Ibn Al-Haitham Journal for Pure and Applied Sciences
Journal homepage: jih.uobaghdad.edu.iq
PISSN: 1609-4042, EISSN: 2521-3407
IHJPAS. 2026; 39(1)

Evaluation of the Inhibitory Activity of Tea Tree Leaves Essential Oils Against *Cryptococcus Neoformans* and *Candida Tropicalis*

Sajad Hyder Mohammed[1*] and Ibrahim Jabber Abed [2]
[1,2]Department of Biology, College of science, University of Baghdad, Baghdad, Iraq
*Corresponding Author.

Received: 24/July/2025
Accepted:30/November/2025
Published: 20/January/2026
doi.org/10.30526/39.1.4259

Abstract

Tea tree oil, extracted from the leaves of *Melaleuca alternifolia*, is known for its antibacterial and antioxidant properties. This study aimed to investigate its chemical composition, antifungal activity against *Candida tropicalis* and *Cryptococcus neoformans*, and free radical scavenging capacity. Evaluating the inhibitory activity of tea tree essential oil . Essential oil was obtained via steam distillation from air-dried leaves. Chemical constituents were analyzed using Gas chromatography-mass spectrum analysis. Antifungal activity was assessed by agar dilution assays at concentrations of 200–2,000 µg/ml. Antioxidant activity was determined using the DPPH assay within the 12.5–200 µL range. GC–MS analysis identified terpinen-4-ol (18.3%), γ-terpinene (11.2%), α-pinene (7.2%), terpinolene (4.0%), sabinene (3.2%), and camphene (1.1%) as major components. Complete inhibition of both yeast species occurred at 500 ppm, establishing the minimum inhibitory concentration. Determination of antioxidant activity by 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) free radical scavenging assay revealed a dose-dependent increase in radical scavenging, with 50% inhibition achieved at approximately 22 µL of oil. Tea tree oil rich in terpinen-4-ol exhibits moderate antifungal activity and notable antioxidant potential. These findings support its potential use as a natural preservative or adjunct therapeutic agent. Further work is required to optimize formulations and evaluate safety for clinical and industrial applications.

Keywords: *Cryptococcus neoformans, Candida tropicalis,* essential oil , *Melaleuca alternifolia*.

1. Introduction

Cryptococcus neoformans is an encapsulated opportunistic yeast that primarily causes life-threatening infections such as meningitis in immunocompromised individuals, especially people living with HIV. It has evolved virulence factors like a polysaccharide capsule and melanin production that help it evade host immunity and persist in the body[1]. On the other hand, *Candida tropicalis* is a non-albicans *Candida* species increasingly implicated in invasive candidiasis, particularly in hospitalized or immunosuppressed patients[2]. This species is notable for its capacity to form biofilms and develop antifungal resistance, making infections difficult to treat [2]. *Melaleuca alternifolia*, which is often known as tea tree, has been used for medicinal purposes. However, this informal name is also used for other species in the Leptospermum and Melaleuca genera. A small tree that grows to a height of up to 5 meters and has papery bark and thin, tapering leaves that can be up to 20 millimeters long, it blooms in the summer. Tea tree oil, or *Melaleuca alternifolia*, has become more popular as a medicine in the last few years. It is a pale, yellow, and thick liquid with a strong smell that is made up of a mix of monoterpenes, 1-terpinen- 4-ol, cineole, and other hydrocarbons. People know that tea tree oil (TTO) kills bacteria, stops infections, and kills germs[3-6]. Tea tree oil (TTO), derived from the leaves

