Antimicrobial Activity of a Carvacrol-rich *Thymus maroccanus* Ball. Essential Oil: Whole-oil Superiority over its Major Constituent

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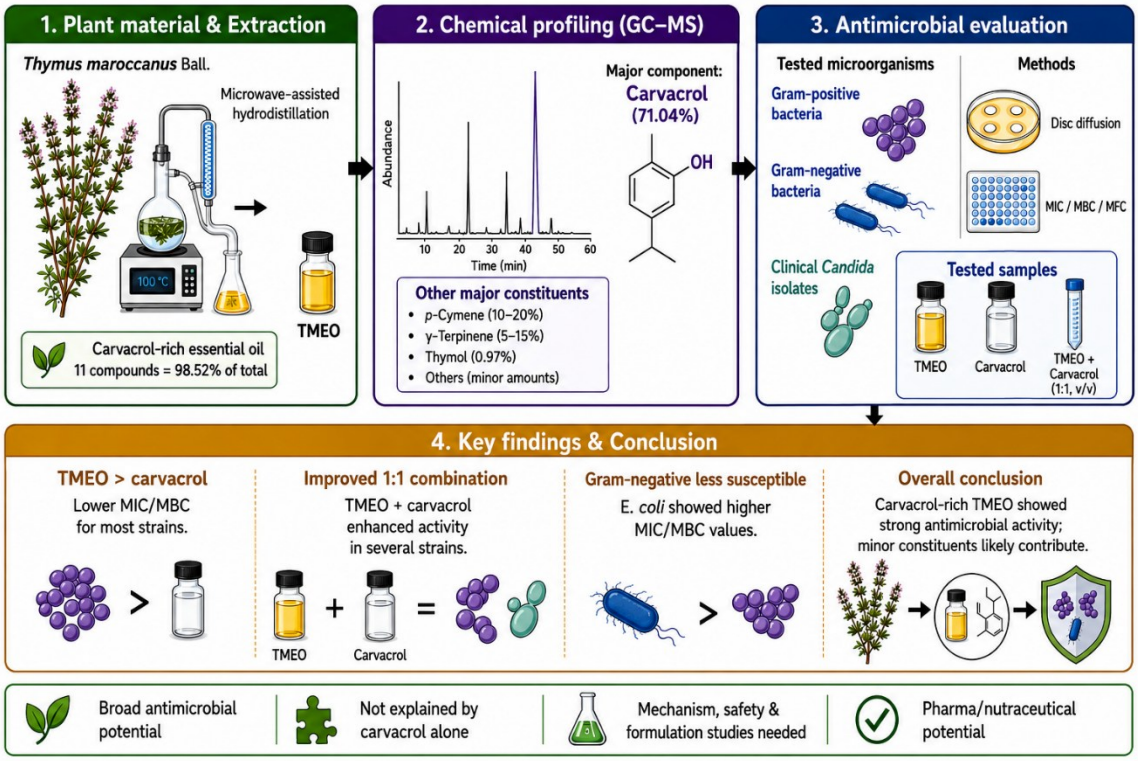
GRAPHICAL ABSTRACT



Antimicrobial Activity of a Carvacrol-rich *Thymus maroccanus* Ball. Essential Oil: Whole-Oil Superiority Over its Major Constituent

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This study characterized *Thymus maroccanus* essential oil (TMEO) and compared its antimicrobial activity with carvacrol and their combination. The TMEO was obtained by microwave-assisted hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC–MS), revealing a carvacrol-dominant profile (71.04%) with 11 compounds representing 98.52% of the volatile fraction. Antimicrobial activity was assessed against Gram-positive and Gram-negative bacteria and clinical *Candida* isolates using disc diffusion minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration **(**MFC) assays. TMEO showed low MIC values and bactericidal activity against *Staphylococcus aureus* and *Micrococcus luteus* (MBC/MIC ≤ 2), while *Escherichia coli* was less susceptible. Notably, TMEO consistently outperformed carvacrol alone, indicating that the activity of the whole oil cannot be explained by its major component only. A 1:1 (v/v) TMEO–carvacrol combination further improved antimicrobial activity against several strains, particularly Gram-positive bacteria and *Candida albicans*. However, in the absence of interaction assays, this effect is interpreted as enhanced combined activity rather than confirmed synergy. The observed differences in susceptibility are consistent with known structural and physiological variation among microorganisms. In conclusion, *T. maroccanus* was found to be a carvacrol-rich chemotype with promising antimicrobial activity, warranting further mechanistic, safety, and formulation studies for practical applications.

