Chemical Composition and Biological Activities of Rosemary Essential oil (*Rosmarinus oficinalis* L.) Collected in Dak Lak, Vietnam

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Article Info
ISSN (online): 3049-0421
Volume: 02
Issue: 02
March-April 2025
Received: 21-02-2025
Accepted: 16-03-2025
Page No: 33-39

Abstract
Rosemary (*Rosmarinus oficinalis* L.) essential oil was analyzed using GC-MS, revealing 60 identified compounds. The major constituents include α-pinene (29.52%), eucalyptol (22.55%), verbenone (9.98%), and geraniol (5.62%). Rosemary essential oil exhibits strong antioxidant activity with an IC_{50} value of 12.44 µg/mL, determined by the DPPH method. However, it shows only weak antibacterial activity against *E.coli*, as evaluated by the disc diffusion method.

DOI: https://doi.org/10.54660/IJPGRR.2025.2.2.33-39

Keywords: Rosmarinus oficinalis L., Rosemary, chemical composition, biological activities, α-pinene

Introduction

Vietnam is a country with abundant and diverse resources of medicinal plants; however, it has not yet been fully exploited. Over 400 plant species with biological activity have been documented, and the search for new plant species remains a key interest for scientists [1].

Rosemary, also known as *Rosmarinus officinalis*, is a flowering plant in the Lamiaceae family.[2] Rosemary emits a strong fragrance, with a minty scent. *Rosmarinus officinalis* L. is an aromatic herb from the Mediterranean region, previously grown extensively in Southern Europe, Western Asia, and Northern Africa. It grows wild along the northern and southern Mediterranean coasts and in the sub-Himalayan region [2]. The parts commonly used from the rosemary plant are the tops and leaves. In traditional medicine, rosemary is considered one of the effective herbs for treating headaches, poor circulation, inflammation, and physical and mental fatigue [3].

Rosemary essential oil possesses antioxidant, antibacterial, and antifungal properties; it can stimulate hair growth, enhance mental activity, alleviate pain, and address respiratory issues [2, 3].

However, there are very few reports on the chemical composition and biological activities of rosemary essential oil in Dak Lak. Therefore, to supplement the existing database on rosemary in Dak Lak and contribute to the exploitation and development of this plant species, this study aims to provide additional insights.

Material and Methods

Chemicals

Ascorbic acid, DPPH, Tween 80, a homologous series of C7-C30 straight-chain hydrocarbons, and various reference chemicals for identification were procured from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). All other chemicals, including those of analytical grade, were acquired from Merck (Darmstadt, Germany). The culture media and standard antibiotic discs were obtained from Oxoid Ltd. (Basingstoke, Hampshire, UK).

Plant Material

Leaves and stems of *Rosmarinus oficinalis* L. were gathered from Tan Tien commune (12°40′34″N 108°2′7″E), Buon Ma Thuot City, Dak Lak Province, Vietnam in January 2023. To serve as a reference, a voucher specimen (No: HT-BMT-01) was deposited at the Faculty of Natural Science and Technology, Tay Nguyen University, Buon Ma Thuot City.

Essential Oil Extraction

The leaves and stems of *Rosmarinus oficinalis* L. were cleaned, cut into smaller pieces, and subjected to steam distillation using a Clevenger-type apparatus for 4 hours. The obtained essential oil was dehydrated with anhydrous sodium sulfate and stored in a sealed vial at 10^0C in the dark before analysis.

Essential Oil Analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil from the leaves and stems of *Rosmarinus oficinalis* L. was conducted using a Thermo Trace GC Ultra - ITQ900 system (Thermo Fisher Scientific, MA, USA). Data interpretation was performed using MassFinder 4.0 software. Separation was achieved using a fused silica capillary TG-SQC column (30 m × 0.25 mm i.d., 0.25 µm film thickness).

GC Operation Conditions

The GC operation conditions included an injector temperature of 250^0C, a detector temperature of 260^0C, and an oven temperature program from 60 to 260^0C at a heating rate of 4^0C/min. Helium served as the carrier gas at a flow rate of 1.0 mL/min. An oil sample (1 µL) was injected using the split mode with a split ratio of 1:10.

