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ORIGINAL ARTICLE

Evaluation of antifungal activity of *Melaleuca alternifolia* and *Curcuma longa* Oils against *Candida albicans*

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Abstract

Background: The increasing incidence of Candida infections and the emergence of antifungal resistance have created a need for alternative antifungal agents with effective and potentially safer profiles. Plant-derived essential oils contain diverse bioactive constituents and have demonstrated considerable antimicrobial potential. *Melaleuca alternifolia* (tea tree oil; TTO) and *Curcuma longa* (turmeric oil; TO) possess reported antimicrobial and therapeutic properties and may provide promising natural sources for antifungal development. **Objective:** The present study aimed to evaluate and compare the *in vitro* antifungal activity of *M. alternifolia* and *C. longa* oils, individually and in a 1:1 combination, against *Candida albicans* (MTCC 3958). The study also aimed to determine the minimum inhibitory concentrations (MICs) of the individual oils and their combination and compare their antifungal activity with the standard antifungal drug, Amphotericin B. **Materials and Methods:** Authenticated *M. alternifolia* and *C. longa* oils were evaluated against *C. albicans* (MTCC 3958). Antifungal activity was assessed by agar well diffusion using individual oils and their 1:1 combination at 62.5, 125, 250, and 500 µL/mL, with Amphotericin B as the positive control. MIC was determined by broth microdilution using two-fold serial dilutions (1.95-500 µL/mL), with appropriate controls. The MIC was defined as the lowest concentration showing complete inhibition of visible fungal growth. **Results:** Both individual oils demonstrated concentration-dependent antifungal activity against *C. albicans*. *M. alternifoli*a oil exhibited inhibition zones of 12.2 ± 1, 13.5 ± 2, 16.2 ± 1, and 18.2 ± 1 mm at 62.5, 125, 250, and 500 µL/mL, respectively, whereas *C. longa* oil produced corresponding inhibition zones of 10.3 ± 2, 12.0 ± 2, 13.8 ± 2, and 14.2 ± 2 mm. The 1:1 combination demonstrated enhanced activity, producing inhibition zones of 13.7 ± 2, 16.5 ± 1, 18.0 ± 1, and 19.0 ± 1 mm at the respective concentrations. Amphotericin B produced an inhibition zone of approximately 22.3 ± 2 mm. In the broth microdilution assay, *M. alternifolia* oil showed an MIC of 15.63 µL/mL, while *C. longa* oil showed an MIC of 31.25 µL/mL. The 1:1 combination exhibited a lower MIC of 7.81 µL/mL, with 92.8% growth inhibition at 3.91 µL/mL and complete inhibition at 7.81 µL/mL and higher concentrations. Amphotericin B produced complete inhibition at 1 µg/mL. Thus, the combination demonstrated greater inhibitory activity than either oil individually under the conditions evaluated, although the standard antifungal remained more potent. **Conclusion:** The findings demonstrate that both *M. alternifolia* and *C. longa* oils possess measurable and concentration-dependent in vitro antifungal activity against *C. albicans*, with M. alternifolia showing greater activity than C. longa when tested individually. Importantly, their 1:1 combination enhanced the antifungal response and reduced the MIC to 7.81 µL/mL, compared with 15.63 and 31.25 µL/mL for *M. alternifolia* and *C. longa*, respectively. These findings support further investigation of the oil combination as a potential basis for natural antifungal formulations.