of *Melaleuca alternifolia*, has been extensively researched for its antifungal efficacy. Antifungal activity is often measured by the minimum inhibitory concentration (MIC) the lowest concentration that inhibits apparent growth, and the minimum fungicidal concentration (MFC)—the lowest concentration that eradicates ≥ 99.9% of the inoculum[7]. Besides the entire oil, terpinen-4-ol, its primary active constituent, is frequently assessed independently due to its significant role in antifungal effectiveness and its application as a benchmark for tea tree oil quality management [8-10]. These properties are primarily due to thymol, terpinen-4-ol, and sabinene found within it. *Melaleuca alternifolia*, also known as tea tree, is the most renowned of the species belonging to the Myrtaceae family, which is native to Australia [11-13]. Tea tree oil primarily consists of monoterpenes and monoterpenoid alcohols; specifically, a limited group of eight constituents—predominantly terpinen-4-ol, γ-terpinene, α-terpinene, 1,8-cineole, terpinolene, p-cymene, α-pinene, and α-terpineol—can comprise approximately 90% of the oil, although the precise composition differs based on chemotype and source. Camphor is often not a predominant component in Melaleuca alternifolia oil according to ISO-compliant evaluations. The oil's lipophilic terpenes integrate into microbial membranes, enhancing permeability, inducing K^+/H^+ leakage, and dissipating the proton motive force, which disrupts respiration and diminishes the functional activity of the F_1F_0-ATPase. This pattern aligns with membrane-targeted lethality and a reduced likelihood of developing classical antibiotic resistance; certain terpenes may also inhibit efflux pumps [14-16]. Direct exposure to pure tea tree oil has been associated with allergic reactions and irritations in some people. When applied topically, tea tree oil is generally considered safe, although side effects may include rashes, redness, burning sensations, itching, and stinging. Injuries to the mucosa may occur when tea tree oil is applied in an undiluted state. Therefore, diluted tea tree oil products are advised for application in sensitive areas such as the face, vagina, or around the eyes. The systemic absorption of tea tree oil is noted following topical applications and is dose-dependent. In children, tea tree oil poisoning has been related to the inappropriate use of tea tree oil products, accidental oral ingestion, or an excessive amount of tea tree oil that was applied topically. In children, tea tree oil poisoning should raise suspicion for preexisting exposure to erogenous hormones or plant extracts with estrogenic properties as well as developmental concerns regarding the adequacy of the educational level of the caregiver [17, 18]. The aim of the current research is to evaluate the efficacy of essential oils from tea tree plants as anti-pathogenic yeasts against the viability of two strains, *Candida tropicalis* (Reference Number: ATCC 66029) and *Cryptococcus neoformans* (Reference Number: ATCC14116). in addition to their efficacy as antioxidants through DPPH radical scavenging.

2. Materials and method

2.1. Collection and extraction of tea tree plant

Melaleuca alternifolia, widely referred to as tea tree, was gathered from the gardens of Baghdad University in March and April of 2024. Subsequent to collecting, the plants were sanitized, rinsed with tap water, air-dried at ambient temperature, and thereafter stored under hygienic conditions until utilization. The essential oils from these plants were extracted from the desiccated leaves of the tea tree. 250 grams of the ground leaves were then placed in a Clevenger (steam distillation apparatus) to obtain the essential oil of the tea tree. The plant material with distilled water (1.2L) was cooked for three hours; the essential oil was stored at 4°C until utilized. [19,20] . To create the stock solution, the concentrated oil extract was combined with Dimethyl sulfoxide (DMSO) and diluted. Subsequently, various concentrations (200-2000 µg/ml) were prepared by mixing specific volumes of the stock solution with specific volumes of DMSO.

2.2.Yeast used in the study

Fungal isolates obtained from the American company Microbiologics, *Candida tropicalis* (Reference Number: ATCC 66029) and *Cryptococcus neoformans* (Reference Number:

ATCC14116), were cultured using the spread plate technique on Sabouraud Dextrose Agar (SDA). The culture plates were prepared using sterile Petri dishes containing freshly poured SDA medium under aseptic conditions.

2.3. Antifungal assays

Evaluation of Minimum Inhibitory Concentration by Agar Dilution Method: The agar dilution technique was used to find the Minimum Inhibitory Concentration (MIC) of the essential oils. We produced the agar plates by adding different amounts of plant essential oils (200 to 2000 µg/ml) to Sabouraud Dextrose agar (SDA) (20 ml per petri dish). A cell suspension of *Candida tropicalis* (ATCC 66029) and *Cryptococcus neoformans* (ATCC 14116) was put on the plates. At 32.5°C for 48 hours, all plates were incubated three times at each dose. The control group consisted of plates that did not have any plant essential oil. Fungal growth on the plates was noted every 24 hours over a 48-hour incubation period. The MIC values were determined as the minimal concentration of essential oil that inhibits the visible growth of *Candida tropicalis* and *Cryptococcus neoformans* [21].

