Synergy between Essential Oils of *Corymbia citriodora*, *Lavandula stricta*, and *Rosmarinus officinalis* and Pure Volatile Compounds against Clinically Isolated *Candida* spp.

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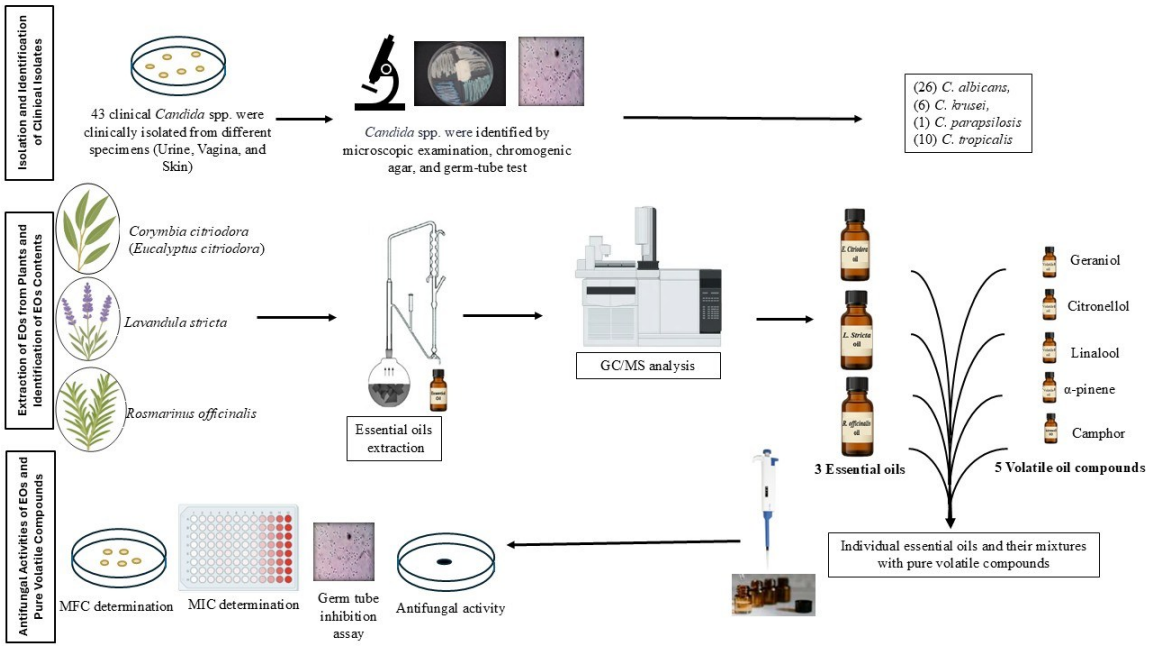
GRAPHICAL ABSTRACT



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Severe complications arise from fungal infections by *Candida* species. The antifungal properties of single and mixed essential oils (EOs) of *Corymbia citriodora*, *Rosmarinus officinalis*, and *Lavandula stricta*, as well as five pure components, against *Candida* species were explored. A total of 43 clinical *Candida* species were clinically isolated and identified as test subjects. The combination of *L. stricta* and *R. officinalis* oils mixture showed the highest activity, displayed from the inhibitory zones of 19.4 mm against *Candida tropicalis* (18K). The highest activity of citronellol with linalool mixtures was 19.4 mm in *C. albicans* (551). The highest activity of the combination of *C. citriodora* oil with camphor, linalool, and α-pinene mixture against *C. parapsilosis* (20K) was 21.9 mm. The minimum inhibitory concentrations (MICs) of *C. citriodora* oil combined with citronellol, linalool, and α-pinene against clinically isolated *C. parapsilosis* (20K), *C. albicans* (551), and *C. tropicalis* (18K) were 0.06, 0.24, and 0.98 µg/mL, respectively. Also, mixing these oils inhibited germ-tube formation in *C. albicans* as compared with the individual oil at the same concentration. The results emphasize the synergistic capabilities of mixtures as antifungal agents, presenting a promising path for creating new therapies from natural sources against *Candida*-based infections.

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Keywords: Essential oils; *Corymbia citriodora*; *Eucalyptus* sp.; *Lavandula stricta*; *Rosmarinus officinalis*; *Volatile compounds*; *Antifungal activity*; *Candida* spp.

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INTRODUCTION

Mycosis, known as fungal infections, can cause severe invasive and systemic disorders, even death. In recent years, epidemiological data have shown an increase in the incidence of severe fungal infections, which is largely attributable to a rising number of immune-compromised individuals and the introduction of fungal pathogenic forms that are increasingly resistant to antimycotic pharmacological therapies (Vitiello *et al.* 2023). Fungal infections, particularly those caused by *Candida* species, have become a major problem in hospital settings because of the rise of drug-resistant forms (McDermott 2022; Macias-Paz *et al.* 2023). *Candida* species are responsible for various clinical manifestations from mucocutaneous overgrowth to bloodstream infections (Eggimann *et al.* 2003). *Candida* spp. are opportunistic fungal pathogens that can cause a variety of

human illnesses, such as oral thrush, vaginal yeast infections, and systemic candidiasis (Vila *et al.* 2020). Candidiasis is a cutaneous, mucosal, or deep-seated organ infection caused by over 20 different *Candida* species, the most common of which is *C. albicans*. These are pathogenic yeasts typically found in the microbiome (Lopes and Lionakis 2022).

Yeast infections, including *Candida,* are treated with many antifungal classes, including azoles, polyenes, and echinocandins. These medications target various metabolic processes that are particular to yeast. For example, azoles inhibit the formation of ergosterol, a unique cell membrane component found only in fungi. These antifungals are either fungistatic (azoles) or fungicidal (echinocandins). Aside from pharmacological efficacy, treatment failures may be caused by the emergence of drug resistance in the infecting *Candida* spp. Antifungal resistance is connected with molecular processes such as membrane transporter overexpression, altered cell wall, and ergosterol production, and gain-of-function mutations in transcription factors that regulate membrane transporters and ergosterol biosynthesis. Ongoing research to better understand these pathways may aid in discovering resistant isolates, identifying novel drug targets, and slowing the rise of drug resistance (Bhattacharya *et al.* 2020). The growing number of drug-resistant *Candida* strains has prompted the search for alternative antifungal agents (de Oliveira Santos *et al.* 2018). In recent years, there has been a growing interest in exploring alternative treatments for candidal infections, particularly natural plant products, which are a promising source of new antifungal medicines that may help battle drug-resistant *Candida* infections (Guevara-Lora *et al.* 2020). There is an increasing interest in researching alternative treatments for these illnesses, such as essential oils, other natural products, and isolated substances that have anticandidal effects. Important sources include essential oils (EOs), as well as pure isolated compounds from natural sources that have demonstrated promising anticandidal action, making them potential candidates for the development of new antifungal agents. Several EOs have been shown to exhibit potent anticandidal activity (Abd Rashed *et al.* 2021; El-Sakhawy *et al.* 2023b).

Highlighting a novel approach combining multiple plant-derived EOs and pure volatile compounds is the gap that still needs to be closed, which could enhance efficacy while minimising resistance development, including a critical concern given the limitations of conventional antifungal drugs. In other words, this work aligns with broader efforts to harness plant-based compounds for combating fungal pathogens while reducing reliance on synthetic drugs (Hleba *et al.* 2024; Patel *et al.* 2025). The research is timely, as *Candida* infections, particularly by drug-resistant strains, pose significant morbidity and mortality risks, especially in immunocompromised individuals, as well as other healthy individuals (Mallick *et al.* 2025).