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Keywords: Thymus essential oil; Carvacrol; Antibacterial activity; Antifungal potential; Microwave essence extraction; GC-MS profiling

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INTRODUCTION

The continuous rise of antimicrobial resistance represents a major global health challenge, significantly reducing the effectiveness of conventional antibiotics and antifungal agents (Fisher *et al.* 2022; Salam *et al.* 2023; Vitiello *et al.* 2023). Pathogenic bacteria and opportunistic yeasts increasingly display resistance to common antimicrobials, leading to persistent infections and limited therapeutic choices (Perlin *et al.* 2017). This situation has renewed interest in plant-derived antimicrobials, particularly essential oils, as complementary sources of membrane-active compounds. However, their value depends on

careful chemical characterization, standardized antimicrobial testing, and cautious interpretation of activity in relation to conventional drugs (Khwaza and Aderibigbe 2025). Essential oils (EOs) are complex mixtures of volatile substances found in various medicinal plants. They are mainly composed of terpenes and phenolic compounds, which are widely known for their broad-spectrum antimicrobial effects (Raut and Karuppayil 2014). Their mechanisms of action often encompass disruption of microbial cell membranes, alteration of permeability, leakage of intracellular components, and interference with enzymatic systems. Because EOs contain chemically diverse constituents, their antimicrobial activity may involve multiple cellular targets, including membrane disruption, altered permeability, and leakage of intracellular materials. Nevertheless, reduced resistance development should be considered a hypothesis rather than a proven advantage unless supported by resistance-selection experiments (Reyes-Jurado *et al.* 2015; Vergis *et al.* 2015 ; Chouhan *et al.* 2017).

The genus *Thymus* (Lamiaceae) includes several species traditionally used in folk medicine for the treatment of respiratory, gastrointestinal, and infectious diseases (Chaachouay *et al.* 2023). Several *Thymus* species are recognized for producing EOs rich in phenolic monoterpenes, especially thymol and carvacrol, components with well-documented antimicrobial potential. Among these, *Thymus maroccanus* Ball. is an endemic Moroccan species that remains relatively underexplored despite its traditional medicinal use and promising phytochemical profile (Abbad *et al.* 2011; Belaqziz *et al.* 2013; Aljaiyash *et al.* 2022).

Carvacrol, one of the major bioactive constituents commonly found in *Thymus* oils, has demonstrated strong antimicrobial activity against a wide range of Gram-positive and Gram-negative bacteria, as well as pathogenic yeasts (Mączka *et al.* 2023). Its antimicrobial efficacy has been attributed to its ability to disrupt cytoplasmic membranes, collapse proton motive force, and induce leakage of cellular contents (Mastelić *et al.* 2008). However, the activity of isolated compounds may differ from that of the whole EO due to potential additive, enhancing, or antagonistic interactions among constituents (Chroho *et al.* 2024).

Previous studies reported synergistic interactions under conditions validated by checkerboard or related assays, leading to enhanced antimicrobial activity, although formal confirmation of synergy requires dedicated interaction assays such as checkerboard analysis, fractional inhibitory concentration indices, or time–kill approaches (Ju *et al.* 2022; Soulaimani 2025). Such combinations may enhance antimicrobial performance compared with individual constituents and, in some contexts, may offer advantages over conventional antibiotics. However, their practical value cannot be assumed from *in vitro* activity alone. Toxicological safety, formulation stability, and any resistance-modulating effects must be experimentally confirmed before therapeutic claims are justified. At present, evidence on the combined antimicrobial activity of *T. maroccanus* EO and carvacrol remains limited.

Therefore, this study aimed to characterize the volatile composition of *T. maroccanus* essential oil obtained by microwave-assisted hydrodistillation and to evaluate its antibacterial and anticandidal activity alone, as pure carvacrol, and as an EO–carvacrol combination. Antimicrobial performance was assessed using disc diffusion, minimum inhibitory concentration (MIC), minimum fungicidal concentration-to-minimum inhibitory concentration (MFC/MIC) ratios, or minimum bactericidal concentration-to-minimum inhibitory concentration (MBC/MIC) ratios. Because no checkerboard or time–kill interaction assay was performed, the combined treatment is interpreted as enhanced

combined activity rather than confirmed synergy. This cautious framing strengthens the biological interpretation and avoids overextension of the available data.

EXPERIMENTAL

Chemicals

Microbiological culture media and reagents used in the antimicrobial assays included Luria–Bertani (LB) medium, nutrient agar, potato dextrose agar (PDA), yeast extract–peptone–dextrose (YPD) medium, erythromycin, fluconazole, p-iodonitrotetrazolium chloride (INT), dimethyl sulfoxide (DMSO), and sodium chloride. Commercial carvacrol (CAS No. 499-75-2; product No. W224511; manufacturer-specified assay, 99%) was purchased from Sigma-Aldrich, France, and used as received without further purification as the antimicrobial test compound. It was not used as a GC–MS identification or quantification standard, and its residual impurity/isomer profile was not independently characterized. INT was prepared at 0.2 mg/mL for use as the microbial viability indicator in the broth microdilution assay.