2.4. Quantitative and qualitative estimation of chemical components in tea tree essential oils

The Chemical Analysis Center in Iraq scientific laboratories conducted Gas Chromatography-Mass (GC-MS) analysis on *Melaleuca alternifolia* essential oil using a Shimadzu gas chromatograph. The procedure for this analysis was performed in the same manner as outlined by [20]. The DB-5 capillary column, measuring 30 meters in length with an inner diameter of 0.2 mm and a thickness of 0.25µm, was the type of column employed. The GC apparatus received an injection of two microliters of plant samples. The GC apparatus was initially set to a temperature of 60°C for a duration of four minutes. It was then raised to 150°C for another four minutes before being further increased to 250°C. The sample material was transported from the injector to the detector by the carrier gas, helium, with a flow ratio of 1.35 ml/min. The mass spectrometry (MS) technique utilized the electron impact (EI) mode [22].

2.5. Antioxidant Activity Solutions

These solutions were formulated in accordance with [22].

Mixture of Methanol and DMSO (9:1 v/v) Solution: A methanol-DMSO mixture (9:1 v/v) was prepared by mixing 9 volumes of methanol with 1 volume of DMSO.

DPPH Solution: To make it, 0.01g of DPPH radical was dissolved in a combination of methanol and DMSO in a 9:1 (v/v) ratio. Ascorbic acid Solution: To make it, 0.01g of ascorbic acid was dissolved in a mixture of methanol and DMSO at a 9:1 (v/v) ratio.

Antioxidant Activity protocol:

The antioxidant properties of several isolated components from the crude extract of *Melaleuca alternifolia* leaves were assessed using the DPPH radical scavenging assay, following the outlined technique [23], as follows:

1. Twofold serial dilutions of Cymbopogon flexuosus crude extracts and ascorbic acid (12.5, 25, 50, and 100 µg/ml) were added to the test tubes in 0.5 ml aliquots.
2. At the same time, 3 ml of a methanol-DMSO combination and 0.3 ml of a DPPH solution were added to each concentration.
3. The samples were kept at 37ºC for an hour.
4. The ELISA reader was used to measure the samples' ability to scavenge radicals against the stable DPPH radical. When DPPH reduction was detected at 517 nm, the colorimetric altered from deep violet to pale yellow.
5. The algorithm below was used to figure out the percentage of radical that the samples stopped:

$$\text{Inhibition}\% = \frac{\text{Absorbance of – ve control} - \text{Absorbance of sample}}{\text{Absorbance of – ve control}} \times 100 \quad \textbf{(1)}$$

The negative control consisted of methanol-DMSO mixture and DPPH solution, while ascorbic acid was used as a reference.

2.6.Replicates and statistical analysis

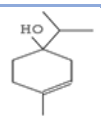
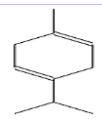
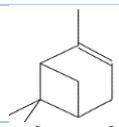
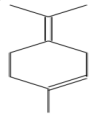
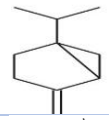
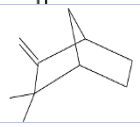
All antifungal and DPPH experiments were conducted in triplicate over three separate days (n = 9 per concentration). Data are presented as mean ± standard deviation. Statistical significance across treatment groups was evaluated using one-way ANOVA, followed by Tukey's post hoc test, with $p < 0.05$ deemed significant.

3. Results

3.1. GC-MS

Six principal components were discovered in the steam-distilled essential oil of *Melaleuca alternifolia* via GC-MS. **Table 1** indicates that the primary components were terpinen-4-ol (18.34%), γ-terpinene (11.24%), α-pinene (7.15%), terpinolene (3.97%), sabinene (3.21%), and camphene (1.09%).

Table 1. Main Compounds present in the oil extract of *Melaleuca alternifolia* using GC-MS analysis.

NO.	Main Components	Area%	Structures
1.	Terpinen-4-ol	18.34	
2.	gamma-Terpinen	11.24	
3.	alpha-Pinene	7.15	
4.	Terpinolene	3.97	
5.	Sabinen	3.21	
6.	Camphene	1.09	

3.2.The antifungal activity

The efficacy of *Melaleuca alternifolia* (tea tree oil) against *C. tropicalis* and *C. neoformans*, as shown in **Table 2**, was evaluated using an agar dilution method throughout a concentration spectrum of 200–2000 µg/ml. At 200 µg/ml, both organisms exhibited normal growth, as evidenced by observed turbidity. At 250 µg/ml, partial suppression occurred; however, growth remained detectable. Complete inhibition (minimum inhibitory concentration, MIC) for both *C. tropicalis* and *C. neoformans* was seen at 500 µg/ml, as evidenced by the absence of turbidity (–) in all replicate wells. No regrowth was seen at elevated concentrations (550–2000 µg/ml), so demonstrating that 500 µg/ml is the minimum inhibitory concentration (MIC) for both species. **Figures 1** and **2**.