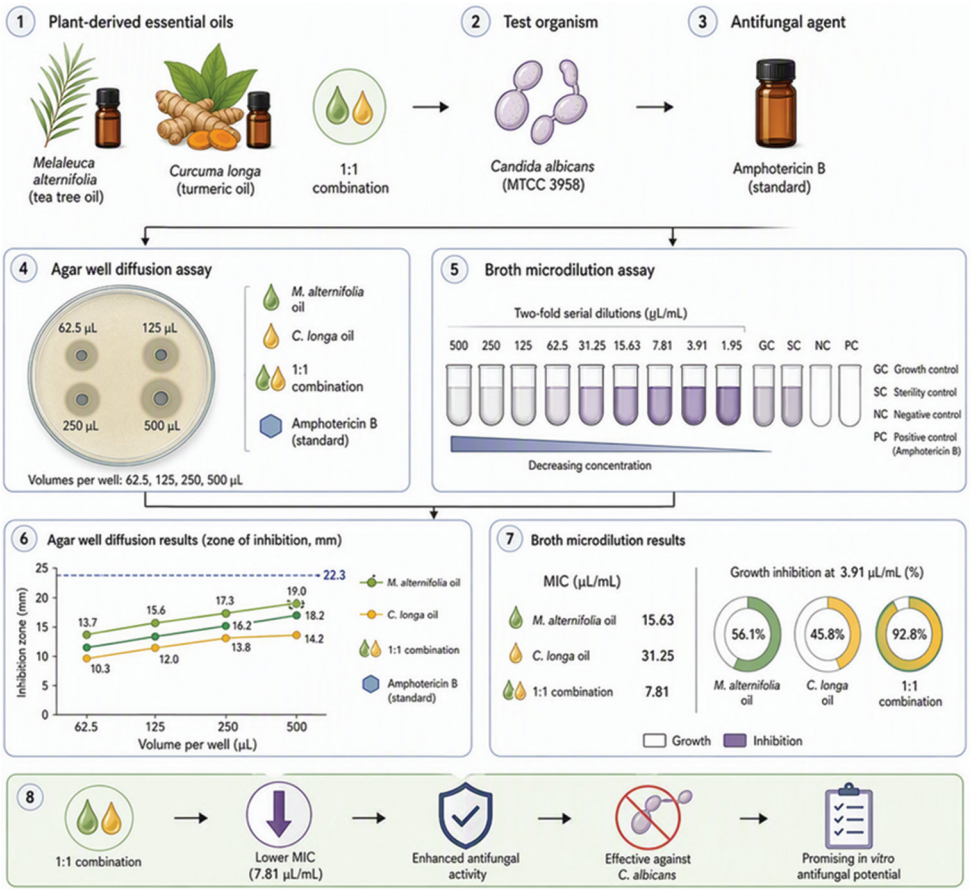
Key words: Antifungal activity, *Candida* species, *Curcuma longa*, *Melaleuca alternifolia*, natural therapeutics, oils

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GRAPHICALABSTRACT



INTRODUCTION

Fungal infections are an increasing public health concern worldwide, especially in immune-compromised individuals such as human immunodeficiency virus/acquired immunodeficiency syndrome-suffering patients, organ transplant patients, and those undergoing prolonged antibiotic or corticosteroid therapy.[1,2] *Candida* strains are considered the most frequent opportunistic fungus among fungal infections and are responsible for a wide variety of diseases ranging from superficial skin candidiasis to life-threatening candidiasis.[3,4] *Candida albicans* is the most common causative species, although the incidence of infection with non-albicans species such as *Candida tropicalis*, *Candida glabrata,* and *Candida krusei* has been rising.[1] These non-albicans species often demonstrate resistance[2,3] to conventional antifungal agents, thereby complicating treatment strategies. Conventional antifungal therapies,[4,5] including azoles such as fluconazole and itraconazole, polyenes such as amphotericin B, and echinocandins, are effective but not without limitations. Long-term and without discrimination use of these drugs has led to the development of resistant *Candida* species, drug–drug interactions, high cost, and undesirable side effects such as nephrotoxicity and hepatotoxicity.[6,7] The rising concern of resistance and limitations of therapy requires a search for novel antifungal medicines with effective, safe, and cost-effective profiles. Plant-origin natural materials, especially oils, have emerged as promising alternatives to synthetic antifungal agents.[8,9] Oils are volatile, aromatic compounds extracted primarily through steam distillation or solvent extraction from various parts of plants such as leaves, bark, flowers, and seeds.[10-13] They are complex mixtures of terpenes,[14,15] terpenoids, phenolic compounds, and other bioactive constituents with diverse pharmacological properties.[16] Several studies have documented their antibacterial, antifungal, antiviral, antioxidant, and anti-inflammatory activities, making them attractive candidates for drug discovery and formulation development.[17,18]

Among the diverse medicinal plants, *Melaleuca alternifolia*[19,20] and *Curcuma longa*[21-25] have attracted considerable attention due to their diverse pharmacological properties and therapeutic potential. The high importance of these molecules is given by their long history of use in traditional medicine and their experimentally proven antibacterial activity. *M. alternifolia* oil, indigenous to Australia, *M. alternifolia* oil contains terpinen-4-ol, γ-terpinene, and α-terpinene[15,26] as major constituents. It has been widely used in dermatological preparations for treating fungal infections such as candidiasis, athlete's foot, and onychomycosis due to its membrane-disruptive action on fungal cells. *C. longa* is a well-known medicinal