MS Operation Conditions

The mass spectrometer was operated in electron-impact (EI) mode, with an ionization energy of 70eV, interface temperature of 280^0C, ion source temperature of 230^0C, MS quadrupole temperature of 200^0C, and scan range of 35-650 amu. The GC operation conditions were identical to those described in the section above, "GC Operation Conditions".

Identification and Quantification of Essential Oil Constituents

The retention indices of the oil constituents were determined on an HP-5 MS column using standard C7-C30 straight-chain hydrocarbons (Sigma-Aldrich Chemical Company, USA). Individual compounds in the oil were identified by comparing their mass spectra and retention indices with those in GC-MS libraries (NIST 08, Wiley 09th Version) and/or with those reported in the literature. The relative percentages of the separated compounds were computed from GC data without the use of correction factors.

Antioxidant Activity

The antioxidant activity of the *Rosmarinus oficinalis* L. essential oil extract was assessed using the DPPH assay. Different concentrations of the extract in methanol (ranging from 1 to 30 mg/mL) and a positive control, ascorbic acid, were mixed with 200 µL of a methanolic solution containing DPPH radicals at a concentration of 150 µmol/L. The resulting mixtures were then vigorously shaken and allowed to stand for 30 minutes in the dark for the reactions to run to completion. Subsequently, the absorbance of the solutions was measured using a Shimadzu UV1800 spectrophotometer (Shimadzu Corporation, Japan) at 517 nm against a blank (a control solution with no extract or ascorbic acid). Each test was performed in triplicate to maintain accuracy and reliability. The scavenging ability was calculated as in Equation 1:

$$Scavenging\ acbility\ (\%) = \frac{A_{517}\ of\ control - A_{517}\ of\ sample}{A_{517}\ of\ control}\ x\ 100 \quad (1)$$

Antimicrobial Activity

The antibacterial activity of the *Rosmarinus oficinalis* L. leaf essential oil was evaluated using a Gram-negative strain - *Escherichia coli* (*E. coli*; ATCC 25922) - obtained from laboratory stock cultures and the agar disc diffusion method. A liquid culture of *E. coli* (at a concentration of 10^7 colony-forming units per milliliter [CFU/mL]) was spread evenly on a solid medium in a Petri dish. Circular pieces of filter paper with a diameter of 6 mm were placed in the center of the dish. The essential oil from *Rosmarinus oficinalis* L. was extracted by steaming and dissolved in 10% dimethyl sulfoxide (DMSO); 40 µL of the essential oil was then applied to the filter paper, using 10% DMSO as a negative control. The Petri dishes were then sealed and incubated. The diameter of the inhibition zones formed around the filter paper was measured and used as an indicator of antimicrobial activity, and the entire assay was conducted in triplicate to maintain accuracy. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of the *Rosmarinus oficinalis* L. essential oil that visibly inhibits the growth of the bacteria [14]. The essential oil was dissolved in ethanol, and two-fold serial dilutions were carried out in a 96-well plate to yield a concentration range of 1.0 to 10.0 mg/mL. A bacterial broth medium (20 µL) was added to each well to produce the different solution concentrations. The pH of the medium was adjusted to a value in the range of 7.4 to 7.6, and the microplates were incubated at 37^0C for 24 hours. Each assay was performed in triplicate to ensure the reliability and accuracy of the results. The MIC value is a measure of the potency of the essential oil as an antimicrobial agent against *E. coli*.

Statistical Analysis

All treatments were carried out in triplicate, and the data were subjected to statistical analysis using the analysis of variance (ANOVA) with Statistica 5.5 software (Stat Soft Inc., Tulsa, OK, USA). The results are presented as the mean ± standard deviation (SD).

Results and Discussion

Results

Chemical composition of Rosmarinus Oficinalis L.

The extracted rosemary essential oil is pale yellow with a mild fragrance, yielding an essential oil content of 0.75 - 0.8% by weight compared to the fresh material.

The chemical composition of the extracted rosemary essential oil was determined using steam distillation. The results of the GS-MS analysis and the chemical composition are shown in Figure 1 and Table 1.