Resistance to any antimicrobial is theoretically possible and may develop in the near term. However, the development of such resistance likely faces a higher barrier against EOs compared to single-target synthetic antifungals. This is due to the complex, multi-component nature of EOs and their combinations, which often act on multiple targets in fungal cells simultaneously. Their mechanisms include disrupting membrane integrity, suppressing efflux pumps, inhibiting cell wall synthesis, causing mitochondrial dysfunction, preventing biofilm formation, and inducing oxidative stress. While the synergy between EOs and established antifungal drugs is one strategy to combat resistance, a promising novel approach is the combination of EOs with other active volatile compounds. This natural-source synergy has significant potential to overcome and combat antifungal resistance (Chouhan *et al.* 2017; Khan 2024; Kowalczyk 2024; Leiva-Mora *et al.* 2025). By exploring synergistic interactions, the study could pave the way for more

effective, natural antifungal therapies, leveraging the known antimicrobial properties of EOs such as citronellal (from *Corymbia*), linalool (from *Lavandula*), and α-pinene (from *Rosmarinus*) (Bassolé and Juliani 2012; Ju *et al.* 2022).

The study of clinically isolated strains of *Candida* has added to connecting laboratory results to practical uses (Hou and Huang 2024). Moreover, the EOs' activity against fungi has been reported in many studies, but the specific mechanism of action for all pure volatile constituents remains uncertain. Several EOs mechanisms have been recorded, including suppression of cell membranes as well as walls, leading to inhibition of sporulation, fungal germination, and hyphal extension. Moreover, the mechanism of mixture activity of volatile oils with EOs has not been reported; therefore, the current study may be the first step to cover this aspect (Cavanagh 2007; Basak and Guha 2018; El-Sakhawy *et al.* 2025). Due to the increased frequency of drug pathogens and the toxicity of existing antifungal compounds, the current study aims to investigate the anticandidal activity of natural products of EOs from *Corymbia citriodora*, *Lavandula stricta*, and *Rosmarinus officinalis*, alone and with pure volatile compounds (VCs), their mixtures, and their potential synergistic antifungal effects as novel agents in medical mycology, especially clinically isolated *Candida* species. This work aligns with broader efforts to harness plant-based compounds for combating fungal pathogens while reducing reliance on synthetic drugs.

MATERIALS AND METHODS

Extraction of EOs from Plants

Plant materials

The aerial parts of *Corymbia citriodora* Hook. (common synonym *Eucalyptus citriodora* Hook., Myrtaceae) leaves, *Lavandula stricta* Delile (Lamiaceae) flowers, and *Rosmarinus officinalis* L. (Lamiaceae) leaves in the flowering stage were collected in Egypt during 2024 from Dakhla Oasis (New Valley Governorate), Saint Catherine (South Sinai), and Wadi Hagul (Suez Governorate), respectively. The identification of these plants was carried out by plant taxonomists at the Desert Research Center (DRC). Voucher specimens of *Corymbia citriodor*a Hook. (common synonym *Eucalyptus citriodora* Hook., Myrtaceae) leaves, *Lavandula stricta* Delile (Lamiaceae) flowers, and *Rosmarinus officinalis* L. were kept in the DRC herbarium, Cairo, Egypt, under numbers CAIH-1268, CAIH-1269, and CAIH-1270, respectively. Leaves of the collected aerial plant parts were washed with running water to remove any dust or dirt before the oil extraction.

Extraction of EOs

EOs from the fresh collected aerial parts of *Corymbia citriodora*, *Rosmarinus officinalis,* and *Lavandula stricta* plants were extracted using hydrodistillation in a Clevenger-type apparatus as follows: Prior to extraction, the leaves were thoroughly washed under running tap water to remove any dust or particulate matter. The cleaned leaves were then cut into small pieces. About 500 g of this prepared plant material was placed into a 2000 mL round-bottom flask containing 1000 mL of water (distilled water), and the mixture was boiled for 5 h. The extracted EOs were collected in small opaque vials. The yields of each obtained EOs were calculated as the weight/weight of the plant material (Achak *et al.* 2009; Mehani and Ladjel 2012; El-Sakhawy *et al.* 2023b). All oil samples were stored in the fridge (at 4 °C) for further investigation.

Identification of EOs Contents

GC/MS analysis

The GC/MS analysis of the prepared EOs samples was carried out on an Agilent 7890A/5975C GC/MS system equipped with a HP-5MS non-polar column. Analyses were carried out using helium as carrier gas at a flow rate of 1.0 mL/min and a split ratio of 1:10 using the following temperature program: 40 °C for 1 min; rising at 4.0 °C /min to 160 °C and held for 6 min; rising at 6 °C/min to 210 °C and held for 1 min. The injector and detector were held at 210 °C. Diluted samples (1:10 hexane, v/v) of 0.2 μL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of *m*/*z* 40 to 450. Most of the compounds were identified using the analytical method: mass spectra (authentic chemicals, Wiley spectral library collection, and NSIT library). The EOs were identified using GC/MS by comparing the National Institute of Standards and Technology (NIST) library data of the peaks with those reported in the computer data bank.

The Commercial Volatile Compounds (VCs)

Based on the GC-MS analysis of extracted EOs of *C. citriodora*, *L. stricta*, and *R. officinalis*, geraniol, and citronellol (*C. citriodora*), linalool (*L. stricta*), α-pinene, and camphor (*R. officinalis),* respectively, were purchased from Sigma-Aldrich Co. as the major compounds in the extracted essential oil.

Isolation and Identification of Clinical Isolates

Yeast isolation

Samples were collected during June 2025 from 43 clinical specimens obtained at different hospitals in Al-Kharj, Saudi Arabia. Physicians and nurses collected samples from various body parts of patients, including the vagina and skin, while urine samples were obtained from clinical laboratories. Of the 43 specimens, 34 were from female patients, and 9 were from male patients. Sabouraud Dextrose Agar (SDA - Scharlau, Spain) and Yeast Malt Agar (YMA - Scharlau, Spain) media were used for the isolation and maintenance of stock cultures. Yeast isolates were identified using chromogenic agar, germ tube tests, microscopic examination, and biochemical characteristics. The study protocols adhered to the ethical guidelines set by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University and were granted ethical approval (Reference No. SCBR-510-2025).

Identification of Yeast Isolates

Microscopic examination

Direct examination and observations of specimens and cultures, with particular emphasis on diagnostic features, were made from material mounted in distilled water. Wet mounts in a 10% potassium hydroxide solution are useful for distinguishing *Candida* sp. A drop of lactophenol cotton blue stain may be added to the potassium hydroxide wet mount. Examination was conducted with a light microscope using bright-field optics. Observations were recorded on a 12-megapixel Panasonic digital camera.

Chromogenic agar

Presumptive identification of isolated *Candida* species obtained with a chromogenic agar medium. CHROM agar *Candida* (Becton Dickinson/BBL) allowed the presumptive differentiation of over 10 species (Odds 1998). The chromogenic agar medium

(chloramphenicol 0.5 g, peptone 10.2 g, chromogenic mix 22 g, and agar 15 g, pH: 6.1±0.2) is based on the differential release of chromogenic breakdown products from various substrates as a result of enzymatic activity.

Germ-tube test

The germ tube test is one of the most rapid and simple tests for the presumptive identification of *C. albicans.* Approximately 1.0 mL of sterile serum was inoculated using a clean Pasteur tip, making a yeast suspension of $10^{5\text{-}10}$ cells/mL, then incubated at 37 °C for no longer than 3 h. Then, one drop of the yeast-serum mixture was placed on a slide with a cover slip and examined microscopically.

Biological Activity

Anticandidal activity

The agar well diffusion method was used for antifungal activity screening. The broth microdilution method was used for the determination of minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of the investigated oils and compounds. The EOs, five pure compound mixtures with EOs, the mixture of commercial compounds, and the mixture of EOs with commercial compounds of the plants under investigation (*Corymbia citriodora*, *Lavandula stricta,* and *Rosmarinus officinalis*) were screened for antifungal activity, MICs, and MFCs against clinically isolated fungi.

