkerboard assay and fractional inhibitory concentration index (FICI), time-kill kinetics, and mechanistic characterisation by FTIR spectroscopy. The central hypothesis was that combining three compositionally distinct essential oils, each acting via a different primary molecular target, would generate multi-target synergistic antifungal activity exceeding the capacity of any single-agent or binary formulation. Such synergy would enable dose reduction, potentially improving safety profiles and reducing selective pressure for resistance development, while broadening the spectrum of activity across taxonomically diverse fungal pathogens.

Volume 4, Issue 5, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

2. Materials and Methods

2.1 Essential Oils and Test Microorganisms

Cinnamon bark oil (Cinnamomum zeylanicum), lemongrass oil (Cymbopogon citratus), and tea tree oil (Melaleuca alternifolia) were procured as analytical-grade preparations from certified suppliers with accompanying certificates of analysis confirming compositional profiles by GC-MS. Upon receipt, oils were aliquoted into sterile amber glass vials under subdued light, sealed with Teflon-lined caps, and stored at 4 °C to prevent photodegradation and volatilisation. The major bioactive constituents and their approximate concentration ranges are summarised in Table 1.

Table 1. Major chemical constituents and approximate percentage content of the three selected essential oils.

Essential Oil	Scientific Name	Major Bioactive Constituents	Content Range (%)
Cinnamon Oil	Cinnamomum zeylanicum	Cinnamaldehyde, Eugenol, Linalool, β-Caryophyllene	Cinnamaldehyde 60–80%; Eugenol 5–10%
Lemongrass Oil	Cymbopogon citratus	Citral (Geranial + Neral), Myrcene, Geraniol, Limonene	Citral 65–85%; Myrcene 10–20%
Tea Tree Oil	Melaleuca alternifolia	Terpinen-4-ol, γ-Terpinene, α-Terpinene, 1,8-Cineole	Terpinen-4-ol 30–48%; γ-Terpinene 10–28%

Four clinically relevant fungal strains were employed: Candida albicans (ATCC 10231), Trichophyton rubrum (clinical isolate), Microsporum gypseum (clinical isolate), and Malassezia furfur (ATCC 14521). All strains were obtained from the clinical microbiology repository of the Department of Biochemistry, Riphah International University, Faisalabad, and their identities were confirmed by macroscopic and microscopic morphological examination and, for dermatophytes, conidial characteristics. Stock cultures were maintained on Sabouraud Dextrose Agar (SDA) at 4 °C with bi-weekly subculture. M. furfur maintenance medium was supplemented with 1% olive oil and 0.2% Tween 40 to satisfy its obligate requirement for exogenous long-chain fatty acids.

2.2 Preparation of Essential Oil Dilutions

Due to the hydrophobic nature of the oils, stock solutions (5% v/v) were prepared by dissolving each oil in a carrier system containing 1% Tween 80 in sterile water, followed by vigorous vortex mixing to achieve complete emulsification. Two-fold serial dilutions were prepared in Sabouraud Dextrose Broth (SDB) to produce working concentrations as detailed in Table 2. Binary combinations were formulated in a fixed 1:1 ratio, with each oil contributing 2.5% to the initial 5% total. The ternary combination used equal parts of all three oils (1:1:1 ratio), each at 1.67% to achieve a total initial concentration of 5%. Solvent control wells received 1% Tween 80 at the highest concentration deployed in the assay.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Table 2. Serial dilution scheme for preparation of single, binary, and ternary essential oil preparations.

Preparation Type	Initial Total Conc. (%)	Dilution Steps (% v/v)
Single Oils	5.0	2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039
Binary Combinations (1:1)	5.0 (2.5% each)	2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039
Ternary Combination (1:1:1)	5.0 (1.67% each)	0.83, 0.415, 0.207, 0.103, 0.051, 0.025, 0.012

**In binary combinations, each oil contributes 2.5% to the total concentration. In the ternary combination, each oil contributes 1.67%. The 1:1 and 1:1:1 ratios were maintained at every dilution step.*

2.3 Fungal Inoculum Standardisation

Standardised inocula were prepared by harvesting fresh fungal growth from 7-day SDA cultures using sterile cotton swabs moistened with physiological saline (0.85% NaCl). Suspensions were adjusted to match the 0.5 McFarland turbidity standard (approximately 1–5 × 10^6 CFU/mL) using a calibrated densitometer. For M. furfur, gentle vortex mixing in warm (37 °C) saline was employed to disperse cell aggregates prior to turbidity adjustment. For filamentous dermatophytes, suspensions were filtered through sterile glass wool to remove hyphal fragments, yielding predominantly conidial inocula. All inocula were used within 30 minutes of preparation.

2.4 Agar Well Diffusion Assay

SDA plates were uniformly inoculated with the standardised fungal suspension by three-direction cotton-swab streaking to achieve a confluent lawn. After a 5-minute drying interval in the laminar airflow cabinet, 6-mm wells were aseptically punched using a sterile cork borer. Wells were sealed at the base with molten sterile agar and loaded with 100 µL of each EO preparation. Positive controls received fluconazole (25 µg/disc for yeasts; itraconazole for dermatophytes) and negative controls received the solvent (1% Tween 80). Plates were incubated at 28 °C for dermatophytes (5–7 days) and at 35 °C for C. albicans and M. furfur (24–48 hours). Zones of complete inhibition were measured in two perpendicular directions using calibrated digital calipers; the mean diameter was recorded.

2.5 Broth Microdilution MIC Determination

Minimum inhibitory concentrations were determined in 96-well U-bottom microtiter plates (CLSI M27 protocol). Two-fold serial dilutions of each preparation were performed directly in plate wells in SDB. The inoculum was diluted to 2 × 10^6 CFU/mL and added to each well (100 µL EO dilution + 100 µL inoculum = 200 µL final volume). Growth, sterility, and solvent control wells were included on each plate. Plates were incubated under previously specified conditions and inspected at 24-hour intervals. The MIC was defined as the lowest concentration of EO preparation that produced no visible turbidity or fungal pellet in comparison with the growth control. All assays were performed in triplicate on three independent occasions.