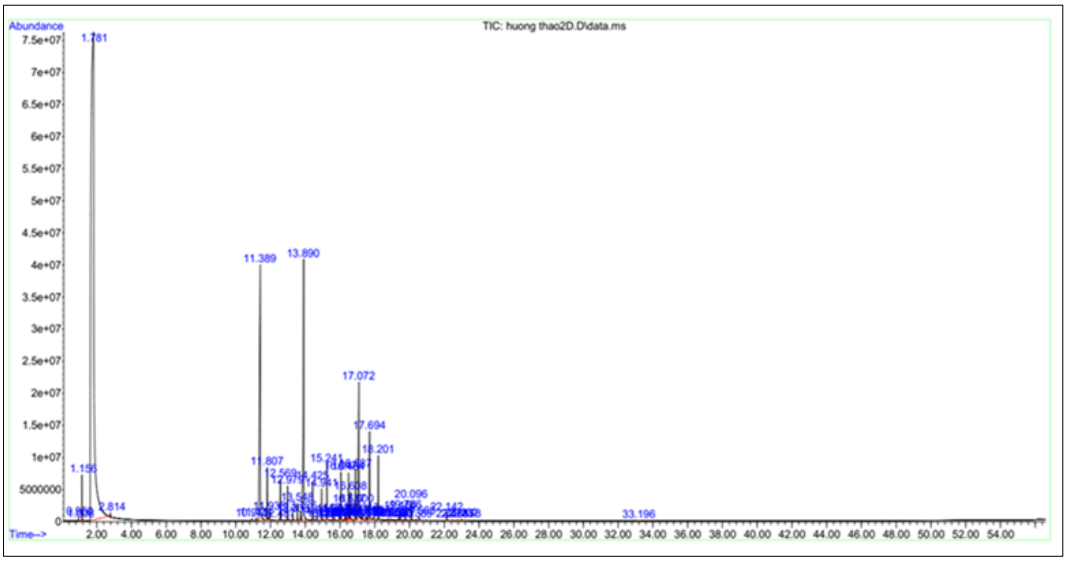


Fig 1: GC-MS total ion chromatogram of *Rosmarinus officinalis L.* essential oil

The analysis of rosemary essential oil cultivated in Dak Lak by GC-MS identified 60 compounds, with the main components being α-pinene (29.52%), Eucalyptol (22.55%), Verbenone (9.98%), Geraniol (5.62%), α-Terpineol (2.92%), Camphene (2.79%), endo-Borneol (2.74%), Linalool (2.61%), Camphor (2.53%), Bornyl acetate (2.53%), and β-pinene (2.03%). Other components were found in smaller quantities (<2%).