Agar well diffusion method

The test organisms were separately seeded in the agar medium. The wells (mm in diameter) were cut from the agar, and 50 µL of each sample was transferred into them (under aseptic conditions). After incubation, the plates were examined, and the inhibition zones were determined (Ohikhena *et al.* 2017; Bubonja-Šonje *et al.* 2020).

Evaluation of MICs and MFCs

MICs of different samples were determined by the broth microdilution method according to Hammer *et al.* (2002). Two-fold serial dilutions (using DMSO) of each sample (stock solution) were placed in Eppendorf tubes labeled A to M. Each tube was then transferred into 96-well microtiter plates. Each well was then filled with 100 µL of fungal suspension to obtain a serial dilution of the test materials. The mixtures were mixed thoroughly and incubated at 37 ºC. The final inoculum size for fungi was 2.5×10^4 CFU/mL, where 1% dimethyl sulfoxide (DMSO) served as a negative control, broth as a sterility control, and broth with fungal suspension as a growth control. Turbidity was taken as an indication of growth, and the lowest concentration at which it remained clear was recorded as the minimum inhibitory concentration (MIC) of the sample. After reading the MIC, the minimum fungicidal concentration (MFC) was determined.

A 100 µL aliquot from the wells in which no growth was observed was transferred to test tubes containing 2 mL of Sabouraud-dextrose broth. A positive control (growth control) and a negative control (sterility control) were included in the test. The tubes were incubated for 2 to 3 days at 28 ºC, and growth was observed visually. MFC was defined as the minimum concentration at which no fungal growth occurred (Donadu *et al.* 2021; Kebede and Shibeshi 2022).

Germ tube inhibition assay

Overnight cell suspensions of *C. albicans* (551) at 37 °C were prepared in serum/albumin 20% and adjusted to obtain a density of $(1.0\pm0.2) \times 10^6$ cells/mL. Extracted

Corymbia oil and mixed oils (*Corymbia*, linalool, α-pinene, and citronellol) were diluted in Tween 80 (polysorbate 80) and added to yeast suspensions with degraded concentrations to obtain appropriate sub-inhibitory concentrations (1/8,1/4,1/2, and the same concentration of the MIC values). Drug-free control suspensions with the same amount of Tween 80 were included for each *C. albicans* (551) strain. After 2 to 3 h incubation at 37 °C, cells were examined under a light microscope

Statistical Analysis

All experiments were designed in a Randomized Complete Block design with four replicates statistically analyzed by ANOVA, using SPSS,21 IPM software (SPSS, Chicago, IL, USA), and treatment means were compared by Duncan at 5% level of probability.

RESULTS

Yeast Isolates

A total of 43 clinical specimens were collected from different hospitals and clinical labs, including female patients 34 (79.1%) and the rest 9 (20.9%) from male patients. The specimens include 19 (44.2%) urine, 20 (46.5%) vaginal swabs, and 4 (9.3%) skin infection samples. On the other hand, out of all clinical specimens, only 43 (19 urine, 20 vaginal, and 4 skin) showed the growth of yeast-like structures on the same media (Table 1).

Table 1. Clinical Specimen Types, Distribution, and Number of Isolated *Candida* Species

Total no. of specimens (%)	Sex no. (%)		Origin no. (%)			Identification of *Candida* species no. (%)			
	Female	Male	Urine	Vagina	Skin	*C. albicans*	*C. krusei*	*C. parapsilosis*	*C. tropicalis*
43 (100)	34 (79.1)	9 (20.9)	19 (44.2)	20 (46.5)	4 (9.3)	26 (60.5)	6 (13.9)	1 (2.3)	10 (23.3)

Identification of Candidal Isolates

Using macroscopic features of the yeast, *Candida* infections were diagnosed by the presence of budding yeasts with hyphae and pseudohyphae on potassium hydroxide examination. Swabs were then streaked onto yeast malt agar and/or Sabouraud dextrose agar media and incubated at 37 °C for 48 h.

a: Yeast growth of *Candida* sp. **b:** Different *Candida* spp. cultured on CHROMagar™ medium **c:** Germ tube of *Candida albicans*

Fig. 1. *Candida* species features and morphological characteristics

Presumptive identification of *Candida* isolates was performed by subculture of the obtained color of colonies on CHROMagar *Candida* (Becton Dickinson/BBL) at 37 ℃ for 24 to 48 h, and germ tube was investigated for presumptive identification of *C. albicans* (Fig. 1 and Table 1).

Based on morphological and biochemical characteristics of the isolated pathogenic yeasts, the 43 candidal isolates were identified as *Candida albicans* (26), *C. krusei* (6), *C. parapsilosis* (1), and *C. tropicalis* (10). These results are recorded in Table 1.

Antifungal Activities of Essential Oils and Pure Volatile Compounds

EOs screening

The antifungal activity of *C. citriodora, L. stricta,* and *R. officinalis* extracted oils were tested against clinically isolated yeasts (unicellular) using the well diffusion agar technique. The obtained results showed that the lowest activity (9 mm; inhibition zone) was detected by *C. citriodora* EOs against *C. tropicalis* (19K), followed by activity (10.4 mm) of *L. stricta* against *C. krusei* (554), and *L. stricta* essential oil activity (11.5 mm) against *C. albicans* (13M). On the other hand, the extracted EOs showed no activity against *C. krusei* (604), as shown in Table 2.

Table 2. Inhibitory Activity of Essential Oils Mixtures of *Corymbia citriodora*, *Lavandula stricta,* and *Rosmarinus officinalis* against Clinically Isolated *Candida* spp.

Clinically isolated *Candida* species	*C. citriodora*	*L. stricta*	*R. officinalis*	*C. citriodora* + *L. stricta*	*C. citriodora* + *R. officinalis*	*L. stricta* + *R. officinalis*	*C. citriodora* +*L. stricta* + *R. officinalis*	Amphotericin B
	Mean of inhibition zone (mm)							
Candida albicans (542)	0	11.7	10.4	15.6	11.6	14.6	16.8	20.9
C. albicans (551)	13.4	12.6	12.6	17.9	14.6	16.3	17.6	19.9
C. albicans (8M)	14.6	10.3	11.2	14.3	15.2	16.9	18.2	20.3
C. albicans (15K)	10.8	12.4	11.9	17.8	14.7	18.9	17.3	17.6
C. albicans (13M)	14.1	8.5	13.8	17.9	16.2	15.8	15.2	16.8
C. krusei (604)	0	0	0	0	0	0	0	17.8
C. krusei (554)	9.4	7.4	0	12.9	10.3	13.3	11.8	18.3
C. tropicalis (2M)	11.6	12.2	10.9	16.2	12.6	13.5	15.2	20.1
C. tropicalis (19K)	7	8.5	10.9	13.4	11.8	0	12.8	16.4
C. tropicalis (510)	15.2	0	0	18.3	16.4	14.9	16.3	16.8
C. tropicalis (18K)	9.5	0	10.5	18.3	12.3	19.4	10.8	15.7
C. albicans (524)	14.4	12.2	12.3	12.4	15.9	17.3	17.4	18.2
C. parapsilosis (20K)	0	0	10.2	10.6	10.2	13.3	16.8	18.9
	F				Sig			
Treatment	1176.0				0.000			
Candida	739.9				0.000			
Treatment X Candida	918.6				0.000			
Well diameter 6 mm, tested sample 30 µL								

Combinations of EOs

The combination of *L. stricta* and *R. officinalis* mixture showed the highest activity (19.4 mm) against *C. tropicalis* (18K), and its activity of 18.9 mm against *C. albicans* (15K), followed by the combination of *C. citriodora* and *L. stricta* EOs activity (18.3 mm) against *C. tropicalis* (18K) and *C. tropicalis* (510). On the other hand, the lowest combination activity (10.2 mm) was recorded by *C. citriodora* and *R. officinalis* mixture oils against *C. parapsilosis* (20K), activity (10.3 mm) of *C. citriodora* and *R. officinalis* oils against *C. krusei* (554), followed by the combination of *C. citriodora* and *L. stricta* oils activity (10.6 mm) against *C. parapsilosis* (20K), while no activity of all EO mixtures against *C. krusei* (604) (Table 2).