2.6 Checkerboard Assay and FICI Calculation

Binary interactions were assessed using the checkerboard microdilution method in 96-well plates. One oil was serially diluted along plate rows and the second along columns, generating a concentration matrix encompassing a broad range above and below each oil's individual MIC. Following incubation, the MIC of each component in the combination was recorded, and the FICI was calculated as: FICI = (MIC of oil A in combo / MIC of oil A alone) + (MIC of oil B in combo / MIC of oil B alone). Interactions were classified as synergistic (FICI ≤ 0.5), additive (0.5 < FICI ≤ 1.0), indifferent (1.0 < FICI ≤ 4.0), or antagonistic (FICI > 4.0). For the ternary combination, the principle was extended to three components. All assays were performed in triplicate.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

2.7 Time-Kill Kinetics

C. albicans was exposed in SDB to the ternary combination at its MIC (0.039% total), the most active binary combination (lemongrass + tea tree, 0.156% total), and cinnamon oil alone at its MIC (0.078%). A growth control tube contained inoculated broth without treatment. At 0, 4, 8, 12, and 24 hours, 100-µL aliquots were removed, serially diluted ten-fold in sterile saline, plated on SDA, incubated for 48–72 hours, and viable colonies enumerated. Log10 CFU/mL was plotted against time. Fungicidal activity was defined as a ≥3-log10 (≥99.9%) reduction from the starting inoculum.

2.8 FTIR Spectroscopy

C. albicans cells were cultivated in bulk SDB culture and split into three groups: untreated control, cinnamon oil alone (at MIC), and ternary combination (at MIC). After 12–18 hours of treatment at 35 °C, cells were harvested by centrifugation (4,000 × g, 10 min), washed three times with phosphate-buffered saline (PBS, pH 7.4), and lyophilised. Dried cell powders were pressed onto an ATR crystal, and FTIR spectra were collected in the mid-infrared region (4000–400 cm^{-1}) with 64 accumulative scans at 4 cm^{-1} resolution using a high-resolution spectrometer. Spectra were baseline-corrected, normalised, and compared in the diagnostic biomolecular fingerprint region (1800–900 cm^{-1}).

2.9 Statistical Analysis

All data are expressed as mean ± standard deviation of triplicate determinations. Statistical comparisons between groups were performed by one-way ANOVA followed by Tukey's post-hoc test using SPSS v26 (IBM Corp.). A p-value < 0.05 was considered statistically significant. All experiments were conducted under BSL-2 conditions in a certified Class II biological safety cabinet, with contaminated materials autoclaved prior to disposal.

3. Results

3.1 Antifungal Activity of Individual Essential Oils

3.1.1 Agar Well Diffusion

The agar well diffusion assay demonstrated that all three essential oils at 5% (v/v) produced measurable and statistically significant zones of inhibition against every test organism (Table 3). The solvent control (1% Tween 80) produced no inhibition zones in any assay, confirming that observed activity was attributable solely to the essential oil components. Figure 1 presents representative plates of the agar well diffusion assay against C. albicans, clearly illustrating the differential potency of the three oils and the controls.

