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Original Article

Verbenone-Rich Rosemary (*Rosmarinus officinalis*) Essential Oil: GC-MS Profiling, Potent Antibacterial and Antifungal Activities, and Cytotoxic Potential

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Abstract: This study investigated the chemical composition, antimicrobial activity, and cytotoxic potential of *Rosmarinus officinalis* L. (syn. *Salvia rosmarinus* Spenn.) essential oil (EO). Fresh leaves were hydrodistilled using a Clevenger apparatus, and the resulting EO was analyzed by GC-MS. Twenty-five compounds representing 96.06% of the oil were identified, dominated by oxygenated monoterpenes (82.34%): verbenone (27.14%), camphor (16.59%), and 1,8-cineole (12.55%), followed by hydrocarbon monoterpenes (9.89%), sesquiterpene hydrocarbons (1.84%), and oxygenated sesquiterpenes (1.00%). Antimicrobial activity was assessed against clinical and reference bacterial and fungal strains using the disc diffusion and broth microdilution methods. The EO exhibited potent antibacterial effects, with inhibition zones up to 20.0 ± 1.3 mm and MICs ranging from 0.9–3.75 mg/mL. Notably, activity against *Staphylococcus aureus* and *Escherichia coli* surpassed that of cefotaxime, while *Staphylococcus epidermidis* showed the lowest susceptibility. Antifungal testing revealed substantial inhibition of *Cryptococcus neoformans* ATCC 90112 (17.3 ± 0.8 mm; MIC 0.9 mg/mL), equivalent to amphotericin B, with moderate activity against *Candida albicans* strains (MIC 1.9–3.75 mg/mL). Cytotoxicity toward normal Vero cells was evaluated at concentrations of 10–100 µg/mL, revealing a dose-dependent inhibition with an IC_{50} of 62.80 µg/mL, classifying the EO as highly cytotoxic under OECD and NIH guidelines. Importantly, the IC_{50} was considerably higher than the MICs observed, and the lowest cytotoxicity test concentration (10 µg/mL) exceeded the antimicrobial effective range, suggesting that antibacterial and antifungal activity may occur at non-cytotoxic levels. These findings highlight verbenone-rich *R. officinalis* EO from Jordan as a promising source of natural antimicrobial agents with potential pharmaceutical and preservative applications. However, its high cytotoxicity warrants further investigation into safe dosing, formulation strategies, and synergistic combinations to optimize its therapeutic index.

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INTRODUCTION

Rosmarinus officinalis L., recently reclassified as *Salvia rosmarinus* Spenn., is an aromatic perennial shrub widely distributed in the Mediterranean basin and cultivated globally for its culinary, medicinal, and ornamental uses (de Macedo et al., 2020). Its essential oil (EO) is a complex mixture of volatile compounds, primarily monoterpenes and sesquiterpenes, that have been extensively studied for their diverse biological activities, including antimicrobial, antioxidant, anti-inflammatory, and anticancer effects (de Sousa et al., 2023). Among these constituents, oxygenated monoterpenes such as 1,8-cineole, camphor, and verbenone have been identified as key bioactive molecules contributing to the oil's pharmacological potential (Aziz et al., 2022).

The increasing prevalence of antimicrobial resistance among pathogenic bacteria and fungi has intensified the search for alternative therapeutic agents from natural sources (Salam et al., 2023). Plant-derived EOs are particularly attractive candidates due to their broad-spectrum antimicrobial properties and low propensity for resistance development (Kovačević et al., 2024). Moreover, certain EO constituents have demonstrated selective cytotoxicity, making them promising leads for anticancer research (Mohamed Abdoul-Latif et al., 2023). However, variations in EO composition, driven by geographic origin, environmental conditions, and plant chemotypes, can significantly influence their biological activity (Ben Arfa et al., 2022).

Verbenone-rich *R. officinalis* EO is a less-studied chemotype compared to the more common 1,8-cineole- or camphor-rich types, yet preliminary evidence suggests that verbenone possesses strong antimicrobial and cytotoxic properties (Al-Hayali et al., 2023; Al-jaafreh, 2024; Soliman et al., 2024). Despite this, comprehensive studies evaluating the antimicrobial and cytotoxic profiles of verbenone-dominant *R. officinalis* EO, particularly from Jordanian flora, remain scarce.