Plant Material and EO Extraction

Thymus maroccanus aerial parts were collected in April 2024 from the High Atlas region of Morocco, at approximately 31.286° N, 7.966° W and an elevation of approximately 1,260 m above sea level. The plant material was taxonomically authenticated by Dr. Hanae Naceiri Mrabti (Laboratory of Drug Sciences, Faculty of Medicine, Pharmacy, and Dental Medicine of Fez, Sidi Mohamed Ben Abdellah University, Fez, Morocco), and a voucher specimen (T8025) was deposited in the Laboratory of Drug Sciences. The aerial parts were shade-dried at room temperature under well-ventilated conditions for approximately two weeks and stored in a tightly sealed container until essential-oil extraction.

For essential-oil extraction, 90 g of dried aerial material was immersed in 1.5 L of distilled water and subjected to microwave-assisted hydrodistillation using a Whirlpool MWD 119 microwave oven (20 L capacity; 2.45 GHz) directly connected to a Clevenger-type apparatus equipped with a water-cooled condenser supplied continuously with cold tap water throughout the extraction to ensure efficient condensation of the volatile components. Extraction was performed at 600 W for 20 min. The recovered essential oil was separated from the aqueous phase, dried over anhydrous sodium sulfate to remove residual moisture, transferred into a tightly sealed amber glass vial, and stored at 4 °C in the dark until GC–MS analysis and antimicrobial testing. Essential-oil yield was calculated as the volume of recovered oil per 100 g of dry plant material and expressed as mL EO/100 g dry weight.

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

The volatile composition of TMEO was analyzed using an HP 6890 gas chromatograph coupled to an HP 5973 mass-selective detector and equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness), following the previously described analytical procedure with minor adaptation (Al-Mijalli *et al.* 2025). The oven temperature was initially maintained at 50 °C for 5 min and then increased to 200 °C at a rate of 4 °C/min. Helium was used as the carrier gas at a flow rate of 1.5 mL/min. A 1 μL aliquot of the diluted essential oil was injected, and mass spectra were

acquired under electron-impact ionization at 70 eV, with the ion-source temperature maintained at 230 °C and a scan range of *m/z* 35 to 450. Volatile constituents were tentatively identified by comparison of their mass spectra with the NIST mass-spectral library and by comparison of their calculated retention indices with published reference values. Retention indices were determined under the same chromatographic conditions using a homologous series of *n*-alkanes (C9–C31). The relative abundance of each constituent was calculated from its chromatographic peak area and expressed as a percentage of the total detected volatile fraction.

Studied Microorganisms

The antimicrobial activity of TMEO was evaluated against six microorganisms comprising three Gram-positive bacterial reference strains, *Listeria innocua* ATCC 33090, *Micrococcus luteus* ATCC 14452, and *Staphylococcus aureus* ATCC 29213; one Gram-negative bacterial strain, *Escherichia coli* ATCC 25922; and two clinical yeast isolates, *Candida albicans* and *Candida tropicalis*. The clinical *Candida* isolates were obtained from the laboratory collection of the Laboratory of Drug Sciences, Faculty of Medicine, Pharmacy, and Dental Medicine of Fez, Sidi Mohamed Ben Abdellah University, Fez, Morocco, and had been previously identified to the species level using routine microbiological identification procedures. Before antimicrobial testing, bacterial strains were subcultured on nutrient agar and incubated at 37 °C for 24 h, whereas the *Candida* isolates were subcultured on potato dextrose agar (PDA) and incubated at 37 °C for 48 to 72 h. Fresh colonies were suspended in sterile physiological saline (0.85% NaCl), and the turbidity of each suspension was adjusted to a 0.5 McFarland standard. The standardized suspensions were subsequently diluted as required to obtain final inoculum concentrations of approximately 1×10^6 CFU/mL for bacterial assays and 1×10^4 CFU/mL for yeast assays. Freshly prepared inocula were used for all antimicrobial experiments.

Disc-diffusion Test

The antimicrobial activity of TMEO, carvacrol, and the TMEO–carvacrol combination was evaluated using the agar disc-diffusion method with minor modifications from a previously described protocol (Al-Mijalli *et al.* 2023). Standardized microbial suspensions were evenly inoculated onto Luria–Bertani (LB) agar for bacterial strains and YPD agar for *Candida* isolates. Sterile paper discs (6 mm diameter) were loaded with 10 µL of undiluted TMEO, undiluted carvacrol, or a TMEO–carvacrol mixture prepared at a fixed 1:1 (v/v) ratio and placed onto the inoculated agar surfaces. DMSO was maintained at a final concentration of 0.5% (v/v) in the corresponding assay system, and discs containing 0.5% DMSO without the test compounds served as negative controls. Erythromycin (15 µg/disc) was used as the positive control for bacterial strains, whereas fluconazole (10 µg/disc) served as the positive control for *Candida* isolates. Plates were incubated at 37 °C for 24 h for bacteria and at 25 °C for 48 h for *Candida* isolates. Following incubation, inhibition-zone diameters, including the 6-mm disc diameter, were measured in millimeters. Each assay was performed in three independent experiments, and the results were expressed as mean ± standard deviation. Antimicrobial activity was classified according to Galovičová *et al.* (2021), with inhibition zones >15 mm considered very strong, >10 mm moderate, and >5 mm weak