Table 3. Antifungal Activity of Volatile Compound Mixtures against Clinically Isolated *Candida* Species

Clinically isolated *Candida* species	Camphor	Citronellol	Geraniol	α-pinene	Linalool	Camphor + Citronellol	Camphor + Geraniol	Camphor + Linalool	Camphor + α-pinene	Citronellol + Geraniol	Citronellol + Linalool	Citronellol + α-pinene	Geraniol + Linalool	Geraniol + α-pinene	Linalool + α-pinene	Amphotericin B
	Mean of inhibition zone (mm)															
Candida albicans (542)	0	0	13.2	19.6	0	0	11.3	12.3	12	0	0	15.2	13.6	16.3	0	20.9
C. albicans (551)	17.6	12.9	14.9	20.2	15.4	16.3	16.7	11.7	15	16.5	19.4	13.4	15.2	19.3	16.3	19.9
C. albicans (8M)	14.8	17.8	15.3	18.3	16.7	14	15.3	0	14.3	17	17.9	17.9	16.7	17.2	17.2	20.3
C. albicans (15K)	18.6	14.9	0	15.7	12.6	17.2	18.2	15.4	13	13.1	18.9	15.3	18.3	15.1	13.4	17.6
C. albicans (13M)	17.3	15.2	12.7	14.2	14.9	16.3	17.3	12.9	15.6	16.8	16.7	17.2	17.1	14.9	12.9	16.8
C. krusei (604)	18.4	0	0	14.6	0	17	16.7	0	0	0	0	15.3	0	16.2	0	17.8
C. krusei (554)	13.5	0	0	0	0	12.9	15.4	14.2	0	13.9	12.3	0	11.6	16.3	0	18.3
C. tropicalis (2M)	17.3	19.8	15.3	16.8	18.3	16.4	16.7	15.7	11.9	15.2	18.4	18.3	16.3	15.2	18.7	20.1
C. tropicalis (19K)	12.6	15.2	14.7	16.7	16.8	12.2	17.3	17.8	12.1	10	16.3	16.1	15.2	14.6	18.9	16.4
C. tropicalis (510)	16.8	17.6	16.2	17.3	19.3	15.4	13.8	16.4	0	17.6	18.4	16.3	17.4	13.7	16.9	16.8
C. tropicalis (18K)	14.7	16.3	13.9	16.4	15.8	15.1	18.2	0	12	11.6	13.6	16.2	18.3	16.8	13.4	15.7
C. albicans (524)	17.3	17.2	11.9	19.4	15.2	14	15.2	0	13.6	15.9	17.8	16.3	19.2	15.3	15.4	18.2
C. parapsilosis (20K)	12.6	12.9	11.7	20.8	13.7	16.3	17.9	13.4	12	16.7	13.1	17.3	14.3	16.1	14.2	18.9
	F			Sig.												
Treatment	4315.9			0.000												
Candida	106.1			0.000												
Treatment X Candida	118.6			0.000												
Well diameter 6 mm, tested sample 30 µL																

Activity of Commercial Pure Compounds

The antifungal activity of five commercial pure compounds, camphor, citronellol, geraniol, linalool, and α-pinene were tested against clinically isolated *Candida* spp fungi. While an activity of 11.7 mm was obtained by geraniol against *C. parapsilosis* (20K), geraniol also showed an activity of 11.9 mm against *C. albicans* (524), followed by camphor compound activity (12.6 mm) against *C. parapsilosis* (20K) and *C. tropicalis* (19K) (Table 3).

Activity of Combinations of Commercial Compound Mixtures

The antifungal activity of the commercial compound's mixtures was obtained highest activity (19.4 mm) by citronellol and linalool mixture against *C. albicans* (551), a combination of geraniol and α-pinene mixture activity (19.3 mm) against *C. albicans* (551), followed by geraniol and linalool mixture activity (19.2 mm) against *C. albicans* (524). On the other hand, the lowest activity (10 mm) was determined by combinations of citronellol and geraniol mixture against *C. tropicalis* (19K), followed by the same mixture activity (11.6 mm) against *C. tropicalis* (18K), and a combination of geraniol and linalool activity (11.6 mm) against *Candida krusei* (554), (Table 3).

Activity of the Mixture of Extracted Oils and Commercial Pure Compounds

The supplemented EOs with commercial compounds represented the highest activity (21.9 mm) of the combinations of *C. citriodora* oil with camphor, linalool, and α-pinene mixture against *C. parapsilosis* (20K), combination activity (21.7 mm) was detected by *C. citriodora* oil supplemented with linalool and α-pinene mixture against *C. albicans* (551), followed by combination activity (21.6 mm) of *C. citriodora* oil supplemented with linalool against *C. tropicalis* (18K). The lowest activity (7.1 mm) was obtained by a mixture of *L. stricta* EO supplemented with camphor and citronellol against *C. albicans* (542), whereas activity (7.5 mm) was obtained by the mixture of *L. stricta* EO supplemented with camphor against *C. parapsilosis* (20K), followed by a mixture of *L. stricta* EO with camphor and citronellol activity (8.0 mm) against *C. krusei* (604), data recorded in Table (4a and 4b).

Table 4a. Antifungal Activity of Mixtures of Extracted Essential Oils with Commercial VCs against Clinically-Isolated *Candida* Species

Clinically isolated *Candida* species	*Corymbia citriodora*	*C. citriodora* + Camphor	*C. citriodora* + Linalool	*C. citriodora* + α-pinene	Lavandula stricta	*L. stricta* + Camphor	*L. stricta* + Citronellol	*L. stricta* + Geraniol	*L. stricta* + α-pinene	Rosmarinus officinalis	*R. officinalis* + Citronellol	*R. officinalis* + Geraniol	*R. officinalis* + Linalool	*C. citriodora* + Camphor + Linalool	*L. stricta* + Citronellol + Geraniol
	Mean of inhibition zone (mm)														
C. albicans (542)	0	15.9	0	16.4	11.7	9.9	0	12.6	17.8	10.4	13.9	12.3	15.6	15.1	0
C. albicans (551)	13.4	16.8	19	18.3	12.6	15.6	13.4	13.4	18.3	12.6	12.4	11.7	13.9	16.3	15.3
C. albicans (8M)	14.6	15.3	20.2	17.3	10.3	12.7	18.3	14.2	17.2	11.2	9.3	0	12.2	15.1	18.6
C. albicans (15K)	10.8	16.8	16.8	18.2	12.4	16.4	15.2	11.7	14.3	11.9	15.5	15.4	16.4	16.1	16.4
C. albicans (13M)	14.1	14.6	20.9	17.3	8.5	12.9	13.4	12.3	13.5	13.8	12.9	12.9	15	14.6	19.2
C. krusei (604)	0	0	19.3	0	0	10	0	0	0	0	0	0	0	0	0
C. krusei (554)	9.4	10.3	21.3	12.6	7.4	9.7	0	13.2	15.2	0	15.2	14.2	15.6	11.1	10.3
C. tropicalis (2M)	11.6	17.2	20.6	19.3	12.2	18.2	20.3	14.4	14.3	10.9	16.9	15.7	16.4	16.1	19.3
C. tropicalis (19K)	7	16.3	21.3	13.4	8.5	9.8	17.33	15.2	15.7	10.9	0	0	17.8	13.4	17.2
C. tropicalis (510)	15.2	16.8	17.3	15.7	0	9.8	17.8	15.3	15.3	0	17.1	16.4	18.3	13.6	16.3
C. tropicalis (18K)	9.5	16.3	21.6	13.6	0	8.6	16.3	12.1	16.4	10.5	10	0	15.4	12.4	15.3
C. albicans (524)	14.4	14.6	16.4	14.2	12.2	18.5	18.3	10.6	18.2	12.3	0	0	16	12.1	17.3
C. parapsilosis (20K)	0	15.2	13.2	15.6	0	7.5	13..5	10	19	10.2	14.6	13.4	12.8	10.6	12.6
Well diameter 6 mm, tested sample 30 µL															