The present study aimed to determine the chemical composition of *R. officinalis* EO from Jordan using gas chromatography–mass spectrometry (GC–MS), evaluate its antibacterial and antifungal activities against clinical and reference strains, determine the minimum inhibitory concentrations (MICs), and assess its cytotoxic potential against normal Vero cells. The findings contribute to understanding the therapeutic potential and safety profile of this chemotype, providing a basis for future pharmacological applications.

MATERIALS AND METHODS

Plant materials

Fresh aerial parts of *Rosmarinus officinalis* L. (syn. *Salvia rosmarinus* Spenn.) were collected from a home garden in the Al-Karak district, Jordan. The plant material was thoroughly washed with distilled water to remove surface dust and debris, and the leaves were carefully separated from the stems. The cleaned fresh leaves were then ground into a fine paste using a laboratory mill and immediately subjected to essential oil extraction.

Essential oil extraction

The freshly ground leaf material was subjected to hydrodistillation using a simple Clevenger-type apparatus to

extract the essential oil. The distillate was collected, and the oil phase was separated from the aqueous phase by liquid–liquid extraction with hexane. The hexane extract was subsequently evaporated under reduced pressure to remove the solvent, yielding the pure essential oil. The obtained oil was collected in amber glass vials and stored at 4 °C until further analysis.

Gas Chromatography–Mass Spectrometry (GC–MS) analysis

The chemical composition of the essential oil was analyzed using a Shimadzu QP2010 Plus GC–MS system (Kyoto, Japan) equipped with a DB-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The oven temperature program was initiated at 50 °C, held for 5 min, and then increased at a rate of 4 °C/min to 290 °C, with a total run time of 68 min. The injector temperature was set at 250 °C, and the injection volume was 1.0 µL of essential oil diluted in an appropriate solvent, with a split ratio of 1:100. The GC–MS interface (transfer line) temperature was maintained at 250 °C. Mass spectrometric detection was performed in electron ionization (EI) mode at 70 eV, with an ion source temperature of 250 °C. Mass spectra were acquired over the appropriate m/z range under full scan mode.

Compound identification was achieved by comparing the retention times and calculated Kovats retention indices (KI), determined using a homologous series of n-alkanes (C_8–C_{20}), with values reported in the literature (Al-Ghoul et al., 2023; Al-jaafreh, 2024; Hudaib et al., 2015; William E. Wallace, 2001). Further confirmation was performed by comparing the acquired mass spectra with those in the NIST mass spectral library and previously published data (William E. Wallace, 2001). Co-chromatography with authentic standards was performed for selected compounds to verify identity. The relative percentage of each identified compound was calculated from its peak area relative to the total peak area of all detected compounds, without applying correction factors.

Antimicrobial activities

Microbes

The microbial strains used in this study were kindly provided by Al Bashir Hospital (Amman, Jordan) and included two Gram-positive bacteria, *Staphylococcus aureus* and *Staphylococcus epidermidis*, two Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, as well as the yeast *Candida albicans*. In addition, *Cryptococcus neoformans* was included for antifungal testing. Microorganism identification was initially performed through microscopic examination and conventional biochemical assays, followed by final confirmation using the automated Biomérieux VITEK® 2 system. The antibiotic resistance profiles of the bacterial strains were also determined using the same platform. Standard reference strains from the American Type Culture Collection (ATCC) were employed: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 10145, *C. albicans* ATCC 10231, and *C. neoformans* ATCC 90112. These well-characterized strains served as quality controls to ensure the reliability and reproducibility of the antimicrobial assays.

Disc diffusion method

The antimicrobial activity of the verbenone-rich *R. officinalis* essential oil was evaluated using the disc diffusion method (ALrawashdeh et al., 2019). Briefly, a single colony of each test microorganism was inoculated into 3 mL of nutrient broth (NB) for bacteria or potato dextrose broth (PDB) for fungi and incubated to obtain the starter culture. The turbidity of each culture was adjusted to match the 0.5 McFarland standard (1×10^8 CFU/mL for bacteria and 1×10^6 CFU/mL for yeasts). For bacterial assays, Mueller–Hinton agar (MHA) plates were used, while potato dextrose agar (PDA) plates were employed for fungal strains. A 100 µL aliquot of the adjusted microbial suspension was evenly spread over the agar surface using a sterile swab.