MIC Assay

The minimum inhibitory concentrations (MICs) of TMEO, carvacrol, and the TMEO–carvacrol combination were determined using a broth microdilution method adapted from Belaqziz *et al.* (2013), with minor modifications. Two-fold serial dilutions of each test preparation were prepared over a concentration range of 16.0 to 0.0625% (v/v) in Luria–Bertani (LB) broth for bacterial strains and YPD broth for *Candida* isolates. This concentration range was selected to encompass the expected antimicrobial activity of the tested preparations, while allowing determination of inhibitory endpoints across a broad range using two-fold serial dilutions. DMSO was maintained at a final concentration of 0.5% (v/v) in the test wells and corresponding solvent controls. Aliquots of 190 µL of each dilution were dispensed into sterile 96-well microplates, followed by 10 µL of the appropriately diluted microbial suspension to obtain final inoculum concentrations of approximately 1×10^6 CFU/mL for bacteria and 1×10^4 CFU/mL for yeasts. Plates were incubated at 37 °C for 24 h for bacterial strains and at 25 °C for 48 h for *Candida* isolates. Wells containing inoculated broth with 0.5% DMSO but without the test preparation served as growth/solvent controls, whereas uninoculated broth served as the sterility control. Following incubation, 25 µL of p-iodonitrotetrazolium chloride (INT; 0.2 mg/mL) was added to each well as a microbial viability indicator. The MIC was defined as the lowest concentration at which no visible color change from yellow to pink occurred, indicating inhibition of detectable microbial growth. For the combination treatment, equal volumes of TMEO and carvacrol were mixed at a fixed 1:1 (v/v) ratio before serial dilution, and the reported concentrations refer to the total concentration of the mixture.

Determination of MBC, MFC, and Killing Profile

Subsequent to MIC determination, the MBC for bacterial strains and the MFC for fungal strains were determined using a standardized agar subculture technique, with minor adaptations from previously reported methods (Hernandes *et al.* 2013). Briefly, 50 µL aliquots were withdrawn from microdilution wells corresponding to the MIC and higher EO concentrations and aseptically spread onto appropriate solid media. The inoculated plates were incubated under optimal growth conditions. Following incubation, plates were inspected for visible colony formation. The MBC or MFC was defined as the lowest concentration of the tested samples resulting in complete absence of microbial growth, thereby confirming irreversible microbial killing rather than growth inhibition. To estimate the killing profile of each treatment, and the killing profiles which are MBC/MIC and MFC/MIC ratios were calculated. Ratios ≤ 4 were considered indicative of bactericidal or fungicidal activity, whereas ratios > 4 suggested a predominantly bacteriostatic or fungistatic effect (García-Vela *et al.* 2025). This ratio-based interpretation provides insight into the killing efficiency and bactericidal or fungicidal tendency of the tested samples.

Statistical Analysis

Inhibition-zone assays were performed in three independent experiments, and the results are expressed as mean ± standard deviation (SD). For each microorganism, differences among TMEO, carvacrol, the TMEO–carvacrol combination, and the reference antimicrobial were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's honestly significant difference (HSD) post hoc test when a significant overall difference was detected. Differences were considered statistically significant at $p < 0.05$. MIC, MBC, and MFC assays were performed in triplicate and reported as discrete two-fold dilution endpoints; these values and the derived MBC/MIC and MFC/MIC ratios were

not subjected to parametric statistical analysis. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 32.0 (IBM Corp., Armonk, NY, USA).

Methodological Limitations

The TMEO–carvacrol combination was evaluated at a fixed 1:1 (v/v) ratio; however, checkerboard, fractional inhibitory concentration index (FICI), and time–kill interaction assays were not performed. Accordingly, improvements observed with the combination are interpreted as enhanced combined activity rather than confirmed synergism. In addition, inhibition-zone comparisons between the essential-oil preparations and reference antimicrobials should be regarded as descriptive because these agents differ substantially in applied dose, agar diffusion behavior, volatility, and physicochemical properties. These limitations should be considered when interpreting the comparative antimicrobial results.

RESULTS AND DISCUSSION

Volatile Content

GC–MS analysis identified 11 volatile constituents in TMEO (Table 1; Fig. 1). The oil was characterized predominantly by oxygenated monoterpenes, with carvacrol as the major constituent, indicating a strongly phenolic monoterpene-rich profile. The contribution of minor constituents should nevertheless be considered when interpreting the biological activity of the whole oil.