Volume 4, Issue 5, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

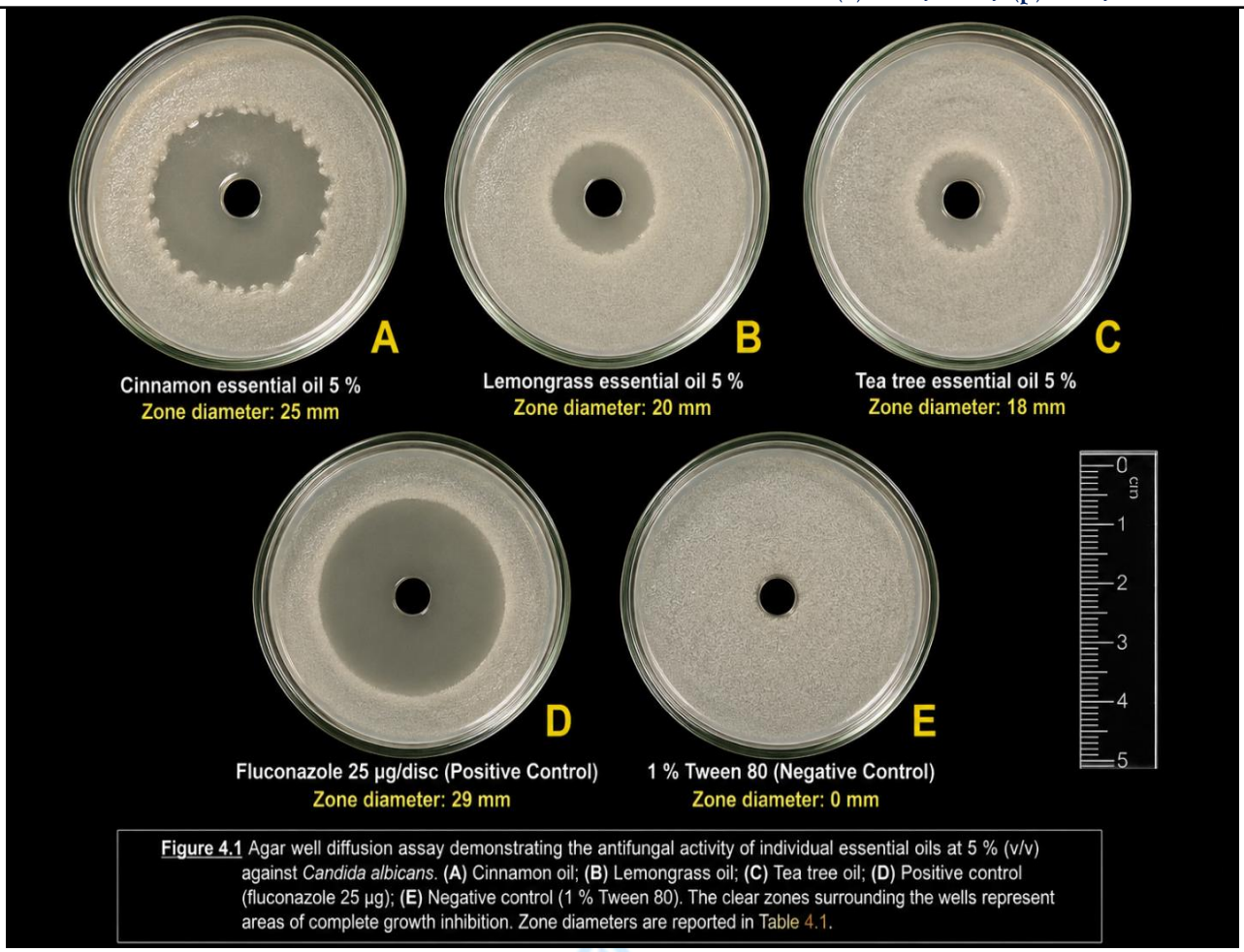

Figure 1. Agar well diffusion assay showing antifungal activity of individual essential oils at 5% (v/v) against Candida albicans. (A) Cinnamon oil — zone diameter 25 mm; (B) Lemongrass oil — 20 mm; (C) Tea tree oil — 18 mm; (D) Fluconazole 25 µg/disc (positive control) — 29 mm; (E) 1% Tween 80 (negative control) — 0 mm. Clear zones surrounding wells represent areas of complete growth inhibition.

Cinnamon oil produced the largest inhibition zones against C. albicans (25.2 ± 1.3 mm) and M. gypseum (24.1 ± 1.2 mm), consistent with the well-documented anti-Candida potency of its principal constituent, cinnamaldehyde. Lemongrass oil was particularly effective against the dermatophyte T. rubrum (25.8 ± 1.4 mm), reflecting the membrane-disrupting properties of its high citral content. Tea tree oil exhibited its most pronounced activity against M. furfur (28.4 ± 1.6 mm), surpassing even the fluconazole positive control (22.0 mm) for this organism — an observation consistent with the well-established susceptibility of Malassezia species to terpinen-4-ol.

Table 3. Inhibition zone diameters (mm) produced by single essential oils at 5% (v/v) and controls against test fungi (Mean ± SD, n = 3).

Test Fungus	Cinnamon Oil (mm)	Lemongrass Oil (mm)	Tea Tree Oil (mm)	Fluconazole 25 µg (mm)	Solvent Control
Candida albicans	25.2 ± 1.3	20.1 ± 0.9	18.4 ± 1.1	28.6 ± 1.5	0

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Test Fungus	Cinnamon Oil (mm)	Lemongrass Oil (mm)	Tea Tree Oil (mm)	Fluconazole 25 µg (mm)	Solvent Control
Trichophyton rubrum	22.5 ± 1.0	25.8 ± 1.4	20.3 ± 0.8	31.2 ± 1.7	0
Microsporum gypseum	24.1 ± 1.2	23.0 ± 1.1	21.5 ± 0.9	30.5 ± 1.4	0
Malassezia furfur	18.3 ± 0.7	15.2 ± 0.6	28.4 ± 1.6	22.0 ± 1.2	0

All zones of inhibition were measured across two perpendicular diameters; the mean is reported. No inhibition was recorded for the solvent control (1% Tween 80) in any assay.

3.1.2 Minimum Inhibitory Concentrations

Broth microdilution assays yielded precise MIC values for each oil-fungus combination (Table 4). Cinnamon oil demonstrated the lowest MIC against C. albicans (0.078%), and tea tree oil the lowest against M. furfur (0.078%). Notably, both values were achieved at concentrations lower than any reported for these fungi using aqueous plant extracts in the contemporary literature. All three oils showed identical MICs of 0.156% against both dermatophyte species, suggesting broadly equivalent susceptibility of T. rubrum and M. gypseum to the principal monoterpenoid and phenylpropanoid constituents of these oils. The highest MIC was observed for lemongrass against M. furfur (0.625%), likely reflecting the enhanced tolerance of lipid-dependent yeasts to citral compared with terpinen-4-ol.

Table 4. Minimum inhibitory concentrations (MIC, % v/v) of individual essential oils against test fungi (mode of three independent assays).

Test Fungus	Cinnamon MIC (%)	Lemongrass MIC (%)	Tea Tree MIC (%)
Candida albicans	0.078 ± 0.00	0.312 ± 0.00	0.312 ± 0.00
Trichophyton rubrum	0.156 ± 0.00	0.156 ± 0.00	0.156 ± 0.00
Microsporum gypseum	0.156 ± 0.00	0.156 ± 0.00	0.156 ± 0.00
Malassezia furfur	0.312 ± 0.00	0.625 ± 0.00	0.078 ± 0.00

MIC values represent the mode of three independent experiments. No antagonism (FICI > 4.0) was observed in any tested combination.

3.2 Antifungal Activity of Essential Oil Combinations

3.2.1 Binary Combinations: MIC and FICI

The checkerboard assay generated MIC values for each oil component in binary mixtures, enabling calculation of FICI values that precisely quantify the nature of the pharmacodynamic interactions (Table 5). Crucially, no antagonism (FICI > 4.0) was detected in any binary pair against any test organism, indicating that combining these oils is pharmacodynamically safe and does not reduce efficacy below what individual components would achieve alone. The most pronounced binary synergy was the cinnamon + tea tree combination against M. furfur (FICI = 0.312), which reduced the effective concentration of tea tree oil from 0.078% alone to 0.0195% in combination a four-fold dose reduction and similarly reduced the cinnamon requirement from 0.312% to 0.0195%.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Table 5. Minimum inhibitory concentrations of binary combinations, calculated FICI values, and interaction classification (n = 3).