Sterile paper discs (6 mm in diameter) were loaded with 10 µL of essential oil and placed onto the inoculated agar plates. Cefotaxime (30 µg/disc) served as the positive control for antibacterial assays, while Amphotericin B (30 µg/disc) was used for antifungal assays. Discs loaded with 10 µL of 10% dimethyl sulfoxide (DMSO) served as the negative control. Plates were incubated at 37 °C for 24 h, after which the diameters of the inhibition zones were measured in millimeters. All experiments were conducted in triplicate, and results were expressed as mean ± standard deviation.

Minimum Inhibitory Concentration (MIC)

The MIC of the verbenone-rich *R. officinalis* essential oil was determined using the broth microdilution method in 96-well microtiter plates (ALrawashdeh et al., 2019). A single colony of each test microorganism was inoculated into 3 mL of nutrient broth (NB) for bacteria or potato dextrose broth (PDB) for fungi and incubated to obtain the starter culture. The turbidity of each culture was adjusted to the 0.5 McFarland standard (1×10^8 CFU/mL for bacteria and 1×10^6 CFU/mL for yeasts). Mueller–Hinton broth (MHB) was used for bacterial assays, and potato dextrose broth (PDB) was used for fungal assays.

The essential oil was serially two-fold diluted in the appropriate broth medium to obtain final concentrations of 15, 7.5, 3.75, 1.9, 0.9, 0.45, 0.2, 0.1, 0.05, and 0.025 µL/mL. Ten microliters of the standardized microbial suspension were added to each well. A 10% DMSO solution was diluted in the same manner as the essential oil and used as the negative control. Plates were incubated at 37 °C for 24 h, and the MIC was recorded as the lowest concentration of essential oil that completely inhibited visible microbial growth. All assays were performed in triplicate.

Cytotoxicity activity

To observe the antiproliferative activity of the essential oil, normal African green monkey kidney Vero cell lines (ATCC CCL-81) were cultured in 10% fetal bovine serum (FBS) DMEM medium with 1% L-glutamine and 1% penicillin–streptomycin, in an incubator with 5% CO_2 at 37 °C (Al Jaafreh, 2024).

Cytotoxicity was assessed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay, a colorimetric method that quantifies viable cells to evaluate cellular growth, survival, and proliferation. Varying concentrations of the essential oil (10, 25, 50, and 100 µg/mL) were tested. A 10% DMSO solution, prepared and diluted in the same manner as the essential oil, was used as the solvent control, while untreated cultures served as the negative control.
The essential oil was prepared as a stock solution in DMSO and stored at 20 °C. To ensure the final DMSO concentration remained below 0.1%, the stock was diluted with DMEM medium before use in the cytotoxicity assay.

For the assay, 10,000 cells were seeded in 96-well plates with 100 µL of medium per well and incubated at 37 °C for 24 h. Cells were then treated with the essential oil for an additional 24 h. After treatment, each well received 10 µL of MTT solution in PBS (5 mg/mL) and was incubated for 4 h at 37 °C. The supernatant was then carefully removed, and 100 µL of DMSO was added to dissolve the formazan crystals. Plates were shaken on a microplate shaker for 5 min, and absorbance was measured at 595 nm using a Thermo Fisher Scientific microplate reader (Waltham, Massachusetts, USA).

Cell viability was calculated relative to the untreated control, and the percentage of inhibition was determined. Data are presented as the mean ± standard deviation (SD) of three independent experiments. The half-maximal inhibitory concentration (IC_{50}) was obtained by plotting the percentage of inhibition against the corresponding sample concentrations and fitting the resulting curve.

Statistical analysis

All experimental data were expressed as mean ± standard deviation (SD) of at least three independent replicates. Statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). Differences between groups were evaluated using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test where applicable. A p value of < 0.05 was considered statistically significant.