herb used in the Ayurvedic traditional system of medicine and in traditional Chinese medicine. The oil contains sesquiterpenes (ar-turmerone, α-turmerone) and other volatile compounds, in addition to curcuminoids present in the rhizome.[27-29] *C. longa* has shown antifungal and anti-inflammatory effects, with potential to inhibit fungal growth and biofilm formation.[30,31] Its efficacy against *Candida* and dermatophyte fungi has been recognized in both traditional and scientific studies. The combination of these oils or their individual application against *Candida* species may provide a natural and effective approach to managing fungal infections. Exploring their antifungal potential not only validates the knowledge of traditional medicine but also contributes to the development of novel plant-based therapeutics. This present study aims to assess and compare the antifungal effectiveness of oils from *M. alternifolia* (tea tree oil [TTO]) and *C. longa* (turmeric oil [TO]) against *Candida* species through *in vitro* techniques, including agar well diffusion and minimum inhibitory concentration (MIC) determination. This study aims to provide significant insights into the antifungal efficacy of these natural oils and their potential as alternatives to conventional antifungal medications.

MATERIALS AND METHODS

Materials

Plant source oils

Oils of *M. alternifolia* and *C. longa* were selected for the present investigation based on their reported antimicrobial properties. The oils were procured from an authenticated herbal supplier. All the oils were stored in tightly closed amber-colored glass containers at 4°C to protect them from light, oxidation, and volatilization until further use. Before experimentation, the oils were brought to room temperature and visually inspected to ensure the absence of turbidity or precipitation.

Chemicals and reagents

Dimethyl sulfoxide (DMSO), Tween 80, sterile distilled water, and normal saline were used for the preparation of oil solutions and microbial suspensions. Amphotericin B was employed as the standard antifungal drug. All chemical substances and reagents used in the study were of analytical grade and purchased from reliable sources to ensure experimental reliability.

Culture media

Candida species were isolated and tested for antifungal susceptibility using Sabouraud dextrose agar (SDA) and Sabouraud dextrose broth 36–38. The media were prepared according to the manufacturer's instructions and sterilized by autoclaving at 121°C at 15 pressures for 15 min. The sterility of the generated medium was checked before use.

Microorganisms

The antifungal potentials of the oils were investigated against pathogenic *Candida* species, namely *C. albicans*. These *C. albicans* (Microbial Type Culture Collection and Gene Bank [MTCC] No. 3958) strains were obtained from an MTCC laboratory and were routinely maintained on SDA slants at 4°C. Before every experiment, the fungal culture was subcultured on fresh SDA plates and incubated at 37°C for 24–48 h to ensure viability and purity. The fungal inoculum was standardized by adjusting the turbidity to match approximately 0.5 McFarland standard, corresponding to about 1×10^6 colony-forming units/mL.[32,33]

Preparation of oil samples

Due to the hydrophobic nature of the oils, *M. alternifolia* and *C. longa* oils were used directly for the agar well diffusion assay without dilution. The oils were brought to room temperature before use and thoroughly mixed to ensure uniformity. The required volumes of undiluted oils were aseptically measured and directly introduced into the prepared agar wells at 62.5, 125, 250, and 500 µL/well. A negative control containing no oil was included, whereas amphotericin B (20 µg and 1 µg) was used as the positive control. The plates were then incubated under the specified conditions, and the zones of inhibition were measured in millimeters.[36,37]

Antifungal activity assessment

Detection of antifungal effect of individual oils

The antifungal activity of the oils was assessed using the agar well diffusion method. A growth control containing no test oil (0 µL/ml) was included, while the test groups contained 62.5, 125, 250, and 500 µL/ml of the respective oil's concentrations. Amphotericin B (20 µg/disc) was used as the positive control (PC). Sterile SDA plates were inoculated with standardized *Candida* suspensions by evenly swabbing over the surface of the agar using sterile cotton swabs. Wells of equal diameter (6 mm) were aseptically punched into the agar using a sterile cork borer. The appropriate volume of each oil solution was added to the respective wells. Plates were allowed to remain standing at room temperature for a short time to allow prediffusion of compounds into the agar matrix.[37,38] The plates were incubated at 37°C for a duration of 24–48 h, after which they were examined for the presence of clear zones surrounding the wells, indicating antifungal activity. The diameter of the inhibitory zones was analyzed in millimeters with a digital Vernier caliper. Each experiment was conducted at least 3 times, and the average zone of inhibition, together with the standard deviation, was computed.[39-41]