Table 2. the concentration of *Melaleuca alternifolia* the *C. tropicalis* and *Cryptococcus neoformans*.

Concentration(µg/ml)	*Candida tropicalis*	*Cryptococcus neoformans*
200	+	+
	+	+
	+	+
250	+	(MIC)
	+	(MIC)
	+	(MIC)
500	(MIC)	-
	(MIC)	-
	(MIC)	-
550	-	-
	-	-
	-	-
600	-	-
	-	-
	-	-
625	-	-
	-	-
	-	-
1000	-	-
	-	-
	-	-
2000	-	-
	-	-
	-	-

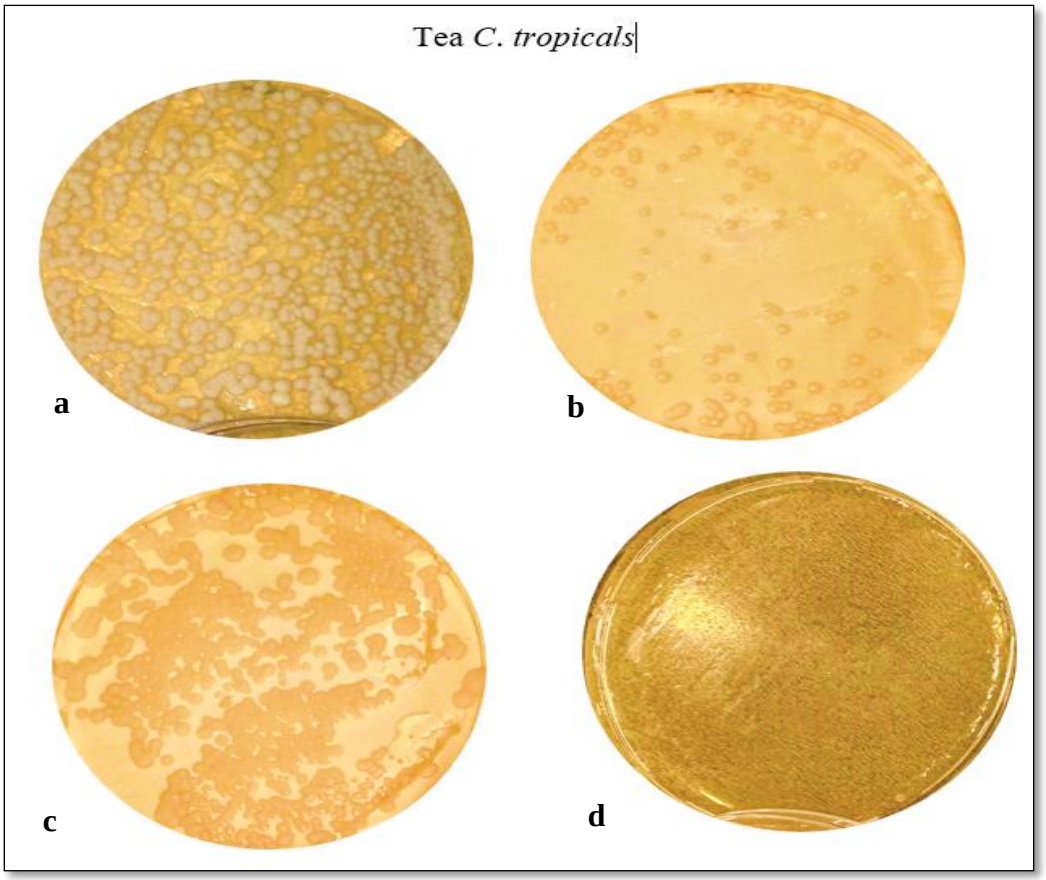


Figure 1. Antifungal effects of tea tree essential oil in different concentrations on *Candida tropicalis.*
a : control ; b : 200 µg/ml ; c : 500 µg/ml ; d : 2000 µg/ml