Table 1: Chemical compositions from Rosmarinus officinalis L. essential oil

Peak No	RT (min)	Compounds	Molecular Formula	%Area
1	10.944	α-Tricyclene	$C_{10}H_{16}$	0.11%
2	11.173	α-Thujene	$C_{10}H_{16}$	0.18%
3	**11.389**	**α-Pinene**	**$C_{10}H_{16}$**	**29.52%**
4	**11.807**	**Camphene**	**$C_{10}H_{16}$**	**2.79%**
5	11.937	2,4-Thujadiene	$C_{10}H_{14}$	0.39%
6	12.486	(-)-β-Pinene	$C_{10}H_{16}$	0.04%
7	**12.571**	**β-Pinene**	**$C_{10}H_{16}$**	**2.03%**
8	12.98	β-Myrcene	$C_{10}H_{16}$	1.57%
9	13.299	α-Phellandrene	$C_{10}H_{16}$	0.29%
10	13.547	(+)-2-Carene	$C_{10}H_{16}$	0.80%
11	13.726	Benzene, 1-methyl-3-(1-methylethyl)-	$C_{10}H_{14}$	0.48%
12	**13.888**	**Eucalyptol (1,8 - Cineole**	**$C_{10}H_{18}O$**	**22.55%**
13	14.423	γ-Terpinene	$C_{10}H_{16}$	1.65%
14	14.661	Cyclofenchene	$C_{10}H_{16}$	0.12%
15	14.94	Terpinolene	$C_{10}H_{16}$	1.30%
16	15.039	m-Cymenene	$C_{10}H_{12}$	0.06%
17	15.187	1R,4R-p-Mentha-2,8-dien-1-ol	$C_{10}H_{16}O$	0.04%
18	**15.241**	**Linalool**	**$C_{10}H_{18}O$**	**2.61%**
19	15.601	Chrysanthenone	$C_{10}H_{14}O$	0.22%
20	15.659	cis-2-p-Menthen-1-ol	$C_{10}H_{18}O$	0.05%
21	15.695	α-Campholenal	$C_{10}H_{16}O$	0.10%
22	15.96	(S)-cis-Verbenol	$C_{10}H_{16}O$	0.24%
23	**16.046**	**Camphor**	**$C_{10}H_{16}O$**	**2.53%**
24	16.113	Cyclopentane, 1,3-bis(methylene)-	C_7H_{10}	0.07%
25	16.271	3-Pinanone	$C_{10}H_{16}O$	0.09%
26	16.302	Pinocarvone	$C_{10}H_{14}O$	0.25%
27	16.365	Oxalic acid, cyclohexyl undecyl ester	$C_{19}H_{34}O_4$	0.07%
28	16.41	3-Cyclopentene-1-ethanol, 2,2,4-trimethyl-	$C_{10}H_{18}O$	0.15%
29	**16.486**	**endo-Borneol**	**$C_{10}H_{18}O$**	**2.74%**
30	16.536	trans-3-Pinanone	$C_{10}H_{16}O$	0.64%
31	16.608	Terpinen-4-ol	$C_{10}H_{18}O$	1.17%
32	16.752	p-Cymen-8-ol	$C_{10}H_{14}O$	0.05%
33	**16.886**	**α-Terpineol**	**$C_{10}H_{18}O$**	**2.92%**
34	16.999	Cyclohexane, 1-(chloromethyl)-4-methylene-	$C_8H_{13}Cl$	0.64%
35	**17.071**	**Verbenone**	**$C_{10}H_{14}O$**	**9.98%**
36	17.21	2-Methyl-4-octenal	$C_9H_{16}O$	0.14%
37	17.318	Citronellol	$C_{10}H_{20}O$	0.23%
38	17.493	2,6-Octadienal, 3,7-dimethyl-, (Z)-	$C_{10}H_{16}O$	0.09%

39	17.552	ξ-Fenchene	$C_{10}H_{16}$	0.11%
40	17.597	D-Carvone	$C_{10}H_{14}O$	0.04%
41	**17.695**	**Geraniol**	**$C_{10}H_{18}O$**	**5.62%**
42	17.938	Citral	$C_{10}H_{16}O$	0.15%
43	17.979	2-Cyclohexen-1-one, 3-methyl-6-(1-methylethenyl)-, (S)-	$C_{10}H_{14}O$	0.09%
44	**18.203**	**Bornyl acetate**	**$C_{12}H_{20}O_2$**	**2.53%**
45	18.738	Myrtenyl acetate	$C_{12}H_{18}O_2$	0.06%
46	18.86	γ-Terpineol	$C_{10}H_{18}O$	0.05%
47	18.959	3-Caren-5-one	$C_{10}H_{14}O$	0.05%
48	19.291	(-)-8-p-Menthen-2-yl, acetate, trans	$C_{12}H_{20}O_2$	0.04%
49	19.386	Cyclohexene, 5-methyl-3-(1-methylethenyl)-, trans-(-)-	$C_{10}H_{16}$	0.09%
50	19.462	cis-Geranyl acetate	$C_{12}H_{20}O_2$	0.32%
51	19.768	Methyleugenol	$C_{11}H_{14}O_2$	0.36%
52	20.096	Caryophyllene	$C_{15}H_{24}$	0.82%
53	20.388	cis-Geranylacetone	$C_{13}H_{22}O$	0.04%
54	20.563	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-	$C_{15}H_{24}$	0.15%
55	22.141	Caryophyllene oxide	$C_{15}H_{24}O$	0.33%
56	22.465	3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	$C_9H_{14}O$	0.04%
57	22.784	(-)-Eudesma-1,4(15),11-triene	$C_{15}H_{22}$	0.04%
58	23.004	9-epi-trans-Caryophyllene	$C_{15}H_{24}$	0.07%
59	23.179	Cyclopentane, cyclopropylidene-	C_8H_{12}	0.05%
60	33.195	Methyl 10,12-octadecadiynoate	$C_{19}H_{30}O_2$	0.03%

Antioxidant activity of *Rosmarinus oficinalis* L. essential oil

The antioxidant activity of rosemary essential oil is presented in Table 2 (with ascorbic acid as the positive control).