RESULTS

GC-MS Analysis of *Rosmarinus officinalis* Essential Oil

Gas Chromatography–Mass Spectrometry (GC–MS) analysis of the essential oil extracted from *R. officinalis* revealed a total of 25 identifiable compounds, representing 96.06% of the total oil composition (Table 1). The chemical profile was dominated by oxygenated monoterpenes (82.34%), followed by hydrocarbon monoterpenes (9.89%), sesquiterpene hydrocarbons (1.84%), oxygenated sesquiterpenes (1.00%), and a small fraction of other compounds (1.00%).

The major constituents were verbenone (27.14%), camphor (16.59%), and 1,8-cineole (12.55%), which together accounted for more than half of the total oil content. Other notable oxygenated monoterpenes included borneol (6.07%), α-terpineol (4.65%), dihydrocarvone (4.22%), carvacrol (4.21%), and terpinen-4-ol (3.66%). Hydrocarbon monoterpenes such as α-pinene (8.74%), sabinene (0.30%), and β-pinene (0.39%) were present in smaller proportions.

The sesquiterpene fraction was relatively low, with caryophyllene (1.56%) as the major hydrocarbon sesquiterpene, while β-ionol (0.46%) and caryophyllene oxide (0.30%) represented the oxygenated sesquiterpene class. Minor constituents, including 3-allyl-6-methoxyphenol (0.92%) and methyl jasmonate (0.08%), fell into the "others" category.

Table 1. Chemical composition of *Rosmarinus officinalis* essential oil as determined by GC–MS.

	Ki_{let}*	Ki_{cal}	Compound	Conc (%)
1.	925	935	α-Thujene	0.01
2.	933	941	α-Pinene	8.74
3.	950	955	Camphene	0.09
4.	979	982	Sabinene	0.30
5.	989	986	β-Pinene	0.39
6.	1018	1029	α-Terpinene	0.30
7.	1031	1034	Limonene	0.06
8.	1034	1042	1, 8-cineole	12.55
9.	1102	1106	Linalool	0.70
10.	1151	1148	Camphor	16.59
11.	1177	1179	Borneol	6.07
12.	1184	1190	Terpinen-4-ol	3.66
13.	1194	1193	Dihydrocarvone	4.22
14.	1199	1199	α-Terpineol	4.65
15.	1213	1218	Verbenone	27.14
16.	1283	1277	Bornyl acetate	1.33
17.	1298	1281	Carvacrol	4.21
18.	1342	1323	Piperitenone	1.22
19.	1360	1366	3-Allyl-6-methoxyphenol	0.92
20.	1422	1419	Caryophyllene	1.56
21.	1508	1509	β-Ionol	0.46
22.	1509	1515	β-Bisabolene	0.28
23.	1587	1586	Caryophyllene oxide	0.30
24.	1611	1653	Methyl jasmonate	0.08
25.	1644	1659	α-Muurolol	0.24
			Total	96.06
			Monoterpenes hydrocarbons (1-7)	9.89
			Oxygenated monoterpenes (8-18)	82.34
			Sesquiterpene hydrocarbons (20, 22)	1.84
			Oxygenated sesquiterpenes (21, 23, 25)	1.0
			Others (19, 24)	1.0

KIcal: Calculated Kovats retention index; *Kilet*: Literature Kovats index. Concentrations are expressed as relative percentages of the total identified components. Compounds are classified into hydrocarbon monoterpenes, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and others.
*(Al-Ghoul et al., 2023; Al-jaafreh, 2024; Hudaib et al., 2015; William E. Wallace, 2001)

Antimicrobial Activity

Disc diffusion assay

The antibacterial activity of the verbenone-rich *R. officinalis* essential oil was assessed against clinical and reference bacterial strains using the disc diffusion method. The oil exhibited varying degrees of inhibition, with the largest inhibition zones observed against *Escherichia coli* (20.0 ± 1.3 mm) and *Staphylococcus aureus* (19.0 ± 1.3 mm). Moderate activity was recorded against *E. coli* ATCC 25922 (17.0 ± 0.9 mm), *Pseudomonas aeruginosa* ATCC 10145 (13.7 ± 0.6 mm), and clinical *P. aeruginosa* (12.2 ± 1.0 mm). The lowest activity was seen against *Staphylococcus epidermidis* (8.5 ± 0.9 mm).