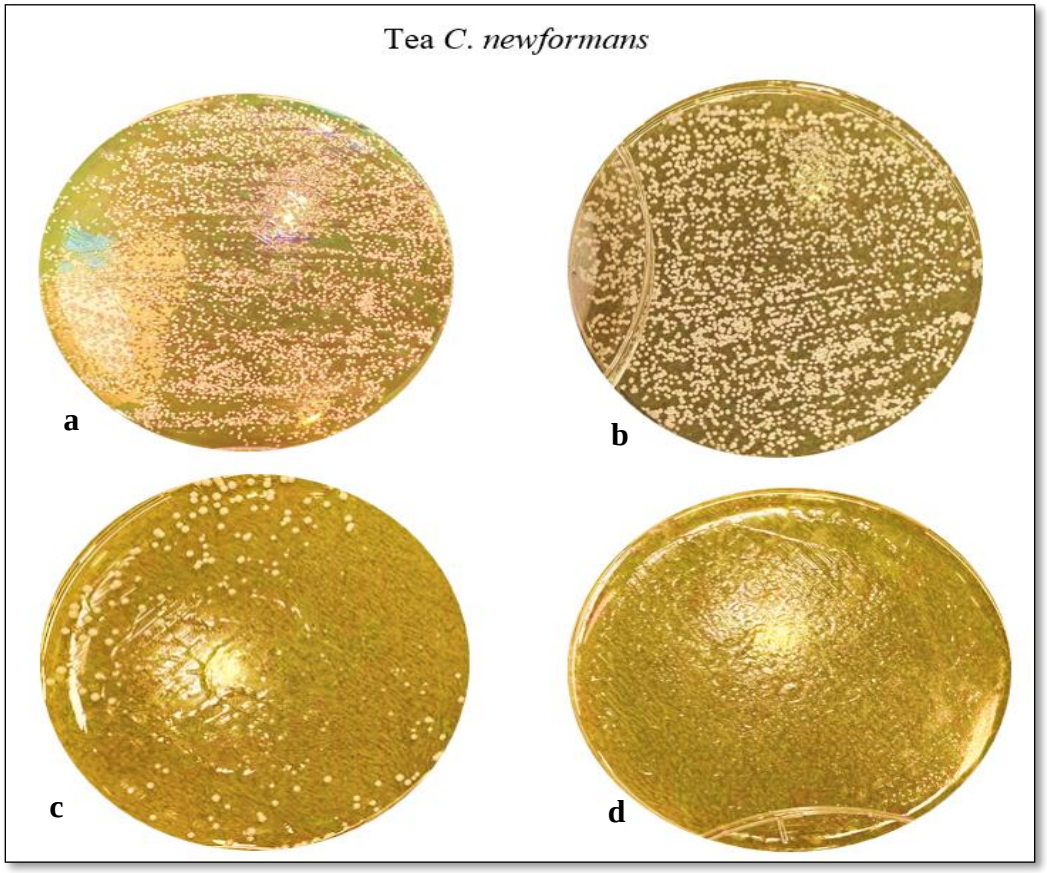


Figure 2. Antifungal effects of tea tree essential oil in different concentrations on *Cryptococcus neoformans.* a : control ; b : 200 µg/ml ; c : 250 µg/ml ; d : 2000 µg/ml

3.3. The Antioxidant activity of tea tree

The free-radical scavenging activity of Melaleuca alternifolia (tea tree oil) was compared to ascorbic acid using the DPPH assay over a concentration range of 12.5–200 µL. All values are presented as mean ± SD, as in **Table 3**.

Table 3. Antioxidant activity of tea tree using DPPH free radical scavenging assay

conc(µl)	Ascorbic acid		Tea tree	
	mean	SD	mean	SD
200	85.64833	1.517818	77.16067	2.601527
100	79.591	0.770839	71.10333	1.771683
50	63.00167	1.159286	62.38433	2.865219
25	54.28233	1.632872	52.31467	2.55387
12.5	40.278	0.530597	40.66367	2.445124