Table 2: The correlation between the concentration of rosemary essential oil and its antioxidant activity

Sample	Concentration (µg/mL)	Inhibition (%)	IC_{50} (µg/mL)
Rosemary essential oil	25	72.60	12.44 ± 0.07
	20	65.46	
	15	55.96	
	10	44.66	
	5	18.98	
Ascorbic acid			44.12 ± 1.08

From Table 2, it can be seen that the antioxidant activity of rosemary essential oil is stronger than that of the positive control, ascorbic acid, with an IC_{50} value of 12.44 ± 0.07 µg/mL, which is lower than that of ascorbic acid (IC_{50} = 44.12 ± 1.08 µg/mL).

Additionally, the experimental results also show that when the concentration of the essential oil ranges from 5 µg/mL to 25 µg/mL, the percentage of DPPH free radical inhibition by the rosemary essential oil increases from 18.98% to 72.60% (Table 2). The antioxidant capacity is best demonstrated in the test sample with an essential oil concentration of 25 µg/mL, with a DPPH free radical inhibition percentage of 72.60%.

Antibacterial activity of *Rosmarinus oficinalis* L. essential oil

The agar diffusion method, based on the D-d(mm) value, with D being the diameter of the antibacterial zone (mm) and d being the diameter of the well (mm), is illustrated in Figure 2.

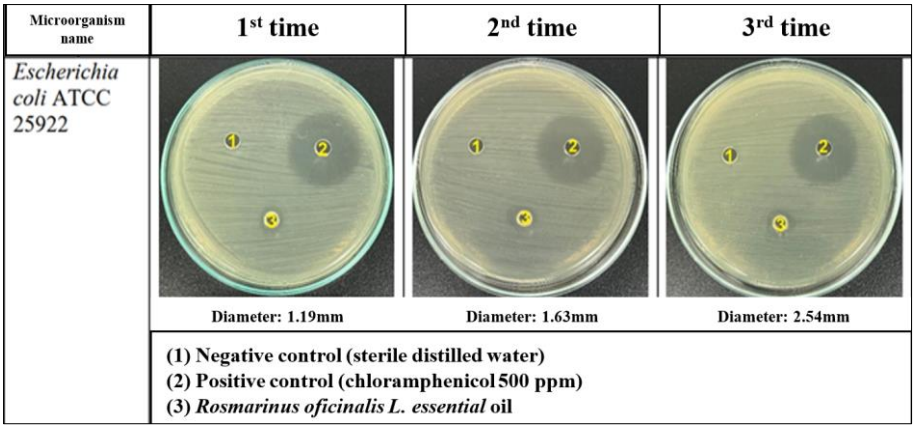


Fig 2: Antibacterial ability of Rosmarinus officinalis L. essential oil

The evaluation of the antibacterial activity of the rosemary essential oil sample cultivated in Buon Ma Thuot shows that the essential oil has weak antibacterial activity against *E. coli*, with inhibition zone diameters of 1.19mm, 1.63mm, and 2.54mm across three trials.

Discussion

Currently, there have been several studies on the chemical composition and biological activities of rosemary (*Rosmarinus officinalis* L.) both domestically and internationally.

Nguyen Ngoc Yen and colleagues surveyed the chemical composition and antioxidant activity of rosemary essential oil.[3] The optimal extraction conditions yielded an oil efficiency of 2.93%, with the major components identified as α-Pinene (26.13%), Eucalyptol (19.41%), and cis-verbenone (17.34%). The rosemary essential oil demonstrated a high antioxidant capacity with an IC_{50} value of 75.7 µg/mL.

Nguyen Dinh Phuc and collaborators studied the steam distillation of rosemary essential oil using specific conditions, yielding an oil efficiency of 3.04%.[4] The main components identified by GC-MS included α-Pinene (23.63%), 1,8-cineole (15.35%), borneol (5.563%), and geraniol (5.517%).