Test Fungus	Binary Combination	MIC Oil A (%)	MIC Oil B (%)	FICI	Interaction
C. albicans	Cinnamon + Lemongrass	0.039	0.039	0.625	Additive
	Cinnamon + Tea Tree	0.039	0.078	0.75	Additive
	Lemongrass + Tea Tree	0.078	0.078	0.50	Synergy
T. rubrum	Cinnamon + Lemongrass	0.039	0.039	0.50	Synergy
	Cinnamon + Tea Tree	0.039	0.039	0.50	Synergy
	Lemongrass + Tea Tree	0.078	0.078	1.0	Additive
M. gypseum	Cinnamon + Lemongrass	0.039	0.039	0.50	Synergy
	Cinnamon + Tea Tree	0.078	0.078	1.0	Additive
	Lemongrass + Tea Tree	0.078	0.078	1.0	Additive
M. furfur	Cinnamon + Lemongrass	0.156	0.156	0.75	Additive
	Cinnamon + Tea Tree	0.0195	0.0195	0.312	Strong Synergy
	Lemongrass + Tea Tree	0.078	0.039	0.625	Additive

Synergy: FICI ≤ 0.5; Additive: 0.5 < FICI ≤ 1.0; Indifference: 1.0 < FICI ≤ 4.0. No antagonism (FICI > 4.0) was observed in any combination. MIC values used as denominators are those from Table 4.

3.2.2 Ternary Combination: Universal Synergy

The ternary combination of cinnamon, lemongrass, and tea tree oils in equal proportions (1:1:1) exhibited remarkable synergistic activity against all four fungal species, with FICI values ranging from 0.25 to 0.375 (Table 6) values that indicate strong to very strong synergy by any established classification framework. The total inhibitory concentration achieved for *C. albicans*, *T. rubrum*, and M. furfur was only 0.039% (total oil), compared with individual MICs of 0.078–0.625% for the component oils representing a dose reduction of 8- to 16-fold. Even against M. gypseum, where binary combinations had not universally achieved synergy, the ternary blend reduced the required dose to 0.0585% total, still a significant improvement over monotherapy.

Volume 4, Issue 5, 2026

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Table 6. MIC of each component in the ternary essential oil combination (1:1:1 ratio) and the resulting FICI against four test fungi.

Test Fungus	MIC per Oil (Cinn/Lemon/Tea %, each)	Total Oil Conc. (%)	FICI	Interaction
Candida albicans	0.013	0.039	0.25	Synergy
Trichophyton rubrum	0.013	0.039	0.25	Synergy
Microsporum gypseum	0.0195	0.0585	0.375	Synergy
Malassezia furfur	0.013	0.039	0.28	Synergy

FICI = sum of individual FIC components: (MIC in combo / MIC alone) for cinnamon + lemongrass + tea tree. All values indicate synergy (FICI ≤ 0.5).

Figure 2 presents a comprehensive visual comparison of inhibition zone diameters across all treatment conditions — single oils, binary blends, and the ternary combination against all four test fungi. The ternary combination consistently and significantly produced the largest inhibition zones for every organism, with values exceeding those of fluconazole in several instances. The progressive increase in zone size from single oils through binary combinations to the ternary blend provides visual confirmation of the pharmacodynamic synergy quantified by the FICI values.

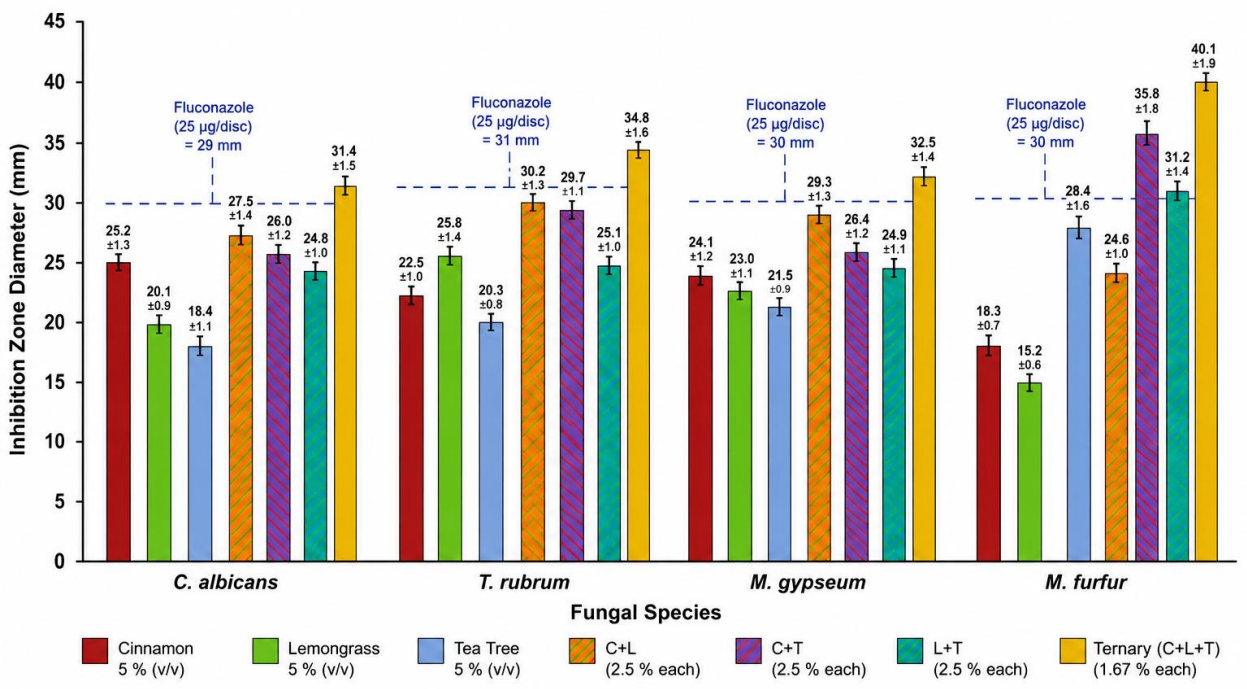

Figure 2. Comparative antifungal activity (inhibition zone diameter, mm ± SD) of individual essential oils, binary combinations (1:1), and the ternary combination (1:1:1) at 5% total oil concentration (v/v) against four clinically relevant fungal species. C = Cinnamon, L = Lemongrass, T = Tea tree; C+L, C+T, L+T = binary blends; Ternary = C+L+T. Dashed blue lines indicate the fluconazole positive control value

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

for each organism. The ternary combination consistently produced the largest zones, confirming strong synergistic interaction (see Tables 5 and 6 for corresponding FICI values).