Detection of antifungal effect of oil combinations

The combined antifungal potentials of oils were investigated by preparing combinations of *M. alternifolia*

Oil and *C. longa* oil (1:1). The agar well diffusion method was employed to evaluate these combinations against *Candida*, and the resulting zones of inhibition were compared to those of individual oils to assess potential combined effects.[42-44]

Determination of MIC

The MIC of the oils was measured through the broth microdilution method. Using sterile 96-well microtiter plates, two-fold serial dilutions of each oil were made in Sabouraud dextrose broth.[45-47] Each well was inoculated with a standardized fungal suspension, and appropriate controls, including growth control and solvent control, were included. Microtiter plates were incubated at 37°C for 24–48 h. The MIC was established as the minimal concentration of oil that entirely prevented apparent fungal growth in comparison to the growth control wells.[48,49]

Comparative study with standard antifungal drug

Amphotericin B was used as the reference antifungal agent for comparative evaluation. The antifungal activity of amphotericin B was assessed in the same agar diffusion and broth dilution assays as the oils. The antifungal effectiveness of the oils was determined by zones of inhibition and MIC values in comparison with amphotericin B and their therapeutic potential.

RESULTS AND DISCUSSION

Antifungal activity of individual oils

The antifungal activity of *M. alternifolia* oil (TTO) and *C. longa* oil (TO) was evaluated against *Candida* species using the agar well diffusion method. Both oils exhibited measurable antifungal activity, with *M. alternifolia* oil showing greater efficacy than *C. longa* oil. *M. alternifolia* oil and *C. longa* oil were tested at concentrations of 62.5, 125, 250, and 500 µL/ml per well, along with a negative control. A progressive increase in the inhibition zone was observed with increasing concentration of the tested oils. *M. alternifolia* oil produced inhibition zones of 12.2 ± 1, 13.5 ± 2, 16.2 ± 1, and 18.2 ± 1 mm at 62.5, 125, 250, and 500 µL/ml, respectively, whereas *C. longa* oil produced corresponding inhibition zones of 10.3 ± 2, 12.0 ± 2, 13.8 ± 2, and 14.2 ± 2 mm at 62.5, 125, 250, and 500 µL/ml. Amphotericin B (20 µg) was used as the positive control and produced the highest inhibition zone of 22.3 ± 2 mm, whereas the negative control showed no inhibition. The results presented in Figures 1 and 2 and Tables 1 and 2 confirm a progressive increase in antifungal activity with increasing volumes of the tested oils, although their activity was lower than that of the standard drug.

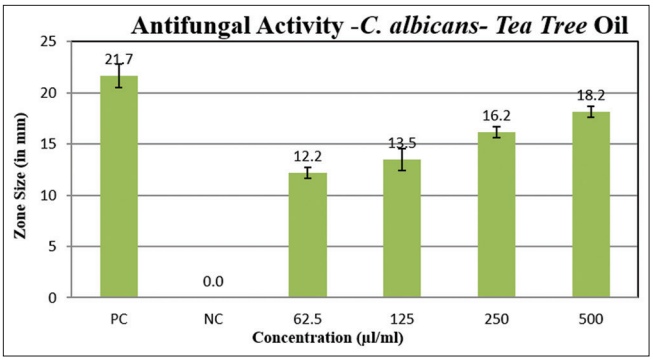


Figure 1: Antifungal activity of *Melaleuca alternifolia* (tea tree) oil against *Candida albicans*

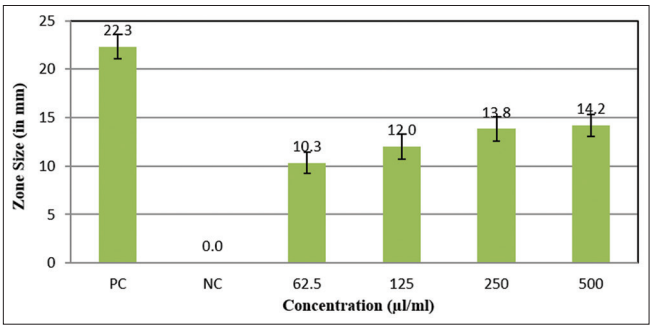


Figure 2: Antifungal activity of *Curcuma longa (turmeric)* oil against *Candida albicans*