Also in 2020, Tran Thi Kim Ngan *et al*. researched the preservation factors affecting rosemary essential oil in Lam Dong province, Vietnam [5]. The oil, extracted via steam distillation from rosemary leaves, showed an extraction efficiency of 1.03%. They identified 30 compounds, with major components such as α-Pinene (25.99%), Eucalyptol (17.989%), and bicyclo [3.1.1] hept-3-en-2-one (10.78%). Their study highlighted the significant impact of temperature and light on oil quality over a 2-month storage period, recommending optimal preservation conditions at 4^0C in dark bottles for best results.

Laiq ur Rahman and colleagues identified the chemical composition of rosemary essential oil from Uttaranchal, India, using GC-MS.[6] They identified 33 compounds comprising 96.29% of the oil. Major constituents included camphor (26.40%), 1,8-cineole (23.40%), α-Pinene (9.94%), Camphene (5.83%), myrcene (4.86%), bornyl acetate (3.97%), verbenone (3.32%), limonene (3.08%), borneol (2.05%), and α-Terpineol (2.68%).

Xiao-qiang Chen and colleagues conducted simultaneous tests on the antioxidant activity of rosemary essential oil preserved at 60^0C, comparing it with synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ). The results indicated that rosemary essential oil exhibited very strong antioxidant properties, approaching those of synthetic antioxidants (BHA and BHT).[7]

Marwa Jardak and colleagues identified the chemical composition, antibacterial activity, and cytotoxicity of rosemary essential oil (*Rosmarinus officinalis* L.) in Tunisia.[8] Analysis by GC-MS revealed 36 compounds, with major constituents including 1,8-cineole (23.56%), Camphene (12.78%), Camphor (12.55%), and β-Pinene (12.3%). Using the micro-dilution method, rosemary essential oil demonstrated inhibitory and bactericidal effects against two strains of bacteria: *Staphylococcus aureus* ATCC 9144 and *Staphylococcus epidermidis* S61. Minimum inhibitory concentrations (MIC) ranged from 1.25 to 2.5 µlml^{-1} for *S. aureus* and from 0.312 to 0.625 µlml^{-1} for *S. epidermidis*, respectively. Minimum bactericidal concentrations (MBC) were 5 and 2.5 µlml^{-1}, respectively. Furthermore, rosemary essential oil exhibited over 57% inhibition of biofilm formation by *S. epidermidis* at a concentration of 25 µlml^{-1}. The study concluded that rosemary essential oil shows potential in treating infections caused by bacteria and in inhibiting the growth of cancer cells.

Gun Binzet and colleagues identified the chemical composition of rosemary essential oil from plants grown in Mersin, Turkey [9].The essential oil was obtained through steam distillation with a yield of approximately 1.2%. Analysis using gas chromatography-mass spectrometry (GC-MS) identified 45 compounds, with the main constituents being Eucalyptol (33.15%) and Camphor (10.31%).

Aoaudi M and colleagues conducted a study on rosemary essential oil (*Rosmarinus officinalis* L.) to evaluate its anthelmintic activity against *Haemonchus contortus* and *Eimeria* spp. under in vitro conditions using small ruminants [10]. GC-MS analysis identified the major compounds as 1,8-cineole (52.06%), α-Pinene (15.35%), and Camphor (7.69%). Rosemary essential oil exhibited activity against Eimeria spp., with an IC_{50} of 1.82 µg/mL against the oocysts of sheep. In the egg hatch test, an inhibitory rate of 73.76% was observed at 16 mg/mL after 2 days of incubation (IC_{50} = 11.41 mg/mL). For the adult worm motility test, a 100% inhibition rate was observed.

Enrique Melero-Bravo and colleagues identified the main components of rosemary essential oil from the Mediterranean region, including Camphor (21.9%), α-Pinene (14.8%), 1,8-cineole (11.6%), β-Pinene + Myrcene (11.3%), and Camphene (8.3%) [11].