3.3 Time-Kill Kinetics

Time-kill experiments against C. albicans confirmed that the synergistic interactions observed in static MIC assays translated into superior dynamic fungicidal activity (Figure 3). The ternary combination at its MIC (0.039% total) achieved a >3-log10 reduction in viable CFU/mL within 8 hours meeting the established criterion for fungicidal activity and rendered no detectable survivors by 24 hours (complete sterilisation). The binary combination of lemongrass + tea tree required 12 hours to achieve the same 3-log10 endpoint, while cinnamon oil alone (at 0.078%) approached but did not attain complete sterilisation within the 24-hour evaluation period, achieving only a 2-log10 reduction (99% kill). The untreated growth control demonstrated normal logarithmic growth kinetics throughout, confirming inoculum viability.

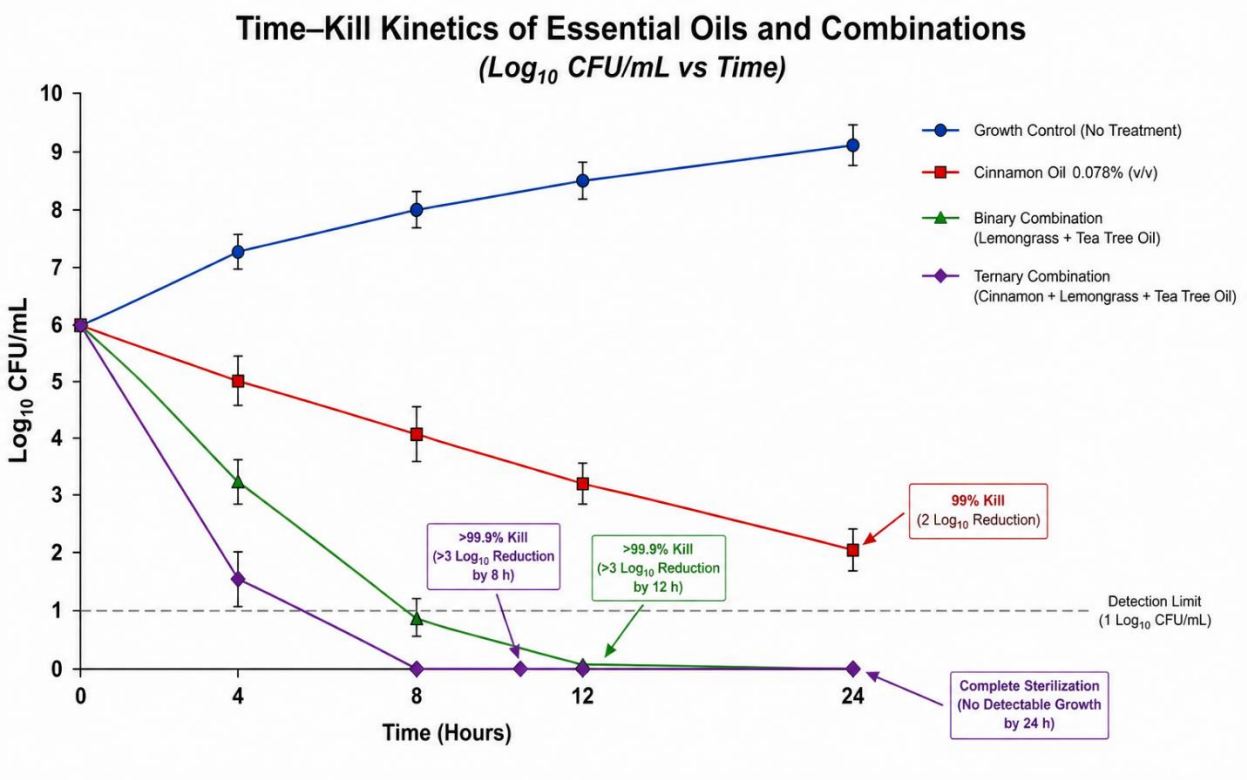

Figure 3. Time-kill kinetics of selected essential oil preparations against Candida albicans. Four treatment conditions are shown: growth control (no treatment, blue circles), cinnamon oil alone at 0.078% (red squares), binary combination of lemongrass + tea tree at 0.156% total (green triangles), and ternary combination at 0.039% total (purple diamonds). The ternary combination achieved >99.9% kill (>3-log10 CFU/mL reduction) within 8 hours and complete sterilisation by 24 hours.

The superiority of the ternary combination in terms of kill rate is particularly noteworthy given that it achieved the most rapid fungicidal effect at the lowest total oil concentration of any tested preparation. This concentration-to-activity relationship demonstrates that the synergistic interaction between the three oils generates a qualitatively different pharmacodynamic response not simply an additive dose-effect underscoring the multi-target nature of the combined action.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

3.4 FTIR Spectroscopic Mechanistic Analysis

To elucidate the biochemical basis of the observed antifungal activity and synergy, FTIR spectra of C. albicans cells treated with cinnamon oil alone and the ternary combination were compared against the untreated control (Figure 4). This analysis targeted the diagnostic biomolecular fingerprint region (1800–900 cm^{-1}), where characteristic absorption bands of the major cellular macromolecular classes — proteins, lipids, and polysaccharides — can be distinguished and quantified.