4. Discussion

Terpinen-4-ol comprised 18.34% of the oil, establishing it as the principal component. This is slightly lower than the 30–40% generally documented for commercial tea tree oils, although it falls within the range (15–25%) observed for wild-grown Australian chemotypes under comparable distillation circumstances[24, 25]. Given that terpinen-4-ol predominantly contributes to the oil's antibacterial properties, its relative concentration indicates a moderate potential for bioactivity in the sample examined in this investigation. γ-Terpinene (11.24%) and α-Pinene (7.15%) are acknowledged precursors in the biosynthetic pathway of terpinen-4-ol. Their aggregate proportion (≈18.4%) signifies active monoterpene oxidation pathways in this plant material [25]. Terpinolene (3.97%) and sabinene (3.21%) have been associated with insect-

repellent characteristics in other Myrtaceae species, indicating possible supplementary applications. Camphene (1.09%), although a minor component, can influence the distinctive scent profile and may exhibit synergistic effects in antioxidant assays [26]. The variability in the relative percentages, particularly of terpinen-4-ol and γ-terpinene, frequently indicates disparities in geographical origin, harvest timing, and drying methods [27].

C. neoformans was entirely inhibited at 250 ppm, but *C. tropicalis* necessitated 500 ppm for complete inhibition, demonstrating an approximate twofold disparity in sensitivity under the same assay conditions. A study by [28] documented minimum inhibitory concentrations (MICs) of 0.03–0.2% (300–2000 ppm) for *C. neoformans* and 0.06–0.4% for other *Candida* species, with *C. tropicalis* frequently positioned at the upper limit of this spectrum. A study that evaluated tea tree oil against oral *Candida* spp. (including *C. tropicalis*) and found MICs between 250 and 500 µg/mL, with the majority of isolates suppressed at 500 µg/mL [29]. A comprehensive test of 82 essential oils revealed that *C. neoformans* was one of the most vulnerable species, with more than one-third of the oils (including tea tree oil) demonstrating MICs < 160 µg/ml [30]. These concordant results confirm that our 500 µg/ml MIC is well within the expected susceptibility window for these opportunistic yeasts. Recent mechanistic studies have identified terpinen-4-ol, the primary component of tea tree oil, as the key antifungal agent. It was employed flow cytometry and electron microscopy to demonstrate that terpinen-4-ol swiftly enhances fungal cell membrane permeability, resulting in cytoplasmic leakage and cell mortality; analogous effects were noted for both *Candida* spp. and *C. neoformans* [31]. These findings support a conserved, membrane-targeting mode of action across diverse yeast pathogens.

It was documented an EC_{50} of 2323.8 mg/L for crude tea tree oil in a DPPH assay, much higher (i.e., less active) than α-tocopherol or terpinen-4-ol alone (EC_{50} = 9.16 and 480.56 mg/L, respectively) [32]. Although units differ (µL vs. mg/L), their work confirms that whole tea tree oil is moderately active, in line with our finding that it achieves 50% scavenging at ~22 µL per assay well. The antioxidant properties of tea tree oil are primarily due to its substantial terpinen-4-ol concentration (generally 30–50% of the oil), supplemented by the synergistic effects of α-terpineol and 1,8-cineole [33]. Terpinen-4-ol Nano emulsions have demonstrated a reduction in EC_{50} values by fifty percent compared to bulk oil, suggesting that formulation may augment efficacy[34].

5. Conclusion

This study emphasizes the dual bioactivity of tea tree oil: a strong antifungal action against opportunistic yeasts at 500 µg/ml and notable antioxidant capacity through DPPH radical scavenging. The prevalence of terpinen-4-ol highlights its essential function in both fungistatic and free-radical neutralization processes. These findings support the integration of tea tree oil in antifungal treatments and as a natural antioxidant component; however, formulation approaches to improve stability and reduce irritation should be investigated. The study collectively endorses the formulation of terpinen-4-ol standardized formulations for medicinal and preservation purposes.

Funding

No funding was received for this study.

Ethical clearance

Ethics approval and consent to participate: Not applicable. This study did not involve human participants or animals.

Consent for publication: Not applicable.

Acknowledgements

The author thanks the staff of the Chemical Analysis Center, Iraq for assistance with GC-MS analysis.