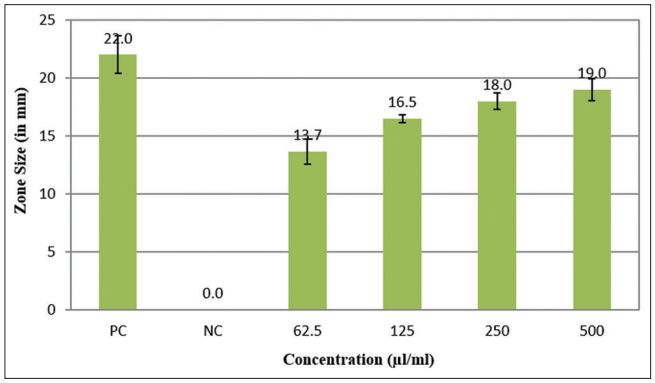


Figure 3: Antifungal activity of *Melaleuca alternifolia* (tea tree) oil and *Curcuma longa* (turmeric) oil (1:1) against *Candida albicans*

Detection of antifungal effect of oil combinations

The combined antifungal activity of *M. alternifolia* oil (TTO) and *C. longa* oil (TO) in a 1:1 ratio was evaluated against *Candida* species using the agar well diffusion method. The combination exhibited a clear progressive increase in the inhibition zone with increasing concentration of oil. The average zones of inhibition increased from 13.7 ± 2, 16.5 ± 1, 18.0 ± 1, 19.0 ± 1 mm at 62.5, 125, 250, and 500 µL/ml, respectively. The negative control showed no inhibition (0.0 ± 0 mm), confirming the absence of antifungal activity in the negative control. Amphotericin B AP (20 µg), used as the positive control, produced an average inhibition zone of

22.0 ± 2 mm. Thus, the TTO-TO combination demonstrated substantial antifungal activity, with the highest concentration producing an inhibition zone close to that of amphotericin B. The enhanced activity of the combination may be associated with the complementary antifungal effects of terpinen-4-ol and other bioactive constituents of *M. alternifolia* oil and turmerones and related compounds present in *C. longa* oil. The results presented in Figure 3 and Table 3 indicate that the combination of the two oils may provide enhanced antifungal activity compared with their individual application.

Determination of MIC

The MICs of *M. alternifolia* oil (TTO), *C. longa* oil (TO), and their 1:1 combination against *C. albicans* were determined using the broth microdilution method. Because the tested oils are hydrophobic, a DMSO-Tween 80 vehicle system was used to facilitate their dispersion in the aqueous culture medium. For preparation of the initial test preparation, 500 µL of undiluted oil was dispersed in 10 µL of DMSO and 5 µL of Tween 80, followed by addition of Sabouraud dextrose broth to a final volume of 1 mL. This preparation corresponded to an oil concentration of 500 µL/mL, with DMSO and Tween 80 present at 1% and 0.5% (v/v), respectively. The preparation was thoroughly mixed to obtain a uniform dispersion. The oil preparations were subsequently subjected to two-fold serial dilution (v/v) to obtain final oil concentrations of 500, 250, 125, 62.5, 31.25, 15.63, 7.81, 3.91, and 1.95 µL/mL. In a two-fold serial dilution, each successive concentration represents one-half of the preceding concentration. Appropriate growth, sterility, and negative controls were included. The percentage inhibition of fungal growth was calculated relative to the growth control. *M. alternifolia* oil demonstrated a progressive increase in antifungal activity with increasing oil concentration. Growth inhibition was 23.5%, 56.1%, and 76.4% at 1.95, 3.91, and 7.81 µL/mL, respectively. Complete inhibition of fungal growth (100%) was observed at 15.63 µL/mL and at all higher concentrations tested. Therefore, the MIC of *M. alternifolia* oil was 15.63 µL/mL. *C. longa* oil also exhibited increasing antifungal activity with increasing oil concentration. Growth inhibition was 18.7%, 45.8%, 83.7%, and 94.3% at 1.95, 3.91, 7.81, and 15.63 µL/mL, respectively. Complete inhibition (100%) was observed at 31.25 µL/mL and at all higher concentrations tested. Accordingly, the MIC of *C. longa* oil was 31.25 µL/mL. The lower MIC of *M. alternifolia* oil compared with *C. longa* oil indicates greater antifungal activity of *M. alternifolia* oil under the conditions of the present assay. The antifungal activity of the 1:1 combination of *M. alternifolia* oil and *C. longa* oil was also evaluated using the broth microdilution method. The combination produced 43.2% and 92.8% inhibition at 1.95 and 3.91 µL/mL, respectively. Complete inhibition of fungal growth (100%) was observed at 7.81 µL/mL and at all higher concentrations tested. Therefore, the MIC of the 1:1 *M. alternifolia* oil – *C. longa* oil combination was 7.81 µL/mL. For the combination, the reported concentration represents the total concentration of the combined oils, with *M. alternifolia* oil and *C. longa* oil present in an equal ratio. The growth control showed 100% fungal growth, whereas amphotericin B with 1µg/mL concentration produced complete inhibition (100%), confirming the expected responses of the growth and positive controls, respectively. The 1:1 TTO–TO combination exhibited a lower MIC (7.81 µL/mL) than either *M. alternifolia* oil (15.63 µL/mL) or *C. longa* oil (31.25 µL/mL)