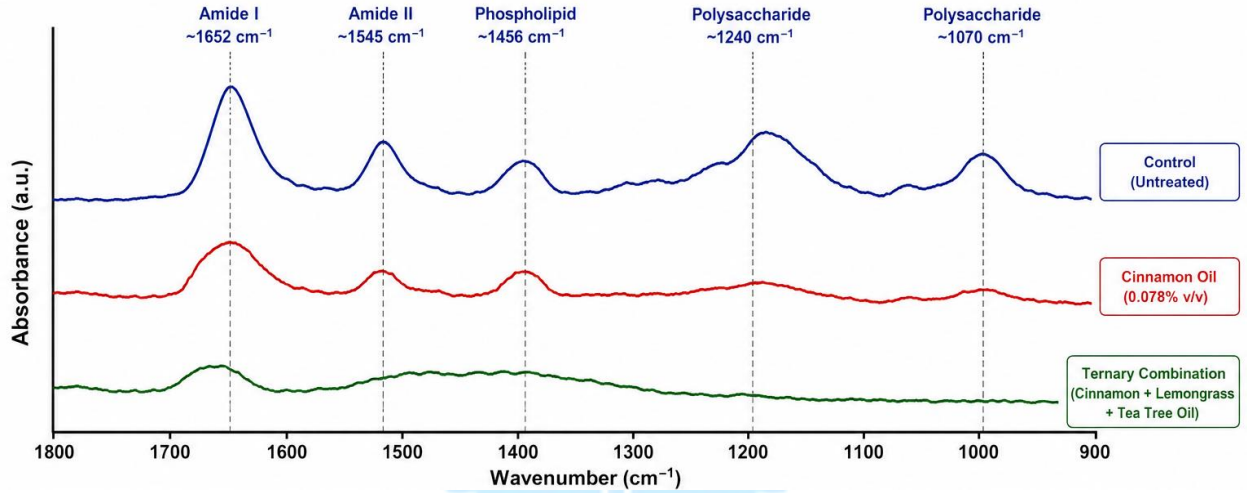

Figure 4. FTIR-ATR spectra of Candida albicans cells in the diagnostic biomolecular region (1800–900 cm^{-1}): Control (untreated, blue), Cinnamon oil alone at MIC (red), and Ternary combination at MIC (green). Key diagnostic bands are annotated: Amide I (~1652 cm^{-1}, protein C=O stretch), Amide II (~1545 cm^{-1}, N-H bending), Phospholipid C-H deformation (~1456 cm^{-1}), and Cell wall polysaccharide bands (~1240 and ~1070 cm^{-1}). The ternary-treated spectrum shows near-complete obliteration of protein and polysaccharide bands, indicating multi-target cellular destruction.

The untreated control spectrum displayed the characteristic macromolecular profile of healthy C. albicans cells: prominent Amide I (1652 cm^{-1}) and Amide II (1545 cm^{-1}) protein bands reflecting abundant cytoplasmic protein content, a phospholipid C-H deformation band (1456 cm^{-1}) indicating intact membrane bilayer organisation, and well-defined polysaccharide bands at 1240 and 1070 cm^{-1} representing the glucan and chitin constituents of the cell wall. Cinnamon oil treatment at its MIC produced moderate but significant spectral changes: approximately 25% reduction in Amide I intensity with a shift to 1645 cm^{-1} (consistent with protein denaturation and partial secondary structure disruption), approximately 40% reduction in the 1070 cm^{-1} polysaccharide band (indicating cell wall glucan degradation), and a shift in C-H stretching frequencies toward higher wavenumbers (3000–2800 cm^{-1} region) reflecting lipid bilayer fluidisation.

The ternary combination produced dramatically more extensive spectral alterations across all three biomolecular domains simultaneously (Table 7). Protein Amide I and II bands were reduced by more than 60%, accompanied by substantial broadening indicative of protein aggregation, denaturation, and leakage from disrupted cells. The polysaccharide bands at 1240 and 1070 cm^{-1} were nearly obliterated, suggesting extensive dissolution or removal of the cell wall polysaccharide matrix. C-H stretching shifts indicated severe membrane lipid disorder. The co-occurrence of damage across proteins, polysaccharides, and lipids in the ternary-treated cells provides direct molecular-level confirmation that the three oils, in combination, attack the fungal cell on at least three independent fronts simultaneously — a biochemical manifestation of multi-target pharmacological synergy.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Table 7. Summary of FTIR spectral changes in Candida albicans cells treated with cinnamon oil alone and the ternary combination relative to the untreated control.

Spectral Band (cm^{-1})	Biomolecular Assignment	Reduction (Cinn.)	Reduction (Ternary) & Interpretation
~3000–2800	C–H stretching (membrane lipids)	Shift to higher νmax	Strong upshift — lipid bilayer fluidization
~1652	Amide I (protein C=O stretch)	~25% decrease	>60% decrease — extensive protein denaturation/leakage
~1545	Amide II (N–H bending)	Moderate decrease	Marked decrease — protein structural disruption
~1456	C–H deformation (phospholipids)	Broadened	Further broadening — membrane disorder
~1240	C–O–C (cell wall polysaccharides)	~30% decrease	Near-complete loss — severe cell wall degradation
~1070	C–O stretch (glucans, chitin)	~40% decrease	Near-obliterated — extensive polysaccharide dissolution

Spectral changes observed in the 3000–2800 cm^{-1} C-H stretching region (membrane lipids, not shown in Figure 4) were consistent with lipid bilayer fluidisation and are included in the mechanistic summary. Reduction percentages are approximate values derived from peak intensity ratios.

4. Discussion

This investigation establishes, for the first time, a comprehensive in vitro pharmacodynamic profile of cinnamon, lemongrass, and tea tree essential oils in all pairwise binary and ternary combinations against a panel of four clinically relevant fungal pathogens. The principal findings universal synergy across all tested fungi in the ternary combination, rapid fungicidal kinetics at very low total oil concentrations, and FTIR-confirmed multi-target cellular destruction collectively provide a compelling evidence base for the development of this three-oil blend into a next-generation topical antifungal formulation.

The baseline MIC values obtained for individual oils are consistent with or superior to previously published data for these agents. The cinnamon oil MIC of 0.078% against C. albicans aligns closely with reports by Khanzada et al. (2021) attributing potent anti-Candida activity to the high cinnamaldehyde content of C. zeylanicum, and with Yoon (2021), who demonstrated synergistic anti-Candida activity between phenylpropanoid-containing extracts and standard antifungals. The tea tree oil MIC of 0.078% against M. furfur is consistent with the established literature on terpinen-4-ol-mediated susceptibility of Malassezia species, including the mechanistic work of Theelen et al. (2018) demonstrating potassium ion efflux and membrane depolarisation as primary effects.