Reference

1. Zhao Y, Ye L, Zhao F, Zhang L, Lu Z, Chu T, Wang S, Liu Z, Sun Y, Chen M, Liao G, Ding C, Xu Y, Liao W, Wang L. *Cryptococcus neoformans*, a global threat to human health. Infect Dis Poverty. 2023;12(1):20. https://doi.org/10.1186/s40249-023-01073-4.
2. Amorim-Vaz S, Sanglard D. *Candida* tropicalis in human disease. Crit Rev Microbiol. 2021;47(3):283–300.
3. Ali H, Abed I, Augul R, Fadhil H. Insecticidal activity of *Eucalyptus* sp. volatile oil against *Anisops sardea*. Iraqi J Agric Sci. 2020;51(1):470–82. https://doi.org/10.36103/ijas.v51i1.946
4. Kairey L, Agnew T, Bowles EJ, Barkla BJ, Wardle J, Lauche R. Efficacy and safety of *Melaleuca alternifolia* oil: a systematic review. Front Pharmacol. 2023;14:1116077. https://doi.org/10.3389/fphar.2023.1116077
5. Bugarcic A, Bowles EJ, Summer K, Agnew T, Barkla B, Lauche R. Australian tea tree oil: updated review of antimicrobial properties. Phytomedicine Plus. 2025;100846. https://doi.org/10.1016/j.phyplu.2025.100846
6. Fatin H. Al-Dulaimi; Morphological and chemical study, with historical importance of *Cucurbita pepo*. AIP Conf. Proc. 2023; 2839 (1): 070002. https://doi.org/10.1063/5.0167785
7. Ward C, Edwards A, Flores Z, Morris J, Bell L, Hughes D, Cox A, Rogers C, Emmanuel M. Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of selected essential oils on cocoa fungi. 2025.
8. Francisconi RS, Bordini EAF, Nogueira MNM, Fontana A, Bedran TBL, Correia MF. Effect of Melaleuca alternifolia and its components on *Candida albicans* and *Candida tropicalis*. J US-China Med Sci. 2015;12:91–98. https://doi.org/10.17265/1548-6648/2015.03.001.
9. Dontje AE, Schuiling-Veninga CC, van Hunsel FP, Ekhart C, Demirci F, Woerdenbag HJ. The Therapeutic Potential of Essential Oils in Managing Inflammatory Skin Conditions: A Scoping Review. Pharmaceuticals. 2024;17(5):571. https://doi.org/10.3390/ph17050571.
10. Upadhyay P, Malik P, Upadhyay S. Tea tree (*Melaleuca alternifolia*) Essential Oil Concentration in Microemulsion with Antibacterial and Antifungal Activity: An Overview. Curr Drug Ther. 2023;18(4):298–311. https://doi.org/10.2174/1574885518666230228103854.
11. Lam NS, Long X, Su XZ, Lu F. Melaleuca alternifolia (tea tree) oil and its monoterpene constituents in treating protozoan and helminthic infections. Biomed Pharmacother. 2020;130:110624.https://doi.org/10.1016/j.biopha.2020.110624
12. Nascimento T, Gomes D, Simões R, da Graça Miguel M. Tea tree oil for acne: a review. Antioxidants. 2023;12(6):1264. https://doi.org/10.3390/antiox12061264
13. Linge KL, Cooper L, Downey A. Authentication of terpinen-4-ol-type tea tree oils using chiral GC/MS. J Agric Food Chem. 2024;72(15):8389–400. https://doi.org/10.1021/acs.jafc.4c00960
14. Singh K, Kaloni D, Sehgal K, Pan S, Sarethy IP. Essential Oils: An Update on Their Biosynthesis and Genetic Strategies to Overcome the Production Challenges. In: Plant-derived bioactives: production, properties and therapeutic applications. 2020; 33–60. https://doi.org/10.1007/978-981-15-1761-7_2.
15. Cox S, Mann C, Markham J, Bell HC, Gustafson J, Warmington J. The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). J Appl Microbiol. 2000;88(1):170–175. https://doi.org/10.1046/j.1365-2672.2000.00943.x.
16. Nazzaro F, Fratianni F, De Martino L, Coppola R, De Feo V. Effect of Essential Oils on Pathogenic Bacteria. Pharmaceuticals. 2013;6(12):1451–1474. https://doi.org/10.3390/ph6121451.
17. Kumari KU, Yadav NP, Luqman S. Promising Essential Oils/Plant Extracts in the Prevention and Treatment of Dandruff Pathogenesis. Curr Top Med Chem. 2022;22(13):1104–1133. https://doi.org/10.2174/1568026622666220531120226.