Table 1. Volatile Composition of TMEO identified with GC-MS*

No.	Compounds	Molecular Formula	RI	Relative Peak Area (%)
1	α-Thujene	C10H16	928	0.33
2	α-Pipene	C10H16	938	3.02
3	Myrcene	C10H16	958	1.47
4	p-Cymene	C10H16	1026	8.15
5	γ -Terpinene	C10H16	1054	3.91
6	Linalool	C10H18O	1082	0.51
7	α-Terpineol	C10H18O	1185	4.68
8	Thymol	C10H14O	1290	0.97
9	Carvacrol	C10H14O	1301	71.04
10	(E)-Caryophyllene	C15H24	1421	3.9
11	ß-Bisabolene	C15H24	1511	0.54
	Total identified %		98.52	
	Monoterpene hydrocarbons		16.88	
	Oxygenated monoterpenes		77.2	
	Sesquiterpene hydrocarbons		4.44	

***Note:** RI, retention index calculated relative to a homologous series of *n*-alkanes (C9–C31) under the stated GC–MS conditions

The present TMEO showed a clearly carvacrol-rich profile with a comparatively low thymol contribution (Table 2). Considerable intraspecific variation has been reported for *T. maroccanus*, including differences associated with plant part and phenological stage

(Belaqziz *et al.* 2013; Aljaiyash *et al.* 2022). The present profile therefore reinforces the marked chemical variability reported within this species.

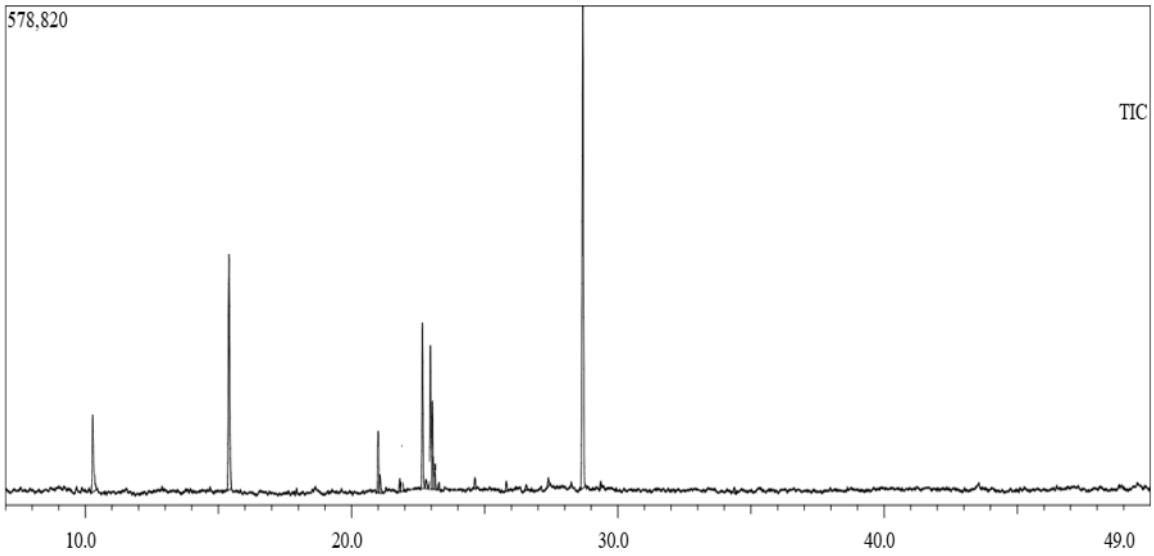

Fig. 1. Chromatogram of GC analysis of TMEO

Table 2. Comparison of Major Volatile Constituents of the Present TMEO with Selected *Thymus* Essential Oils Reported in Previous Studies *

Species/Sample	Carvacrol (%)	Thymol (%)	*p*-Cymene (%)	γ-Terpinene (%)	Dominant profile	Reference
T. maroccanus (present study)	71.04	0.97	8.15	3.91	Carvacrol-rich	Present study
T. maroccanus (different phenological stages)	63.70 to 68.19	ND	6.09 to 9.67	3.67 to 8.49	Carvacrol-rich	Aljaiyash *et al.* (2022)
T. maroccanus (leaf oil)	33.0	—	25.3	—	Carvacrol/*p*-cymene-rich	Belaqziz *et al.* (2013)
T. vulgaris	5.5	48.1	11.7	6.1	Thymol-rich	Galovičová *et al.* (2021)
T. zygis	8.1	5.0	50.6	—	*p*-Cymene-rich	Tantaoui-Elaraki *et al*. (1993)
T. broussonetii	53.3	—	13.5	—	Carvacrol-rich	Tantaoui-Elaraki *et al*. (1993)
T. capitatus	65.38	1.35	—	—	Carvacrol-rich	Zaïri *et al.* (2019)

* **Note:** Values represent relative essential-oil composition (%) as reported in the respective studies. ND, not detected; —, not reported or not included in the source data used for comparison. Essential-oil composition may vary according to plant part, geographic origin, phenological stage, environmental conditions, and analytical methodology.