Table 1: Antifungal activity of *Melaleuca alternifolia* (*tea tree*) oil against *Candida albicans*

Concentration (µl/ml)	Inhibition zone (mm) plate A	Inhibition zone (mm) plate B	Inhibition zone (mm) plate C	Average zone of inhibition
Negative control	0	0	0	0.0±0
62.5	13	12	11.5	12.2±1
125	12	13.5	15	13.5±2
250	15.5	16	17	16.2±1
500	17.5	18	19	18.2±1
Amphotericin-B AP 20 µg (PC)	21	23.5	20.5	21.7±2

Table 2: Antifungal activity of *Curcuma longa* (*turmeric*) oil against *Candida albicans*

Concentration (µl/ml)	Inhibition zone (mm) plate A	Inhibition zone (mm) plate B	Inhibition zone (mm) plate C	Average zone of inhibition
Negative control	0	0	0	0.0±0
62.5	9	10	12	10.3±2
125	10	12.5	13.5	12.0±2
250	12	14	15.5	13.8±2
500	13	13.5	16	14.2±2
Amphotericin B AP 20 µg (PC)	20.5	22.5	24	22.3±2

Table 3: Antifungal activity of *Melaleuca alternifolia* (tea tree) oil and *Curcuma longa* (turmeric) oil (1:1) against *Candida albicans*

Concentration (µl/ml)	Inhibition zone (mm) plate A	Inhibition zone (mm) plate B	Inhibition zone (mm) plate C	Average zone of inhibition
Negative control	0	0	0	0.0±0
62.5	12	14	14.5	13.7±2
125	16.5	16	17	16.5±1
250	17	18	19	18.0±1
500	17.5	19.5	20	19.0±1
Amphotericin-B AP 20 µg (PC)	24.5	20	21.5	22.0±2

Table 4: Minimum inhibitory concentration (MIC) of individual *Melaleuca alternifolia* (tea tree) oil and *Curcuma longa* (turmeric) oil against *Candida albicans*

S. No.	Tea tree oil concentration (µL/mL)	% Organism growth	Calculated % growth inhibited	Turmeric oil concentration (µL/mL)	Organism growth	Calculated % growth inhibited
1	Negative control (0)	100.00	0.00	Negative control (0)	100.00	0.00
2	1.95	76.50	23.50	1.95	81.30	18.70
3	3.91	43.90	56.10	3.91	54.20	45.80
4	7.81	23.60	76.40	7.81	16.30	83.70
5	15.63	0.00	100.00	15.63	5.70	94.30
6	31.25	0.00	100.00	31.25	0.00	100.00
7	62.5	0.00	100.00	62.5	0.00	100.00
8	125	0.00	100.00	125	0.00	100.00
9	250	0.00	100.00	250	0.00	100.00
10	500	0.00	100.00	500	0.00	100.00
11	Amp-B 1 µg/mL (PC)	0.00	100.00	Amp-B 1 µg/mL (PC)	0.00	100.00

Table 5: Minimum inhibitory concentration of combined *Melaleuca alternifolia* (tea tree) oil and *Curcuma longa (turmeric)* oil (1:1) against *Candida albicans*

S. No.	Tea tree oil and turmeric oil combined concentration (1:1) (µL/mL)	% Organism growth	Calculated % growth inhibited
1	Negative control (0)	100.00	0.00
2	1.95	56.80	43.2
3	3.91	7.20	92.8
4	7.81	0.00	100.00
5	15.63	0.00	100.00
6	31.25	0.00	100.00
7	62.5	0.00	100.00
8	125	0.00	100.00
9	250	0.00	100.00
10	500	0.00	100.00
11	Amp-B 1 µg/mL (PC)	0.00	100.00