18. Bertocchi M, Rigillo A, Elmi A, Ventrella D, Aniballi C, Scorpio DG. Preliminary Assessment of the Mucosal Toxicity of Tea Tree (*Melaleuca alternifolia*) and Rosemary (Rosmarinus officinalis) Essential Oils on Novel Porcine Uterus Models. Int J Mol Sci. 2020;21(9):3350. https://doi.org/10.3390/ijms21093350.
19. Peña EF. *Melaleuca alternifolia* oil its use for Trichomonal vaginitis and other vaginal infections. Obstet Gynecol. 1962;19(6):793–795.
20. Mady MS, Elsayed HE, Tawfik NF, Moharram FA. Volatiles extracted from *Melaleuca rugulosa* (Link) Craven leaves: comparative profiling, bioactivity screening, and metabolomic analysis. BMC Complement Med Ther. 2024;24:394. https://doi.org/10.1186/s12906-024-04439-2
21. Alexander B, Procop G, Dufresne P, Espinel-Ingroff A, Fuller J, Ghannoum M. CLSI standards for antifungal susceptibility testing of yeasts. 2017.
22. Abd-alkadhem AHJ, Mohammed NA. GC–MS analysis of tea tree oil. Iraqi J Sci. 2021;62(4):789–97.
23. Al-Saffar A, Al-Shanon A, Al-Brazanchi S, Sabry F, Hassan F, Hadi N. Phytochemical Analysis, Antioxidant and Cytotoxic Potentials of Pelargonium graveolens Extract in Human Breast Adenocarcinoma (MCF-7) Cell Line. Asian J Biochem. 2017;12(1):16–26. https://doi.org/10.3923/ajb.2017.16.26.
24. Liu Y, Tang X, Zhang H, Zheng L, Lai P, Guo C, Ma J, Chen H, Qiu L. Terpinen-4-ol improves lipopolysaccharide-induced macrophage inflammation by regulating glutamine metabolism. Foods. 2024;13(12):1842. https://doi.org/10.3390/foods13121842.
25. Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia*(Tea Tree) Oil: a Review of Antimicrobial and Other Medicinal Properties. Clin Microbiol Rev. 2006;19(1):50–62. https://doi.org/10.1128/cmr.19.1.50-62.2006.
26. Cox S, Mann C, Markham J. Interactions between components of the essential oil of Melaleuca alternifolia. J Appl Microbiol. 2001;91(3):492–497. https://doi.org/10.1046/j.1365-2672.2001.01406.x.
27. Nerio LS, Olivero-Verbel J, Stashenko E. Repellent activity of essential oils: A review. Bioresour Technol. 2010;101(1):372–378. https://doi.org/10.1016/j.biortech.2009.07.048.
28. Southwell I, Lowe R. *Tea Tree: The Genus Melaleuca*. London: CRC Press; 1999.
29. Mondello F, De Bernardis F, Girolamo A, Salvatore G, Cassone A. Tea tree oil against azole-resistant yeasts. J Antimicrob Chemother. 2003;51(5):1223–1229. https://doi.org/10.1093/jac/dkg202.
30. Dalben Y, Grão-Velloso T, Tavares S. *In Vitro* Antifungal Activity of *Melaleuca alternifolia* Essential Oil against Clinical Isolates of *Candida* Species from the Oral Mucosa: Current Status and Challenges. J Mycol Mycolog Sci. 2021; 4(1): 1-4. https://doi.org/10.23880/oajmms-16000139.
31. Powers CN, Satyal P, Mayo JA, McFeeters H, McFeeters RL. Antifungal activity of essential oils against *Aspergillus*, *Candida*, *Cryptococcus*. Molecules. 2019;24(16):2868. https://doi.org/10.3390/molecules24162868
32. Tuan DA, Uyen PVN, Khuon NV, Binh LA, Masak J. Enhanced biofilm inhibition by modified tea tree oil. Front Microbiol. 2025;15:1518598. https://doi.org/10.3389/fmicb.2024.1518598
33. Badr AM, Hassan HM, Ali SA, Khalil AH. Antioxidant activity of tea-tree oil and terpinen-4-ol. J Essent Oil Res. 2023;35(4):345–54. https://doi.org/10.1080/10412905.2023.2195301
34. Malik P, Upadhyay P. GC-MS profile & antioxidant activity of tea tree and rosemary oils. Orient J Chem. 2022;38(5): 1266-1275. https://doi.org/10.13005/ojc/380524.