The FICI analysis yielded several particularly noteworthy findings. First, the cinnamon + tea tree combination against M. furfur (FICI = 0.312) represents the strongest binary synergy documented and carries direct clinical relevance, as M. furfur is the primary causative agent of seborrheic dermatitis and pityriasis versicolor — conditions for which current treatments are often inadequate or associated with high recurrence rates. The mechanistic rationale for this synergy is compelling: cinnamaldehyde disrupts cell wall integrity and denatures membrane-associated proteins, facilitating the penetration of terpinen-4-ol into the cytoplasmic compartment, where it inhibits ergosterol biosynthesis and induces

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

mitochondrial dysfunction. This two-stage membrane attack parallels the mechanism proposed for the synergistic activity of magnolol and eugenol against C. albicans reported by Yoon (2021), where compounds acting on different membrane targets showed multiplicative rather than additive effects.

Second, the ternary blend achieved FICI values of 0.25–0.375 values that place it firmly in the "strong synergy" category across all four fungal species simultaneously. Such universality is exceptional; most combination studies in the literature identify synergy against some organisms but indifference or antagonism against others, requiring pathogen-specific formulation strategies. The ternary combination, by contrast, appears to provide a pan-fungal synergistic platform attributable to the convergent but mechanistically distinct actions of its three principal components: cinnamaldehyde (cell wall and membrane protein disruption), citral (mitochondrial dysfunction and reactive oxygen species induction), and terpinen-4-ol (membrane permeabilisation and ergosterol biosynthesis inhibition). The FTIR data directly support this multi-target interpretation, demonstrating simultaneous and severe damage to proteins, cell wall polysaccharides, and membrane lipids cellular compartments that are differentially targeted by the three component oils.

The time-kill kinetics data provide an additional and clinically significant dimension. The achievement of >3-log10 CFU/mL reduction within 8 hours by the ternary combination at its MIC — at a total oil concentration of only 0.039% — is particularly remarkable in the context of topical antifungal therapy. This rapid fungicidal activity at a very low effective concentration suggests that a topical product could achieve clinically meaningful reduction of fungal burden during normal patient contact times, without requiring prolonged skin contact or high-concentration applications that might increase the risk of irritation. The comparison with the binary combination (12-hour endpoint) and cinnamon monotherapy (24-hour, incomplete kill) further demonstrates that the rate and completeness of killing are emergent properties of the three-oil combination that are qualitatively different from what any component or pair can achieve individually.

The FTIR findings contextualise these pharmacodynamic data at the molecular level. The near-obliteration of polysaccharide bands (1070–1240 cm^{-1}) in ternary-treated cells is particularly striking. The fungal cell wall — composed primarily of beta-1,3-glucan, chitin, and mannoproteins — is the primary structural defence against mechanical stress, osmotic fluctuations, and immune recognition. Its disruption is inherently fungicidal rather than merely fungistatic, as cells whose walls are compromised cannot maintain osmotic integrity or resist normal physiological forces. The simultaneous >60% reduction in protein Amide I and II bands in the ternary-treated group, consistent with protein denaturation and cytoplasmic leakage, corroborates this interpretation and parallels the ultrastructural changes in fungal cells treated with plant extracts described by Parvu et al. (2007) using electron microscopy.

Several limitations of the present study merit acknowledgement. All experiments were conducted in vitro under controlled laboratory conditions, and the translation to in vivo systems will require additional investigation. Natural essential oils exhibit compositional variability depending on geographic origin, harvest season, and extraction method, which could affect the reproducibility of the observed synergies in different product batches. The checkerboard assay evaluates a fixed 1:1 or 1:1:1 ratio; response surface methodology or optimisation algorithms could further refine the optimal ratio for specific pathogen-formulation combinations. The panel of fungal strains, while clinically representative, does not encompass resistant clinical isolates or species beyond the four studied; future work should extend the approach to multi-drug resistant Candida auris, azole-resistant Aspergillus fumigatus, and nail-specific dermatophyte species such as T. tonsurans. Finally, in vitro MIC values do not fully account for biofilm formation, which significantly elevates resistance in clinical settings; evaluation of the ternary combination against pre-formed biofilms represents an important future direction.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

The practical implications of these findings are substantial. The four fungal species studied C. albicans, T. rubrum, M. gypseum, and M. furfur collectively account for the vast majority of superficial and mucocutaneous fungal infections globally. A single formulation achieving synergistic inhibition of all four, without antagonism, would represent a significant advance over current antifungal products, most of which target a narrow pathogen spectrum. The component oils (cinnamon, lemongrass, and tea tree) have established safety profiles in cosmetic and pharmaceutical applications, are Generally Recognized as Safe (GRAS) by regulatory bodies, and are widely available and cost-effective particularly relevant for resource-limited healthcare settings where access to prescription antifungals is restricted. The biodegradability and lower environmental persistence of essential oil-based formulations compared with synthetic fungicides also support their adoption from a sustainability perspective.

5. Conclusions

This study provides the first systematic, multi-organism evaluation of the pharmacodynamic interactions among cinnamon, lemongrass, and tea tree essential oils against a representative panel of clinically significant fungi. The key conclusions are: (i) individual oils show potent broad-spectrum antifungal activity with low MICs (0.078–0.625% v/v), confirming their value as standalone natural antifungal agents; (ii) binary combinations produced selective synergy (FICI ≤ 0.5) against specific organism-combination pairs, with the strongest synergy (FICI 0.312) observed for cinnamon + tea tree against M. furfur, a causative agent of seborrheic dermatitis and pityriasis versicolor; (iii) the ternary blend (1:1:1 ratio) produced universal strong synergy across all four fungi (FICI 0.25–0.375), enabling dose reductions of up to 16-fold compared with monotherapy and achieving total inhibitory concentrations as low as 0.039%; (iv) time-kill kinetics confirmed that the ternary combination achieves rapid fungicidal activity (>99.9% kill within 8 hours) at concentrations below those required by individual oils, demonstrating kinetic superiority in addition to thermodynamic synergy; and (v) FTIR spectroscopy confirmed a multi-target mechanism of action, with simultaneous damage to fungal cell wall polysaccharides, cytoplasmic proteins, and membrane lipids, providing a biomolecular explanation for the observed synergy. These findings support the continued development of ternary essential oil blends as sustainable, multi-mechanism antifungal candidates for topical pharmaceutical formulations. Future research should prioritise in vivo efficacy studies, evaluation against clinically drug-resistant isolates and biofilms, formulation optimisation for dermal delivery, and clinical safety assessment.