Clear chemotypic differences are also evident across the genus. *T. vulgaris* is frequently represented by thymol-rich oils, whereas strongly carvacrol-dominant profiles

have been reported for *T. capitatus* and some *T. maroccanus* oils (Jordán *et al.* 2006; Abdelli *et al.* 2017; Schött *et al.* 2017; Galovičová *et al.* 2021; Gonçalves *et al.* 2017; Zaïri *et al.* 2019). Similar inter- and intraspecific variability has been described for *T. zygis*, *T. satureioides*, *T. broussonetii*, and *T. algeriensis* (Tantaoui-Elaraki *et al.* 1993; Ben El Hadj Ali *et al.* 2015). These differences may reflect geographic origin, altitude, phenological stage, and other environmental factors (Aljaiyash *et al.* 2022; El-Jalel *et al*. 2018). Importantly, despite the predominance of carvacrol, minor constituents such as *p*-cymene, γ-terpinene, and α-terpineol may also contribute to the biological activity through additive or enhancing interactions.

Antibacterial Activity

The antibacterial activity of TMEO, its major constituent carvacrol, and their combination TMEO + carvacrol was evaluated against both Gram-positive and Gram-negative bacterial strains and compared with erythromycin (15 µg/disc).

Disc-diffusion testing showed a clear strain-dependent antibacterial pattern (Table 3). According to the adopted inhibition-zone classification, TMEO showed very strong activity against *S. aureus* and *M. luteus* and moderate activity against *L. innocua* and *E. coli*. Carvacrol alone was less active than TMEO across all tested strains, supporting the contribution of minor essential-oil constituents to the overall antibacterial effect. Although carvacrol predominates in TMEO, compounds such as *p*-cymene may enhance its activity by modifying membrane properties and facilitating the action of more active phenolic constituents.

Table 3. Antibacterial Activity of TMEO, Carvacrol, and TMEO + Carvacrol *

Bacterial Strains	Mean Zone of Inhibition (mm ± SD)			
	TMEO (10 µL/disc)	Carvacrol	TMEO + Carvacrol	Erythromycin (15 µg/disc)
Micrococcus luteus ATCC 14452	20.00 ± 1.07^{c}	14.00 ± 0.64^{a}	28.11 ± 1.36^{d}	15.32 ± 1.12^{b}
Listeria innocua ATCC 33090	14.46 ± 0.28^{b}	12.18 ± 0.71^{a}	14.52 ± 0.11^{b}	14.23 ± 0.14^{b}
Staphylococcus aureus ATCC 29213	21.13 ± 0.34^{c}	17.25 ± 0.16^{a}	23.05 ± 1.54^{d}	19.40 ± 0.3^{b}
Escherichia coli ATCC 25922	11.28 ± 0.16^{b}	10.42 ± 0.13^{a}	11.00 ± 0.48^{b}	12.67 ± 0.09^{c}

* Values are expressed as mean ± SD from three independent experiments (n = 3). Within each row, values with different superscript letters are significantly different according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

The fixed 1:1 (v/v) TMEO–carvacrol combination enhanced antibacterial activity most clearly against *M. luteus* and *S. aureus*, whereas little improvement was observed against *L. innocua* and *E. coli*. This pattern indicates that the effect of the combination was strain dependent.

The TMEO produced inhibition zones comparable to or larger than those of erythromycin under the assay conditions; however, direct potency comparison is limited due to differences in concentration, diffusion behavior, and physicochemical properties between essential oils and standard antibiotics.

The microdilution findings (Table 4) were consistent with the disc-diffusion results. Gram-positive bacteria were generally more susceptible to TMEO than *E. coli*, and TMEO required lower inhibitory concentrations than carvacrol alone. The fixed TMEO–carvacrol combination further improved inhibitory and bactericidal activity in most tested strains, with the clearest effects observed among the Gram-positive bacteria and an improved killing profile against *E. coli*.

Table 4. MIC, MBC, and Killing Profile (Based on MBC/MIC or MFC/MIC Ratios) of Tested Bacteria *

Bacteria	TMEO (%v/v)			Carvacrol (%v/v)			TMEO + Carvacrol (%v/v)		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
M. luteus	0.25	0.25	1	0.5	1	2	0.125	0.125	1
L. innocua	0.5	1	2	1	2	2	0.25	0.25	1
S. aureus	0.125	0.25	2	1	2	2	0.125	0.125	1
E. coli	0.5	4	8	2	8	4	0.5	1	2

* MIC and MBC determinations were performed in triplicate and are reported as discrete two-fold dilution endpoints. MBC/MIC ratios were calculated from the corresponding endpoint values.

The antibacterial activity of TMEO is likely influenced by its high carvacrol content; however, the greater activity observed for TMEO compared to carvacrol alone indicates that additional constituents contribute to the overall effect, a phenolic monoterpene widely recognized for its ability to disrupt bacterial cell membranes, increase permeability, and induce leakage of intracellular constituents (Kachur and Suntres 2020). However, the superior activity of TMEO compared to pure carvacrol indicates that other molecules within the EO likely contribute through additive or enhancing interactions.