Table 4b. Antifungal Activity of Mixtures of Extracted Essential Oils with Commercial Volatile Compounds against Clinically Isolated *Candida* Species

Clinically isolated *Candida* species	*L. ula stricta*+ Citronellol + α-pinene	*L. stricta* + Geraniol + α-pinene	*L. strictab* +Citronellol + Geraniol +	*L. stricta* + Camphor + Citronellol + Geraniol +	*R. officinalis* + Citronellol + Geraniol	*R. officinalis* + Citronellol + Linalool	*R. officinalis* + Geraniol + Linalool	*R. officinalis* + Citronellol + Geraniol + Linalool	*C. citriodora* + Camphor + α-pinene	*C. citriodora* + Linalool + α-pinene	*C. citriodora*+ Camphor +	*L. stricta* + Camphor + Citronellol	*L. stricta* + Camphor + Geraniol	*L. stricta*+ Camphor + α-pinene	Amphotericin B
	Mean of inhibition zone (mm)														
C. albicans (542)	17.3	15.2	17.3	10.2	15.4	13.4	18.6	15.6	14.7	20.3	20.6	7.1	10.9	15.7	20.9
C. albicans (551)	18.1	16.7	18.2	18.1	14.6	12.7	17.3	13.9	19.1	21.7	21.2	16.5	15.9	17.2	19.9
C. albicans (8M)	18.5	18.3	16.7	15.8	13.1	10.6	16.5	12.2	18.2	19.8	20.1	17.9	14.9	14.2	20.3
C. albicans (15K)	17.6	16.4	15.3	16.6	15.2	13.6	13.6	16.4	21.1	17.3	19.9	17.4	8.4	16.6	17.6
C. albicans (13M)	16.3	15.2	16.8	15.3	13.1	11.4	15.2	15	18.3	15.8	18.3	16.1	13.9	14.2	16.8
C. krusei (604)	0	0	0	9.8	0	0	17.4	17.3	14.8	0	0	8	0	15.6	17.8
C. krusei (554)	12.4	18.6	14.6	13.9	15.4	15.1	19.2	17.8	13.9	17.3	19.3	0	0	0	18.3
C. tropicalis (2M)	15.6	17.3	17.3	18.3	16	17	19.4	19.8	21.3	18.3	20.4	19.11	18.8	17.2	20.1
C. tropicalis (19K)	13.4	16.3	16.2	14.6	0	0	13.6	18.4	14.4	16.8	0	16.2	10.8	14.7	16.4
C. tropicalis (510)	17.2	18.3	17.2	18.3	13.5	15.8	18.9	12.4	15.8	17.8	19.1	16.5	16.6	15.7	16.8
C. tropicalis (18K)	17.2	19.3	18.1	15.3	20.3	9.6	15.7	15.6	13.19	18.9	20.3	10.3	10.1	12.7	15.7
C. albicans (524)	18.2	19.7	16.3	16.8	18.6	0	14.7	13.9	15.2	20.3	21.3	19.9	13.5	19.9	18.2
C. parapsilosis (20K)	17.8	20	14.2	13.2	17.8	14.3	12.8	12.2	16.8	21.4	21.9	8.9	9.5	13.6	18.9
				F			Sig.								
Treatment				3379.3			0.00								
Candida				842.3			0.000								
Treatment X Candida				121.2			0.000								
Well diameter 6 mm, tested sample 30 µL															

Minimum Inhibitory Concentration

Extracted essential oils

The minimum inhibitory concentrations (MIC) of *C. citriodora, L. stricta,* or *R. officinalis* extracted EOs were tested using the broth microdilution technique. The lowest MIC (7.8 µL/mL) was detected by *C. citriodora* EO against *C. tropicalis* (510), followed by MIC (15.63 µL/mL) achieved by *C. citriodora* EO against *C. albicans* (8M, 13M, and 551). These results are recorded in Table 5. On the other hand, the lowest MIC of *C. citriodora* EO was calculated 7.8 µL/mL against *C. tropicalis* (510). The lowest minimum inhibitory concentration of *L. stricta* EO was calculated by 31.25 µl/mL against *C. albicans* 15K and 551 isolates. Meanwhile, the lowest minimum inhibitory concentration of *R. officinalis* EO was calculated 31.25 µl/ml against *C. albicans* 13M and 551 isolâtes. On the other hand, no minimum inhibitory concentration of EOs was detected against *C. krusei* under the usage concentrations (Table 5).

Table 5. Minimum Inhibitory Concentration (µL/mL) of the Extracted Essential Oils

Clinically-isolate *Candida* species	*C. citriodora* oil	*L. stricta oil*	*R. officinalis* oil	Amphotericin B
Candida albicans (542)	0	125	500	0.98
Candida albicans (551)	15.63	31.25	31.25	1.95
Candida albicans (8M)	15.63	500	62.5	1.95
Candida albicans (15K)	250	31.25	125	15.63
Candida albicans (13M)	15.63	500	31.25	15.63
Candida krusei (604)	0	0	0	7.8
Candida krusei (554)	250	1000	0	7.8
Candida tropicalis (2M)	62.5	62.5	500	1.95
Candida tropicalis (19K)	1000	500	125	31.25
Candida tropicalis (510)	7.8	0	0	15.63
Candida tropicalis (18K)	500	0	250	31.25
Candida albicans (524)	31.25	62.5	62.5	7.8
Candida parapsilosis (20K)	0	0	250	3.9
	F		Sig.	
Treatment	1753.368		0.000	
Candida	1034.129		0.000	
Treatment X Candida	906.387		0.000	

Mixtures of Extracted Essential Oil with Commercial Volatile Compounds

The MIC activity of most active mixtures of essential oils supplemented with commercial volatile compounds of camphor, citronellol, geraniol, linalool, and α-pinene was tested against *C. albicans* (551), *C. parapsilosis* (20K), and *C. tropicalis* (18k) using the broth microdilution technique. The lowest MIC (0.03 µL/mL) was recorded by camphor, citronellol, geraniol, linalool, and α-pinene mixture against *C. parapsilosis* (20K), followed by *Corymbia* oil supplemented with citronellol, linalool, and α-pinene mixture MIC (0.06 µL/mL) against *C. parapsilosis* (20K), followed by the same mixture MIC (0.24 µL/mL) against *Candida albicans* (551). These data are listed in Table 6. Whereas, the highest MIC (7.80 µL/mL) activity was achieved by citronellol, geraniol, linalool, and α-pinene mixture against *C. tropicalis* (18K), followed by MIC (3.90 µL/mL) activity of *Corymbia* oil and linalool mixture against *C. albicans* (551) and MIC (3.90 µl/ml) of *Corymbia* oil supplemented with linalool and α-pinene mixture against *C .tropicalis* (18K), as shown in Table (6).