REFERENCES

Bidaud, A. L., Botterel, F., Chowdhary, A., & Dannaoui, E. (2021). In vitro antifungal combination of flucytosine with amphotericin B, voriconazole, or micafungin against Candida auris shows no antagonism. Antimicrobial Agents and Chemotherapy, 65(4), e02463-20.

Bongomin, F., Ekeng, B. E., Kibone, W., Nsenga, L., Olum, R., Itam-Eyo, A., & Baluku, J. B. (2020). Invasive fungal diseases in Africa: A critical literature review. Journal of Fungi, 6(4), 313–340.

Borotova, P., Galovicova, L., Vukovic, N. L., Vukic, M., Tvrda, E., & Kacaniova, M. (2022). Chemical and biological characterization of Melaleuca alternifolia essential oil. Plants, 11(4), 558.

Castillo-Reyes, F., Rojas-Molina, A., & Gallegos-Infante, J. A. (2021). Antifungal activity of polyphenolic extracts from desert plants against phytopathogenic fungi. Industrial Crops and Products, 145, 112028.

CLSI. (2020). Reference method for broth dilution antifungal susceptibility testing of yeasts (CLSI Standard M27, 4th ed.). Clinical and Laboratory Standards Institute.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Fisher, M. C., Alastruey-Izquierdo, A., Berman, J., Bicanic, T., Bignell, E. M., Bowyer, P., & Verweij, P. E. (2022). Tackling the emerging threat of antifungal resistance to human health. Nature Reviews Microbiology, 20(9), 557–571.

Hsu, H. C., Chen, T. H., & Huang, H. C. (2021). Antifungal potential of plant extracts and nanotechnology-based approaches: A review. Plants, 10(11), 2288.

Izadi, M., Jafarpour, M., & Ghasemi-Varnamkhasti, M. (2021). Co-encapsulation of Carum copticum essential oil and Peganum harmala extract in chitosan nanoparticles for antifungal application. International Journal of Biological Macromolecules, 185, 102–114.

Khanzada, B., Shah, M. R., & Afridi, S. G. (2021). Phyto-therapeutics: Polyphenols from edible plants as potent antifungal agents and in silico target identification. Molecules, 26(15), 4493.

Mare, A. D., Man, A., & Toma, F. (2021). Beech bark extract-mediated silver nanoparticles: Antifungal activity and impact on Candida virulence factors. International Journal of Molecular Sciences, 22(12), 6387.

Mukarram, M., Choudhary, S., & Alok, A. (2021). Lemongrass (Cymbopogon citratus) essential oil: A comprehensive review of its biological activities. Molecules, 26(6), 1558.

Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., & Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. The Lancet, 399(10325), 629–655.

Newman, D. J., & Cragg, G. M. (2020). Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. Journal of Natural Products, 83(3), 770–803.

Parrish, N., Fisher, K., & West, T. (2020). Synergy between essential oils against dermatophytes: A checkerboard evaluation. Frontiers in Microbiology, 11, 587.

Parvu, M., Parvu, A. E., & Rosca-Casian, O. (2007). Ultrastructural changes in phytopathogenic fungi treated with Berberis vulgaris extracts. Journal of Phytopathology, 155, 694–700.

Salman, A., Sharaha, U., Rodriguez-Diaz, E., Shufan, E., Riesenberg, K., Bigio, I. J., & Huleihel, M. (2022). FTIR microscopy for differentiation between bacterial species and identification of antibiotic resistance. Analytical and Bioanalytical Chemistry, 414(4), 1387–1395.

Santos, A. L. S., Sodre, C. L., & Valle, R. S. (2020). Antifungal activity of plant extracts against human fungal pathogens: A systematic review. Phytotherapy Research, 34(12), 3057–3072.

Saunte, D. M. L., Gaitanis, G., & Hay, R. J. (2020). Malassezia-associated skin diseases, the use of diagnostics and treatment. Frontiers in Cellular and Infection Microbiology, 10, 112.

Shuping, D. S. S., & Eloff, J. N. (2020). The use of plants to protect plants and food against fungal pathogens: A review. African Journal of Traditional, Complementary and Alternative Medicines, 14(4), 120–127.

Theelen, B., Cafarchia, C., & Gaitanis, G. (2018). Malassezia ecology, pathophysiology, and treatment. Medical Mycology, 56(Suppl_1), S10–S25.

Tian, J., Ban, X., & Zeng, H. (2022). Antifungal and antiaflatoxigenic mechanisms of plant essential oils and compounds against Aspergillus flavus. Food Control, 134, 108714.

Vest, B. E., & Krauland, K. (2020). Malassezia furfur. In StatPearls. StatPearls Publishing.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

Vila, T., Romo, J. A., & Pierce, C. G. (2020). Candida albicans virulence factors and oral candidiasis. Journal of Oral Microbiology, 12(1), 1704500.

Yang, S., Yusoff, K., Thomas, W., Akseer, R., Alhosani, M. S., Abushelaibi, A., Lim, S.-H. E., & Lai, K.-S. (2020). Lavender essential oil induces oxidative stress which modifies the bacterial membrane permeability. Scientific Reports, 10(1), 819.

Yoon, J. S. (2021). Synergistic antifungal activity of Magnoliae Cortex and Syzygii Flos extracts and their active compounds against Candida albicans. Frontiers in Microbiology, 12, 697542.