The enhanced activity observed for the TMEO + carvacrol combination, particularly against *M. luteus* and *S. aureus*, suggests an enhancing or additive interaction between TMEO constituents and carvacrol. Such interactions may facilitate improved partitioning of carvacrol into bacterial membranes or modify membrane fluidity, thereby enhancing permeability and antimicrobial action. The pronounced increase in activity against *M. luteus* may reflect higher membrane susceptibility to phenolic monoterpenes. The reduced efficacy observed against *E. coli* is consistent with the intrinsic resistance of Gram-negative bacteria, which possess an outer membrane rich in lipopolysaccharides (LPS) that limits the diffusion of hydrophobic compounds including volatile molecules (Kim *et al.* 2010; Ce pas *et al.* 2019).

Importantly, the TMEO + carvacrol combination demonstrated antibacterial activity comparable to or exceeding that of erythromycin against selected Gram-positive bacteria. This finding highlights the potential of TMEO-based formulations as natural antibacterial agents or as adjuvants to conventional antibiotics, particularly in the context of rising antibiotic resistance.

Anticandidal Activity

The anticandidal activity of TMEO, carvacrol, as well as TMEO + carvacrol was assessed against clinical isolates of *C. albicans* and *C. tropicalis* and compared with fluconazole (10 µg/disc) (Table 5).

Disc-diffusion testing (Table 5) showed greater susceptibility of *C. albicans* than *C. tropicalis* to TMEO. Carvacrol alone was less active than TMEO against both isolates. The TMEO–carvacrol combination significantly increased activity against *C. albicans*, whereas its activity against *C. tropicalis* did not differ significantly from TMEO alone.

Table 5. Anticandidal Effect of TMEO, Carvacrol, and TMEO + Carvacrol *

Fungal Strains	*TMEO (10 µL of EO/disc)*	*Carvacrol*	Mean Zone of Inhibition (mm)	
			TMEO + Carvacrol	Fluconazole (10 µg/disc)
Candida albicans (Clinical isolate)	22.65 ± 2.93^{b}	16.24 ± 1.07^{a}	24.30 ± 1.84^{c}	16.25 ± 0.33^{a}
Candida tropicalis (Clinical isolate)	15.50 ± 0.62^{b}	12.00 ± 0.16^{a}	15.78 ± 0.56^{b}	20.15 ± 1.27^{c}

* Values are expressed as mean ± SD from three independent experiments (n = 3). Within each row, values with different superscript letters are significantly different according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$).

Compared with fluconazole, TMEO and TMEO + carvacrol displayed superior activity against *C. albicans*, whereas fluconazole remained more effective against *C. tropicalis*.

The microdilution results (Table 6) supported the disc-diffusion findings. TMEO required lower inhibitory concentrations than carvacrol alone against both *Candida* isolates. The fixed TMEO–carvacrol combination further reduced the inhibitory concentrations and produced the most favorable fungicidal profile against *C. albicans*, whereas *C. tropicalis* remained comparatively less susceptible.

Table 6. MIC, MFC, and Killing Profile (Based on MBC/MIC or MFC/MIC Ratios) of Tested Yeasts *

Yeast	TMEO (%v/v)			Carvacrol (%v/v)			TMEO + Carvacrol (%v/v)		
	MIC	MFC	MFC/MIC	MIC	MFC	MFC/MIC	MIC	MFC	MFC/MIC
C. albicans	1	2	2	4	8	2	0.5	0.5	1
C. tropicalis	4	4	1	8	16	2	2	4	2

* MIC and MFC determinations were performed in triplicate and are reported as discrete two-fold dilution endpoints. MFC/MIC ratios were calculated from the corresponding endpoint values.

The pronounced anticandidal activity of TMEO, particularly against *C. albicans*, can be ascribed primarily to its high carvacrol content, combined with the contribution of minor terpenoid compounds that may contribute through additive or enhancing interactions (Kachur and Suntres 2020; Chroho *et al.* 2024). Carvacrol is known to exert antifungal effects through disruption of fungal cell membranes, interference with ergosterol

biosynthesis, and induction of oxidative stress, ultimately compromising membrane integrity and cellular viability (Lima *et al.* 2013; Nóbrega *et al.* 2016).

The enhanced activity observed for the TMEO + carvacrol combination against *C. albicans* suggests the presence of an enhanced interaction between carvacrol and other TMEO constituents. These interactions may increase membrane permeability or facilitate deeper penetration of carvacrol into fungal cells, thereby amplifying its antifungal effect. The relatively high standard deviation observed for TMEO against *C. albicans* may reflect biological variability among clinical isolates, which often differ in membrane composition, efflux activity, and antifungal susceptibility profiles.