MIC and MFC of the Most Potent Active EO and Mixtures

The MIC (minimum inhibitory concentration) of *Corymbia* oil and a mixture with citronellol, linalool, and α-pinene by the broth microdilution method revealed that the mixture oil exhibited the lowest MIC, followed by amphotericin B against selected clinically isolated *C. albicans* (551), *C. parapsilosis* (20K), and *C. tropicalis* (18k). The minimum inhibitory concentrations (MICs) of *C. citriodora* oil with citronellol, linalool, and α-pinene mixtures against clinically isolated *C. parapsilosis* (20K), *C. albicans* (551), and *C. tropicalis* (18k) were 0.06, 0.24, and 0.98 µg/mL, respectively. The MFC (minimum fungicidal concentration) of *Corymbia* EO alone was significantly higher than that of the mixed oil (synergistic combination of *Corymbia* EO with the volatile compounds linalool, α-pinene, and citronellol).

Table 6. MIC of the Mixture of Extracted Essential Oils Mixed with Volatile Commercial Compounds against Clinically Isolated *Candida* spp.

Clinically isolated selected *Candida* spp.	*C. citriodora*	*L. stricta*	*R. officinalis*	*C. citriodora* + Linalool	*C. citriodora* + Linalool + α-pinene	*C. citriodora* + Citronellol + Linalool + α-pinene	*C. citriodora* + Citronellol + Geraniol + Linalool + α-pinene	Camphor + Citronellol + Geraniol + Linalool + α-pinene	Amphotericin B
	Minimum Inhibitory Concentration (µL/mL)								
Candida albicans (551)	15.63	31.25	31.25	3.9	0.49	0.24	0.98	0.24	1.95
Candida tropicalis (18K)	500	0	250	0.49	3.9	0.98	1.95	7.8	31.25
Candida parapsilosis (20K)	0	0	250	1.95	0.98	0.06	0.49	0.03	3.9
	F			Sig.					
Treatment	1556.489			0.000					
Candida	1404.206			0.000					
Treatment X Candida	943.248			0.000					

The minimum fungicidal concentrations of *C. citriodora* oil supplemented with citronellol, linalool, and α-pinene mixture against clinically isolated *C. parapsilosis* (20K), *C. albicans* (551), and *C. tropicalis* (18k) were 0.06, 0.24, and 1.95 µg/mL, respectively, while the MFCs of *Corymbia* oil were 62.5 and 1000 µg/mL against *C. albicans* and *C. tropicalis* and inactive against *C. parapsilosis* isolate at the tested concentrations (Tables 6 and 7). According to MIC and MFC results, the antifungal activity of *Corymbia* EO became most potent when combined with the volatile compounds linalool, α-pinene, and citronellol, thereby achieving a synergistic effect.

Table 7. Minimum Fungicidal Concentration (µg/mL) of *Corymbia* Oil and Its Combinations with Commercial Volatile Compounds against Three Selected *Candida* spp

Clinically isolated selected *Candida* species	Minimum Fungicidal Concentration (µL/mL)	
	Corymbia oil	Mixed oil (*Corymbia* + Linalool + α-pinene + Citronellol)
Candida albicans (551)	62.5	0.24
Candida tropicalis (18K)	1000	1.95
Candida parapsilosis (20K)	0.00	0.06
	F	Sig.
Treatment	12.44	0.001
Candida	0.8035	0.4632
Treatment X Candida	0.8129	0.5333

Detection and Inhibition of Virulence Factors

Germ tube formation

The ability of three *Candida sp.* to form germ tubes in human serum was investigated at 35 to 37 °C. Only *C. albicans* (551) cells generated germ tubes in serum at 37 °C, and the germ tubes were true germ tubes without constrictions at their origins. It took at least 2 h for *C. albicans* cells to generate the germ tubes sufficiently in human

serum, while both *C. tropicalis* and *C. parapsilosis* did not form germ tubes under the same conditions (Fig. 1).

Germ tube inhibition assay

Mixed oils inhibit germ-tube formation by the blastospores of *C. albicans* at a concentration 0.24 µL/mL, while the *Corymbia* oil does not inhibit germ-tube formation at the same concentration. The production of germ tubes and subsequent mycelial formation, when exposed to either human serum or 20% albumen, is an *in vitro* correlation of the *in vivo* tissue-invasive capabilities of the pathogenic strains of *C. albicans.*

Table 8. Chemical Composition of *C. citriodora, L. stricta,* and *R. officinalis* Extracted Essential Oils

Peak number	*C. citrodra*	Retention time	%	*L. stricta*	Retention time	%	*R. officinalis*	Retention time	%
1	2-Methylpropyl-2-methylpropionate	3.2	0.75	(2E)-Hexenal	0.51	4.22	Tricyclene	0.3	0.54
2	α-Pinene	3.5	0.67	α-Thujene	0.92	1.53	α-thujene	0.45	2.53
3	β-Pinene	4.0	2.08	α-Pinene	1.03	3.54	α-pinene	0.58	7.16
4	Myrcenol	4.5	2.88	Camphene	1.04	1.30	Camphene	1.2	5.97
5	Eucalyptol	4.6	1.60	Thuja-2,4(10)-diene	1.58	4.11	Verbenene	1.5	0.40
6	2,6-Dimethyl-5-heptenal	4.9	4.74	Sabinene	1.88	7.92	β-Pinene	1.6	4.85
7	Linalool	5.5	0.83	β-Pinene	1.99	0.88	1-Octen-3-ol	1.8	0.43
8	Isopulegol	6.5	12.77	6-methyl-5-Hepten-2-one	2	0.83	3-Octanone	2.0	0.94
9	Menthone	6.7	0.98	Myrcene	2.24	5.06	β-myrcene	2.2	8.25
10	5-Caranol	6.8	2.83	ρ-Cymene	2.5	2.33	β-terpinene	2.6	3.26
11	Dihydrocarveol	6.9	1.00	Limonene	2.8	7.52	δ-3-Carene	2.8	1.41
12	Cyclohexylacetone	7	2.84	1,8-Cineole	3.01	0.87	p-cymene	2.9	6.48
13	Citronellol	3.3	12.42	(Z)-β-Ocimene	3.41	1.44	Limonene	3.2	5.10
14	7-Methyl-1,6-octadiene	7.5	1.70	Benzene acetaldehyde	3.66	0.57	1,8-cineole	3.54	3.98
15	Geraniol	7.7	10.56	(E)-β-Ocimene	3.88	0.72	γ-terpinene	3.8	2.53
16	3-Tetradecanol	7.9	8.28	Terpinene	4.5	0.46	α-Terpinolene	4	0.69
17	Citronellyl formate	8.1	0.78	trans-Linalool oxide	5.1	0.51	Linalool	4.1	1.37
18	9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	8.6	1.51	cis-Linalool oxide	5.6	0.77	2,3-Dimethyl-2,3-Dihydropyridine	4.32	0.54
19	Bicyclo[3.3.1]nonan-9-ol,9-methyl-	6.7	3.18	Linalool	6.21	22.53	Pinocarveol	4.5	0.94
20	Citronellic acid	6.8	7.17	3-Methyl butyl 2-methyl butanoate	6.58	18.75	Camphor	4.88	10.38
21	Citronellol epoxide	9	0.90	Isopentylisovalerate	6.81	1.92	Isoborneol	5.1	1.09
22	p-Methane-3,8-diol	6.1	6.37	3-Methyl-3-butenyl-3-methyl butanoate	7.01	0.98	Borneol	5.8	4.74
23	Citronellyl acetate	9.2	4.44	α-Campholenal	8.05	1.05	Isopinocamphone	6.2	0.40
24	p-Methane-1,8-diol	9.3	2.96	trans-Verbenol	8.65	1.42	Terpinen-4-ol	7.2	1.52
25	Geranyl acetate	10	0.58	iso-Menthol	9.54	0.72	Cuminol	7.8	0.65
26	Methyleugenol	10.3	0.63	Verbenone	12.07	0.48	α-terpineol	8.8	6.73
27	Caryophyllene	10.6	2.34	Methyl thymol	12.45	1.73	Endo-isocamphonone	9	0.54
28	Caryophyllene oxide	11	2.23	Linalyl acetate	12.61	3.07	α-terpinene	9.45	0.76
29				Thymol	12.94	1.10	Verbenone	9.79	2.89
30				Carvacrol	13	0.92	Linalyl acetate	10	0.36
31				(E)-Caryophyllene	14.19	0.77	Bornyl acetate	10.64	2.68
32							β-caryophyllene	11.3	4.59
33							Aromadendrene	11.64	1.30
34							α-Humulene	12.4	1.12
35							Caryophyllene oxide	13.6	2.53
36							Humulene epoxide	14.65	0.33
30	Total identified		100.00			100			100