When compared with the positive control (cefotaxime, 30 µg/disc), the essential oil demonstrated superior inhibition against *S. aureus* and *E. coli*, comparable activity against *E. coli* ATCC 25922, and lower inhibition against *S. epidermidis* and both *P. aeruginosa* strains. No inhibition was observed for any strain with the negative control (10% DMSO).

Table 2: Antibacterial activity of *Rosmarinus officinalis* essential oil determined by the disc diffusion method.

Bacteria	Rosemary Essential oil[a]	Positive control[b]	Negative control[c]
Staphylococcus aureus	19.0±1.3	15.0±0.5	0.0±0.0
Staphylococcus epidermidis	8.5±0.9	9.5±0.0	0.0±0.0
Escherichia coli	20.0±1.3	16.5±1.5	0.0±0.0
Pseudomonas aeruginosa	12.2±1.0	16.0±0.5	0.0±0.0
E. coli 25922	17.0±0.9	17.5±0.3	0.0±0.0
P. aeruginosa 10145	13.7±0.6	19.0±0.0	0.0±0.0

Values represent mean inhibition zone diameters (mm) ± standard deviation (SD) from three independent replicates. [a](10 µL/disc), [b]cefotaxime (30 µg/disc), [C]10% DMSO (10 µL/disc)

The antifungal activity of the verbenone-rich *R. officinalis* essential oil was evaluated against both clinical and reference fungal strains using the disc diffusion method. The essential oil showed the highest inhibitory effect against *Cryptococcus neoformans* ATCC 90112, with a mean inhibition zone of 17.3 ± 0.8 mm, comparable to the positive control amphotericin B (17.0 ± 0.0 mm). *Candida albicans* exhibited a zone of inhibition of 15.2 ± 1.3 mm, while *C. albicans* ATCC 10231 showed lower sensitivity, with a mean inhibition zone of 12.2 ± 0.3 mm. When compared with amphotericin B (30 µg/disc), the essential oil displayed slightly lower activity against *C. albicans* strains but was equivalent against *C. neoformans*. No inhibition was observed in any fungal strain for the negative control (10% DMSO).

Table 3. Antifungal activity of verbenone-rich *Rosmarinus officinalis* essential oil determined by the disc diffusion method.

Fungi	Rosemary Essential oil	Positive control	Negative control
C. albicans	15.2±1.3	17.5±0.5	0.0±0.0
Candida albicans ATCC10231	12.2±0.3	19.0±0.0	0.0±0.0
Cryptococcus neoformans ATCC90112	17.3±0.8	17.0±0.0	0.0±0.0

Values represent mean inhibition zone diameters (mm) ± standard deviation (SD) from three independent replicates. a: 10 µL/disc essential oil; b: Amphotericin B (30 µg/disc) as positive control; c: 10% DMSO (10 µL/disc) as negative control.

Minimum Inhibitory Concentration (MIC)

The MIC values of the verbenone-rich *R. officinalis* essential oil against bacterial and fungal strains are summarized in Table 4. Among bacteria, the lowest MIC (0.9 mg/mL) was observed for *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *P. aeruginosa* ATCC 10145, indicating strong antimicrobial potency. *E. coli* ATCC 25922 showed moderate sensitivity (1.9 mg/mL), while *Staphylococcus epidermidis* was the least susceptible, with an MIC of 2.5 mg/mL.

For fungi, *Cryptococcus neoformans* ATCC 90112 was the most sensitive strain, with an MIC of 0.9 mg/mL, matching the potency observed for the most susceptible bacteria. *Candida albicans* showed intermediate susceptibility (1.9 mg/mL), whereas *C. albicans* ATCC 10231 required the highest concentration for growth inhibition (3.75 mg/mL).