In contrast, the limited improvement observed against *C. tropicalis* suggests a lower susceptibility of this species to membrane-active phytochemicals. This reduced sensitivity may be related to differences in cell wall composition, efflux pump activity, or intrinsic resistance mechanisms that diminish the efficacy of oil substances. The observation that TMEO and its combination with carvacrol produced larger inhibition zones than fluconazole against *C. albicans* under the experimental conditions; however, this observation should be interpreted cautiously due to differences in diffusion properties and standardized dosing between essential oils and antifungal drugs.

Although carvacrol was the dominant constituent, the biological activity of TMEO should not be interpreted solely from its abundance. Several minor constituents identified in the oil have independent or interaction-dependent activities. In a direct antioxidant comparison, thymol showed a higher oxygen radical absorbance capacity than carvacrol (47 versus 33 μmol Trolox equivalents/mg, respectively), although this difference was assay-dependent because the two compounds produced comparable results in other antioxidant assays (Miguel *et al.* 2010). In an antimicrobial model, p-cymene enhanced the activity of carvacrol against planktonic *Gardnerella* spp., whereas carvacrol–linalool combinations at sub-MIC concentrations were particularly effective against biofilm cells (Sousa *et al.* 2022). These observations provide a plausible context for the greater antimicrobial activity of TMEO than carvacrol alone in the present study, without establishing the contribution of any individual minor constituent or confirming synergy.

Other constituents detected in TMEO also possess biological activities beyond antimicrobial action. α-Terpineol inhibited several human tumour cell lines, with NCI-H69 small-cell lung carcinoma being the most sensitive (IC50 = 0.26 mM); this effect was associated with suppression of NF-κB signalling (Hassan *et al.* 2010). Likewise, (E)-caryophyllene, also known as β-caryophyllene, exhibited antioxidant activity and selective antiproliferative activity against HCT116 colorectal cancer cells (IC50 = 19 μM), together with antibacterial activity against *S. aureus* (Dahham *et al.* 2015). These antioxidant and anticancer findings cannot be quantitatively compared with the inhibition-zone and MIC endpoints measured in the present study because they were obtained using different models, concentrations, and assay procedures. Nevertheless, they demonstrate the broader biological relevance of non-carvacrol constituents and support future component-specific investigation of TMEO.

CONCLUSIONS

1. Under the tested *in vitro* conditions, *Thymus maroccanus* essential oil (TMEO) showed greater antimicrobial activity than carvacrol alone against all tested bacterial strains and *Candida* isolates. This superiority was demonstrated by consistently

larger inhibition zones and lower minimum inhibitory concentration (MIC) values for TMEO than for its major isolated constituent.

2. The fixed 1:1 (v/v) TMEO–carvacrol combination further improved antimicrobial activity against several tested microorganisms. Relative to TMEO alone, the combination reduced the MIC against *Micrococcus luteus* from 0.25 to 0.125% (v/v), *Listeria innocua* from 0.50 to 0.25% (v/v), *Candida albicans* from 1.0 to 0.50% (v/v), and *Candida tropicalis* from 4.0 to 2.0% (v/v). It also reduced the minimum bactericidal concentration (MBC) against *Staphylococcus aureus* from 0.25 to 0.125% (v/v) and against *Escherichia coli* from 4.0 to 1.0% (v/v). Because no interaction assay was performed, these results demonstrate enhanced combined activity, not confirmed synergy.
3. Among the bacterial strains, TMEO was most active against the Gram-positive organisms. *S. aureus* and *M. luteus* showed the lowest MIC values, 0.125 and 0.25% (v/v), respectively, with MBC/MIC ratios ≤ 2. In contrast, *E. coli* was less susceptible to TMEO, with an MBC/MIC ratio of 8.
4. In the anticandidal assays, TMEO was more active against *C. albicans* than against *C. tropicalis*, with MIC values of 1.0 and 4.0% (v/v), respectively. The TMEO–carvacrol combination produced its strongest antifungal effect against *C. albicans*, with an MIC of 0.50% (v/v), a minimum fungicidal concentration (MFC) of 0.50% (v/v), and an MFC/MIC ratio of 1.
5. Gas chromatography–mass spectrometry identified 11 volatile constituents accounting for 98.52% of TMEO. Carvacrol, an oxygenated phenolic monoterpene, was the dominant constituent at 71.04%. Oxygenated monoterpenes, including carvacrol, collectively represented 77.20% of the oil composition, confirming the analyzed sample as a carvacrol-rich TMEO.

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Conflict of Interest

Authors declare no conflict of interest exists.

Use of Generative AI

During the preparation of this manuscript, the authors used ChatGPT (OpenAI, San Francisco, CA, USA) solely for language editing, including improvement of grammar, sentence clarity, readability, and overall presentation. The scientific design, experimental data, statistical analysis, interpretation of results, conclusions, and verification of references were conducted and critically reviewed by the authors. The authors take full responsibility for the accuracy, integrity, and originality of the final manuscript.

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