Composition of *C. citriodora*, *L. stricta*, and *R. officinalis* Essential Oils by GC/MS

The constituent analysis presented a number of 28, 32, and 37 compounds of *C. citriodora*, *L. stricta*, and *R. officinalis*, while these EOs were quantified by 0.55%, 36%, and 0.67% (v/w), respectively. The major compounds in *C. citriodora* were isopulegol, citronellol, 3-tetradecanol, citronellic acid, and *p*-methane-3, 8-diol reached 12.77%, 12.42%, 8.28%, 7.17%, and 6.37%, respectively. It was found that *L. stricta* EO contained sabinene, myrcene, limonene, linalool, and 3-methyl butyl 2-methyl butanoate in the amounts of 7.92%, 5.06%, 7.52%, 22.53%, and 18.75%, respectively. The major compounds in *R. officinalis* were α-pinene, camphene, *p*-cymene, limonene, camphor, and α-terpineol, reaching amounts of 7.16%, 5.97%, 6.48%, 5.10%, 10.38%, 6.73%, respectively, as determined by GC/MS (Table 8).

DISCUSSION

In recent decades, the incidence of invasive fungal infections has continuously grown, resulting in mortality and considerable morbidity. Candidiasis and other fungal infections are increased in immunosuppressive disorders, including AIDS and certain chemo- or radiotherapies, as well as smokers' oral cavities (Horn *et al.* 2012; El-Sakhawy *et al*. 2023a). Pathogenic yeasts invade the body at certain sites. If the infection spreads through the bloodstream to the kidneys, lungs, brain, or other organs, it can cause serious systemic complications. These develop only in people who are seriously ill or who have other health problems that weaken the immune system (Rodrigues *et al*. 2019; Talapko *et al*. 2021).

Changes in host defenses, even minor ones, are important for allowing yeasts to invade the skin, respiratory tract, or mucous membranes. For instance, oropharyngeal candidiasis is very common as the yeast *Candida* is a normal oral commensal (Davidson *et al.* 2018; Talapko *et al*. 2021). *Candida albicans* is the most common hospital-acquired infectious agent, and the number of clinical *C. albicans* infections in hospitals has risen significantly in recent years (Pappas *et al.* 2018; Ciurea *et al.* 2020). However, 80% of women have clinical *Candida* infections over their lifetime, and approximately 5% of these thrush infections are recurring, with some infections becoming resistant to antifungal medication. Premature and small babies have an infection rate of up to 7%, and more than half of these patients may die (Krcméry *et al.* 2000; Rosati *et al*. 2020). Generally, Amphotericin B is one of the few treatments that truly kill fungus cells, but it can cause considerable renal damage in individuals. In the late 1980s and 1990s, imidazoles and triazoles were key developments that function by blocking activities of the fungal cell, although they have been proven to result in the recurrence of the infection and the development of drug resistance (Rex *et al.* 1995).

Clinically isolated fungi, including *Candida albicans* and non-*Candida albicans* species, have become increasingly resistant to antifungal medications due to their toxicity and limited effectiveness, as well as the growing side effects of these drugs (Citak *et al.* 2005; Costa-de-Oliveira and Rodrigues 2020). Moreover, resistance to antifungal agents is widely recognized, requiring continuous development of new antifungal agents (Faria *et al.* 2011; Cui *et al.* 2022). As a result, there is a need to look for new, safer, and more effective treatments to combat dangerous fungal infections (Zain *et al.* 2012; Cui *et al*. 2022). On the other hand, the intensive work revealed that the plants are an important source of potentially useful structures for the development of new chemotherapeutic

agents. The first step towards this goal is the *in vitro* antimicrobial activity assay (Zain *et al.* 2012). Plants have been used for medicinal purposes since time immemorial (Chaieb *et al.* 2007). In addition to their reported antimicrobial effects against various pathogens, plant extracts are gaining significant research interest for their anti-insect properties, including oviposition deterrent activity (El-Sakhawy *et al.* 2023b; Abu El-Ghiet *et al*. 2024). A wide range of natural products, especially plant extracts, have antibacterial, antimycotic, and anti-inflammatory properties. Plants are significant because of their applications in traditional medicine and their potential for economic exploitation. They are utilized as scent and flavor enhancers, cosmetics, and medications (Kadri *et al.* 2011; Abd-Elghany *et al*. 2022; El-Hashash *et al*. 2023).

Studies have demonstrated the enormous potential of these natural compounds as antifungal agents (Lima *et al.* 2011). Therefore, *C. citriodora*, *L. stricta*, and *R. officinalis* extracted EOs were selected to test against *Candida* spp. Such attention is justified by their current use in many pharmaceutical, food, and cosmetic goods. As a result, it is not surprising that EOs are one of the most promising classes of natural items for the creation of broad-spectrum, safer, and cheaper antifungal medicines (Lima *et al.* 2011). In the current study, the highest percentage of yeast isolates was *C. albicans* (60.5 %), followed by *C. tropicalis* (23.3%) and *C. krusei* (13.9%). The lowest incidence (2.3%) was for *C. parapsilosis.* This high percentage of *C. albicans* among the investigated patients is in close agreement with findings reported by Pfaller *et al.* (2001), Zhang *et al*. 2020, and El-Sakhawy *et al.* (2023a) who demonstrated that *C. albicans* remains the first yeast pathogen of human and the most common cause of mucosal and systemic fungal infection, While *C. albicans* remains the most frequent fungal pathogen among hospitalized patients, non-albicans *Candida* species are becoming more widespread (Fridkin 2005, Li *et al*. 2023). Unfortunately, many of these fungal species are resistant to the currently available therapeutic options.