Table 6: Comparative antifungal activity of individual oils and combination

Treatment group	Zone of inhibition (mm)
Melaleuca alternifolia oil	18.2±1
Curcuma longa	14.2±2
Melaleuca alternifolia oil and *Curcuma longa*	19.0±1
Amphotericin-B AP 20 mcg (PC)	22.3±2

Table 7: Comparative MIC determination of individual oils and combination

Treatment group	MIC (µL/mL)
Melaleuca alternifolia oil	15.63
Curcuma longa oil	31.25
Melaleuca alternifolia oil and *Curcuma longa* oil	7.81
Amp-B 1 µg/mL (PC)	1 µg/mL

MIC: Minimum inhibitory concentrations

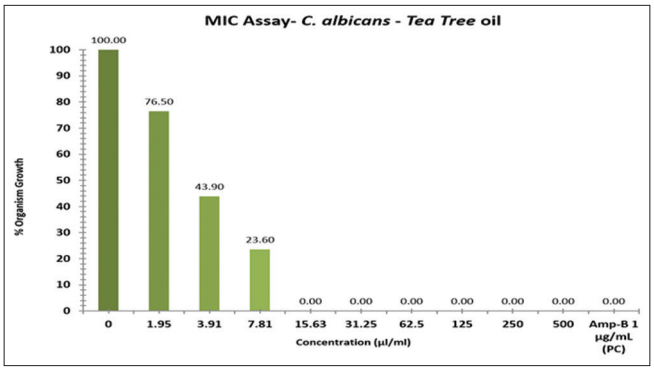


Figure 4: Minimum inhibitory concentration of *Melaleuca alternifolia (tea tree)* oil against *Candida albicans*

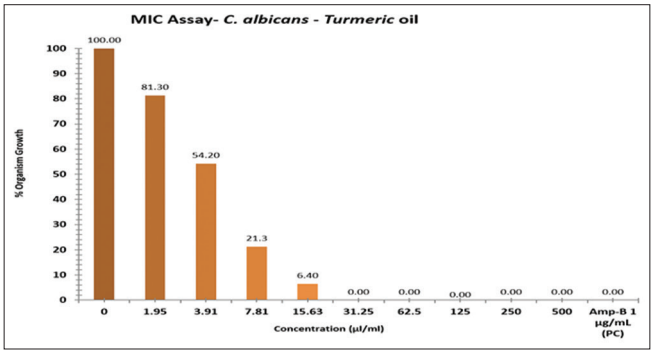


Figure 5: Minimum inhibitory concentration of individual *Curcuma longa* (turmeric) oil against *Candida albicans*

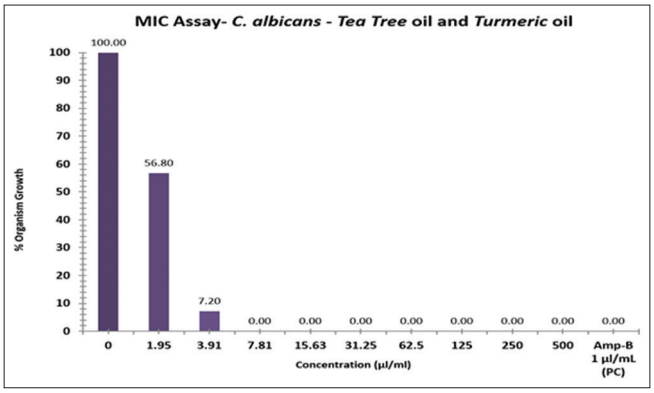


Figure 6: Minimum inhibitory concentration value of the oil combination in 1:1

tested individually, indicating greater inhibitory activity of the combination under the conditions evaluated. The MIC results presented in Tables 4, 5 and Figures 4-6 demonstrate the concentration-dependent inhibitory activity of the tested oils against Candida albicans. The combined treatment exhibited enhanced inhibitory activity, as evidenced by a lower MIC compared with the individual oils..

Comparative evaluation with standard antifungal drug

The antifungal efficacy of the oils and their combined formulations was compared with that of the standard antifungal agent, Amphotericin B. The standard drug exhibited strong and consistent antifungal activity, producing well-defined zones of inhibition across all experiments. Among the tested oils, *M. alternifolia* oil and *C. longa* oil in combination showed antifungal activity closest to that of Amphotericin B, particularly at higher concentrations. The tested oil combination, *M. alternifolia* oil and *C. longa* oil, produced measurable zones of inhibition under the experimental conditions. The findings presented in Tables 6 and 7 indicate enhanced antifungal activity of the oil combination, supporting its potential as a natural antifungal agent.