Cytotoxicity activity

As shown in Figure 1, the cytotoxicity assay shows a dose-dependent increase in the percentage of inhibition of Vero cell viability following exposure to verbenone-rich *Rosmarinus officinalis* essential oil. At the lowest tested concentration (10 µg/mL), inhibition was minimal, around 8.3%**.** This increased to

approximately 25.1% at 25 µg/mL, then to 40.7% at 50 µg/mL. The highest tested concentration (100 µg/mL) resulted in a marked inhibitory effect of 76.9%**.**

Based on these results and linear interpolation between the 50 µg/mL and 100 µg/mL data points, the IC_{50}**,** the concentration required to inhibit 50% of cell viability—was calculated to be approximately 62.80 µg/mL. This value indicates that the essential oil exhibits considerable cytotoxicity toward normal Vero cells at relatively low concentrations.

Table 4. Minimum inhibitory concentrations (MICs) of *Rosmarinus officinalis* essential oil against bacterial and fungal strains.

Bacteria	mg/mL
Staphylococcus aureus	0.9
Staphylococcus epidermidis	3.75
Escherichia coli	0.9
Pseudomonas aeruginosa	0.9
E. coli 25922	1.9
P. aeruginosa 10145	0.9
Fungi	
C. albicans	1.9
Candida albicans ATCC10231	3.75
Cryptococcus neoformans ATCC90112	0.9

MIC values are expressed in mg/mL and were determined by the broth microdilution method in 96-well microtiter plates.

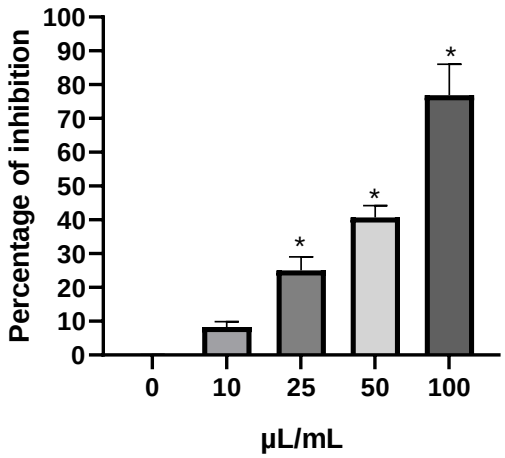

Figure 1: Cytotoxic activity of *Rosmarinus officinalis* essential oil against Vero cells. Vero cells were treated with different concentrations of essential oil (10–100 µg/mL) for 24 h, and cell viability was assessed using the MTT method. Data are expressed as the mean ± SD of three independent experiments. The plot shows a dose-dependent increase in the percentage of inhibition, with an IC_{50} value calculated at approximately 62.80 µg/mL.

DISCUSSION

The present study revealed that the *R. officinalis* essential oil (EO) from Jordan is predominantly composed of oxygenated monoterpenes (82.34%), with verbenone (27.14%), camphor (16.59%), and 1,8-cineole (12.55%) as the major constituents. This chemotypic profile differs from the more frequently reported cineole- or camphor-dominant rosemary oils from other Mediterranean regions, reflecting the influence of genetic, environmental, and geographical factors on EO composition (Barra, 2009). This chemotypic profile is in agreement with a recent Jordanian report by (Al-jaafreh, 2024), which also described a verbenone-rich type containing verbenone (31.8%), camphor (21.7%), and 1,8-cineole (14.6%) as the dominant components. In contrast, (Al-Ghoul et al., 2023) reported a 1,8-cineole-rich chemotype (1,8-cineole 37.7%, and α-pinene 31.3%) with negligible verbenone, while (Hudaib et al., 2015) identified 1,8-cineole (31.1%), α-pinene (16.53%), and camphor (11.7%) as the main constituents, again lacking significant verbenone (3%) content.