In this study, the anti-*Candida* activities of plant EOs were assessed because there are limitations in the use of antifungal drugs, such as resistance (Cosentino *et al.* 1999), toxic effects, and allergic reactions (Duarte *et al.* 2005; Yang *et al*. 2021). Further, in several countries, plants are used in traditional medicine (Kasilo *et al.* 2019). For these reasons, there is a need to test the plant-derived ones, which are cheaper, safer, and more nature-friendly (Brauer *et al.* 2019). According to the results, the essential oils of *C. citriodora*, *L. stricta*, and *R. officinalis* revealed very good antifungal activity against clinically isolated unicellular *Candida* spp. Moreover, *C. citriodora* oil revealed antifungal activity against *Candida* sp., including, *C. albicans* (551), *C. albicans* (8M), *C. albicans* (15K), *C. albicans* (13M), *C. albicans* (524), *C. krusei* (554), *C. tropicalis* (2M), *C. tropicalis* (19K) *C. tropicalis* (510), and *C. tropicalis* (18K). These findings are in the same line with the findings of Ramezani *et al*. (2002) and Luqman *et al.* (2008), who reported the antifungal activity of *C. citriodora* EO against pathogenic *Candida* species. EO of *C. citriodora* is a potent inhibitor of *C. albicans* growth, leading to deleterious morphological changes in cellular structures and cell surface alterations, as demonstrated by Tyagi and Malik (2010) and de Sousa *et al*. (2023). *R. officinalis* EOs revealed antifungal activity against all *C. albicans* isolates. These results are in agreement with the results of Shafaghat *et al.* (2012), Gauch *et al*. (2014), and El-Sakhawy *et al.* (2023b), who stated that EOs from *R. officinalis*, *L. officinalis*, and extracts of *L. officinalis* were shown to have antifungal activities against *C. albicans* or virulence factor (germ tube formation). EOs have many individual components; each of these components adds to the positive or negative effects of these oils. As a result, a thorough understanding of EOs composition enables a better

and more targeted application (Lahlou 2004). However, camphor (1), citronellol (2), geraniol (3), linalool (4), and α-pinene (5) were chosen because they are major components in *C. citriodora* (2 and 3), *L. stricta* (4), and *R. officinalis* (1 and 5), respectively. Zini *et al*. (2003) stated that *C. citriodora* oil is usually rich in citronellal and citronellol, and Egyptian *C. citriodora* oil was found to be rich in geraniol (Abd El Mageed *et al*. 2011). Phytochemical studies have reported the occurrence of α-pinene (43.9 to 46.1%) and camphor (24% to 5.3%) in *Rosemary* EO (Azfali *et al*. 2009; Derwich *et al.* 2010). Linalool (41.2%) was found to be the major constituent in flowering tops and leaves of *Lavandula* sp. (Aburjai *et al.* 2005). The obtained results revealed that the antifungal activity of EOs of *C. citriodora*, *L. stricta*, *and R. officinalis* may be attributed to the volatile components. On the other hand, EOs are not only used in monotherapy but have been used in combination for many years (Lawless 1995; De Rapper *et al.* 2013).

Interestingly, the current study screened the antifungal activities of EOs of *C. citriodora*, *L. stricta*, and *R. officinalis* and the five commercial pure compounds as well as their mixtures. The results revealed that the EO of *C. citriodora* supplemented with citronellol, linalool, and α-pinene showed remarkable antifungal activity against the clinically isolated *C. albicans* (551) and *C. tropicalis* (18k). Accordingly, a new drug(s) for the treatment of *Candida* infections may be used. The synergy is clearly achieved when combining *C. citriodora* essential oil with the volatile compounds linalool, α-pinene, and citronellol against *Candida* spp. In the Al-Mijalli (2023) study, interesting synergistic effects were observed between lemon eucalyptus EO in combination with sweet orange and lentisk EOs. The highest efficient combinations were exhibited against *Candida albicans* and several bacteria, including *Staphylococcus aureus*, *Salmonella enterica*, *E. coli*, and *Bacillus cereus*.

In many cases, the antifungal activity may be attributed to the complex interaction between different classes of compounds, such as phenols, aldehydes, ketones, alcohols, esters, ethers, or hydrocarbons found in EOs (Kim *et al.* 1995; Lambert *et al.* 2001; Burt 2004). However, in some cases, the bioactivities of EOs are closely related to the activity of the main components of oils (Juliani *et al.* 2002). Several studies have found that a number of these compounds exhibited significant antimicrobial properties when tested separately (Bassolé *et al.* 2010; Bajpai *et al.* 2012).

The antifungal activity of EOs has been reported in numerous studies, but the specific mechanism of action for all pure volatile constituents remains uncertain. The diversity of volatile constituents and their concentrations results in a broad spectrum of antifungal activity and diverse mechanisms. The primary reported mechanism of action involves disruption of cell membranes, which appears to be facilitated by the lipophilic nature and varied chemical structures of EOs, which allow them to penetrate and cause leakage in fungal cell membranes. Other antifungal mechanisms of EOs include disruption of cell walls, inhibition of fungal enzymes, and damage to fungal nucleic acids, proteins, and lipids. These effects collectively lead to the inhibition of sporulation, hyphal extension, and spore germination (Cavanagh 2007; Basak and Guha 2018; El-Sakhawy *et al*. 2025). Due to their lipophilic nature and variety of chemical structures, which enable them to pierce and damage the fungal cell membrane, EOs inhibit fungi. They produce cell leakage and increase permeability by their interaction with ergosterol, a crucial component of mycotic membranes. While EOs' hydrophobic nature ensures their preferential partitioning into lipid membranes or may induce membranes to depolarise, phenolics' structure (Nazzaro *et al.* 2013; El-Sakhawy *et al.* 2025).

The results confirmed this postulate when mixing essential oils and/or commercial volatile compounds showed antifungal activities better than the separate ones. Germ tube formation in the current study was strongly inhibited by the mixture of *Corymbia* EO supplanted with linalool, α-pinene, and citronellol pure volatile compounds at concentrations well below the MIC of this mixture against *C. albicans* (551) that may explain the remarkable antifungal activity of this mixture since, germ tube formation was regarded as an important step of pathogenicity of *C. albicans* as demonstrated by (Hammer *et al*. 2000; Calderone and Fonzi 2001; Pinto *et al*. 2008). Ellepola and Samaranayake (1998) demonstrated that most antifungal agents are described as germ tube formation inhibitors; this attribute may well constitute a relevant therapeutic advantage, considering the importance of filamentation in the evolution from commensality to pathogenicity in *C. albicans*.

According to certain research, some EOs have both direct and indirect effects on fungal mycelia by permeating the growth medium and diffusing into cells. The mechanism of action of EOs has been demonstrated, with EOs preferentially absorbed onto the lipophilic surface of mycelia, and the amount of inhibition rises with mycelial surface area. It is theorized that EOs establish irreversible cross-links with components of the fungal cell membrane, resulting in intracellular leaking; furthermore, studies have shown that EOs of some medicinal plants have demonstrated considerable reductions in mold sporulation. (Dhifi *et al*. 2016; Atabati *et al*. 2020; Abdi-Moghadam *et al*. 2023).

Furthermore, while it is theoretically possible for resistance to any antimicrobial to emerge, the complex, multi-component nature of EOs and their combinations may pose a higher barrier to the development of increasing resistance compared to single-target synthetic antifungals. This is because they often target multiple fungal cellular processes simultaneously, including disrupting membrane integrity, suppressing efflux pumps, inhibiting cell wall synthesis, inducing mitochondrial dysfunction, preventing biofilm formation, and promoting oxidative stress. A promising antifungal strategy involves combining EOs with other pure volatile compounds to create synergistic effects that combat resistance (Chouhan *et al*. 2017; Khan, 2024; Kowalczyk 2024; Leiva-Mora *et al*. 2025). This synergy represents a novel, naturally derived approach to overcoming the emergence of fungal resistance.

It is worth noting that the effects of plant EOs and pure volatile compounds against clinically isolated *Candida* research align and support sustainable development goals (SDGs) by improving good health and well-being (SDG 3).

CONCLUSIONS

Results of this study indicated the synergy between essential oils (EOs) of *Corymbia citriodora*, *Rosmarinus officinalis*, and *Lavandula stricta*, as well as five pure volatile compounds, as alternative antifungal agents against *Candida* spp.

1. The results showed that blending two or more EOs or pure volatile compounds can enhance their antifungal properties. For example, a mixture of pure volatile compounds has been demonstrated to have a remarkable synergistic effect against *Candida* species, potentially by targeting multiple cellular pathways simultaneously.

2. Overall, natural plant products are a promising source of new antifungal medicines that could help combat drug-resistant *Candida* infections in the future. In addition to their potential applications in the food packaging industry, further research is needed to elucidate the mechanisms underlying their antifungal effects and to explore their future uses.

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