CONCLUSION

The present study provides evidence of the antifungal potential of *M. alternifolia* (TTO) and *C. longa* (TO) against *C. albicans* (MTCC 3958), with particular emphasis on the comparative activity of the individual oils and their 1:1 combination. The agar well diffusion results demonstrated that both oils exhibited measurable and concentration-dependent antifungal activity. *M. alternifolia* oil consistently showed greater inhibitory activity than *C. longa* oil at all tested volumes. At 62.5, 125, 250, and 500 µL/ml per well, *M. alternifolia* produced inhibition zones of 12.2 ± 1, 13.5 ± 2, 16.2 ± 1, and 18.2 ± 1 mm, respectively, whereas *C. longa* produced corresponding inhibition zones of 10.3 ± 2, 12.0 ± 2, 13.8 ± 2, and 14.2 ± 2 mm. These findings indicate a progressive increase in antifungal activity with increasing oil concentration and demonstrate the comparatively stronger activity of TTO under the experimental conditions of the study. The broth microdilution assay further supported the antifungal activity observed in the agar diffusion study. *M. alternifolia* oil exhibited increasing inhibition with increasing concentration, producing 23.5%, 56.1%, and 76.4% growth inhibition at 1.95, 3.91, and 7.81 µL/mL, respectively, followed by complete inhibition of fungal growth at 15.63 µL/mL and higher concentrations. Accordingly, the MIC of *M. alternifolia* oil was determined to be 15.63 µL/mL. In comparison, *C. longa* oil showed 18.7%, 45.8%, 83.7%, and 94.3% inhibition at 1.95, 3.91, 7.81, and 15.63 µL/mL, respectively, with complete inhibition observed at 31.25 µL/mL and above. Thus, the MIC data further established the greater antifungal potency of *M. alternifolia* oil compared with *C. longa* oil. A particularly important finding of the study was the enhanced antifungal activity observed when the two oils were combined in a 1:1 ratio. In the agar well diffusion assay, the combination produced inhibition zones of 13.7 ± 2, 16.5 ± 1, 18.0 ± 1, and 19.0 ± 1 mm at 62.5, 125, 250, and 500 µL/ml, respectively. The inhibition zone obtained at 500 µL/ml was close to that produced by the amphotericin B positive control (22.0 ± 2 mm in the combination experiment), demonstrating substantial antifungal activity of the combined oils. More importantly, the broth microdilution results showed a marked improvement in inhibitory potency. The 1:1 combination produced 43.2%

inhibition at 1.95 µL/mL and 92.8% inhibition at 3.91 µL/mL, whereas complete inhibition of fungal growth was achieved at 7.81 µL/mL and all higher concentrations. Therefore, the MIC of the combined oils was 7.81 µL/mL, which was lower than the MIC values of both individual oils, i.e., 15.63 µL/mL for *M. alternifolia* and 31.25 µL/mL for *C. longa*. This finding indicates enhanced inhibitory activity of the combination under the conditions evaluated. The comparative evaluation with amphotericin B further demonstrated that the standard antifungal drug remained more potent than the tested oils. Amphotericin B produced an inhibition zone of 22.3 ± 2 mm and showed complete inhibition at 1 µg/mL in the MIC assay. Nevertheless, the 1:1 oil combination demonstrated considerable antifungal activity, particularly at higher concentrations, and showed a substantially lower MIC than either oil individually. The enhanced activity of the combination may be associated with the complementary effects of the bioactive constituents present in tea tree and TOs; however, the present study does not establish a specific synergistic mechanism, and such an interpretation requires dedicated combination and mechanistic studies. Overall, the findings demonstrate that both *M. alternifolia* and *C. longa* oils possess promising *in vitro* antifungal activity against *C. albicans*, with *M. alternifolia* showing superior activity among the individual oils. Most importantly, their 1:1 combination improved the antifungal response and reduced the MIC to 7.81 µL/mL, highlighting the potential advantage of combining plant-derived oils rather than relying solely on either oil individually. The results provide a useful experimental basis for further development of natural oil-based antifungal preparations. However, the present findings are limited to *in vitro* evaluation against *C. albicans* under the specified experimental conditions. Further studies involving detailed phytochemical characterization, assessment of the interaction between the oils, mechanism-of-action investigations, formulation development and optimization, safety and toxicity evaluation, and *in vivo* efficacy studies are required to confirm the therapeutic potential and establish the applicability of this oil combination as a plant-based antifungal formulation.

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