The EO demonstrated notable antibacterial activity, with inhibition zones comparable to or exceeding those of the standard antibiotic cefotaxime against *S. aureus* and *E. coli*. The MIC results further confirmed its potency, with low inhibitory concentrations (0.9–3.75 mg/mL) against most tested bacteria. These findings are consistent with those reported by (Al-jaafreh, 2024) for a verbenone-rich *R. officinalis* EO of similar composition, which exhibited strong inhibitory effects against *S. aureus* (21.0 mm, MIC 0.78 mg/mL) and *E. coli* (20.5 mm, MIC 0.78 mg/mL), as well as moderate activity against *P. aeruginosa* (15.0 mm, MIC 1.56 mg/mL) and *S. epidermidis* (12.5 mm, MIC 3.12 mg/mL). These parallel results reinforce the link between high oxygenated monoterpene content, particularly verbenone, camphor, and 1,8-cineole, and potent antibacterial activity. Such compounds are known to disrupt microbial cell membranes, increase permeability, and interfere with enzymatic systems, ultimately leading to cell death (Kashyap, 2024; Yap et al., 2021).

The antifungal activity of the oil was also substantial, particularly against *Cryptococcus neoformans* ATCC 90112, where the inhibition zone was equivalent to that of amphotericin B. This supports previous findings that rosemary EO constituents can alter fungal cell wall integrity and inhibit ergosterol biosynthesis (Yuan et al., 2024). In agreement with our results, (Matsuzaki et al., 2013) reported that rosemary EO chemotypes exhibited notable activity against *Candida albicans*, with MIC values of 5 µL/mL for the cineole-rich type, and 10 µL/mL for both verbenone- and camphor-rich types. The higher MICs observed for *C. albicans* strains in our study and in this previous work suggest that susceptibility may be chemotype-dependent, and also influenced by strain-specific characteristics such as cell wall composition and efflux pump activity (Grigore-Gurgu et al., 2025).

The cytotoxicity results demonstrated a dose-dependent effect on Vero cells, with an IC_{50} of approximately 62.80 µg/mL. While high cytotoxicity may be a desirable property for targeting malignant cells, it also indicates the need for careful consideration of potential toxicity to normal tissues when developing therapeutic applications. Notably, the IC_{50} value was much higher than the MIC values observed in this study, and the lowest concentration tested in the cytotoxicity assay was 10 µL/mL. This suggests that the antibacterial and antifungal activities observed at concentrations below 10 µL/mL may occur at levels that are not overtly cytotoxic to normal cells, indicating a potentially safe and novel antimicrobial effect. This aligns with other reports highlighting that some EO constituents, while potent antimicrobials, can exhibit low selectivity between normal and cancerous cells (Borsoi et al., 2024).

A limitation of this study is that the antimicrobial and cytotoxic activities were assessed only in vitro, which may not fully reflect the oil's behavior in complex biological systems. Factors such as bioavailability, metabolism, and potential interactions with host tissues could influence efficacy and safety in vivo. Additionally, while the GC–MS analysis provided detailed compositional data, the biological effects of individual constituents versus synergistic interactions within the oil were not separately evaluated. Only a limited number of microbial strains were tested, and their resistance profiles may not represent the full clinical spectrum. Finally, the cytotoxicity assay was performed on a single normal cell line (Vero), which does not account for potential variability in sensitivity among different human cell types. These constraints should be addressed in future studies through broader strain screening, mechanistic assays, and in vivo models to better define therapeutic potential and safety margins.

CONCLUSION

Verbenone-rich *Rosmarinus officinalis* essential oil from Jordan demonstrated strong antibacterial and antifungal activity alongside significant cytotoxic potential. These properties suggest promising applications in pharmaceutical and preservative formulations. However, the high cytotoxicity toward normal cells underscores the need for careful formulation strategies, dosage optimization, and potential synergistic use with other antimicrobials to enhance safety. Future studies should focus on elucidating the molecular mechanisms of action

and evaluating its efficacy against multidrug-resistant pathogens.

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Data Availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest
The authors declare no conflict of interest in this study

Author Contribution
The author confirms the sole responsibility for the conception of the study, presented results and manuscript preparation.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process
The author acknowledges that AI-assisted technologies were partially used in the preparation of this manuscript. Specifically, generative AI tools (ChatGPT) were employed to support language refinement and grammar correction. All content was critically reviewed, edited, and approved by the author to ensure accuracy, originality, and alignment with academic standards. No AI tools were used to generate data, interpret results, or replace human intellectual contribution.

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