Nawaf Al-Maharik and colleagues conducted an analysis of the chemical composition of rosemary essential oil cultivated in five different locations in Palestine: Jenin, Tulkarm, Nablus, Hebron, and Ramallah, using GC-MS.[12] The results revealed that the main compounds in the essential oils included: 1,8-cineole (4.81-37.83%), α-Pinene (13.07-51.36%), and Camphor (11.95–24.30%). The antioxidant activity test using DPPH indicated that the rosemary essential oil from Jenin exhibited the highest activity, with an IC_{50} value of 10.23 ± 0.11 µg/mL, followed by samples from Tulkarm (IC_{50} = 37.15 ± 2.3 µg/mL) and Nablus (IC_{50} = 38.9 ± 0.45 µg/mL). Regarding MIC values against MRSA, the rosemary essential oil extracted from Jenin showed the highest antibacterial activity with MIC values of 12.5, 12.5, 6.25, 6.25, and 6.25 µg/mL. Moreover, the oils from Jenin and Nablus demonstrated stronger antifungal effects against Candida compared to Fluconazole, with MIC values of 0.781, 0.781, and 1.56 µg/mL, respectively.

Benchohra Hadria Amel and colleagues studied the chemical composition of rosemary essential oil in Algeria using GC-MS, identifying 32 compounds [13]. The main constituents were Eucalyptol (70.90%), Borneol (16.63%), α-Pinene (10.52%), β-Pinene (5.77%), Camphor (2.15%), and α-terpineol (1.45%). The antioxidant activity evaluated by the DPPH free radical scavenging method showed an IC_{50} value of 18.04 µg/mL.

Majda Elyemnil and co-workers investigated the chemical composition and antibacterial activity of wild rosemary essential oil from two different regions in Morocco (Fez and Figuig) [14]. GC-MS analysis revealed that the rosemary oil from Fez contained 1,8-cineole (32.18%), Camphor (16.20%), α-Pinene (15.40%), Camphene (9.16%), and α-terpineol (7.36%) as the main components. Both oils from these regions exhibited good antibacterial activity, with MIC values ranging from 0.315 to 2.5 mg/L.

The results comparing the chemical composition of rosemary essential oils from Dak Lak with those from Da Lat by Nguyen Ngoc Yen and colleagues (2019) indicate higher levels of α-Pinene (26.13%), Eucalyptol (19.44%), and Geraniol (3%) in Dak Lak. However, cis-Verbenone content is lower (9.98% compared to 17.34%), as shown in Figure 3 [3].

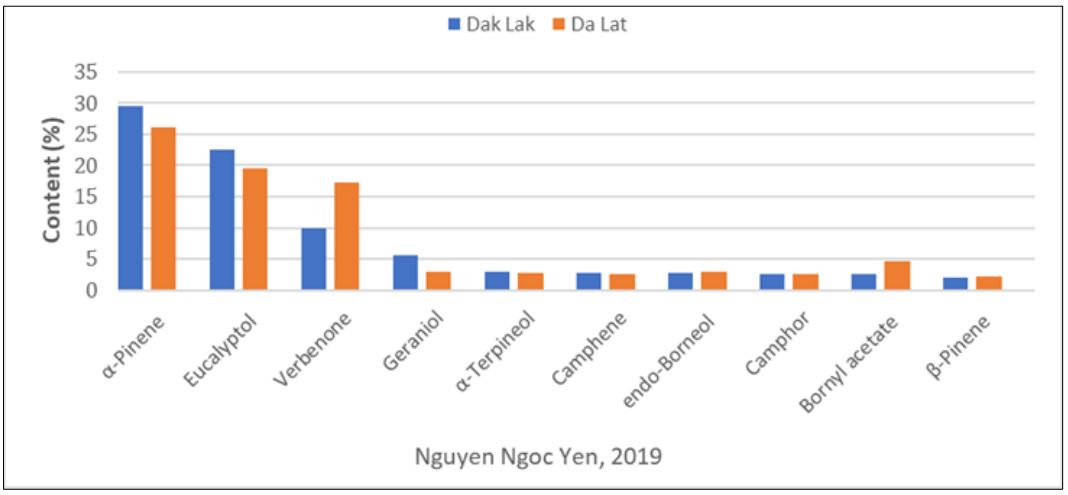
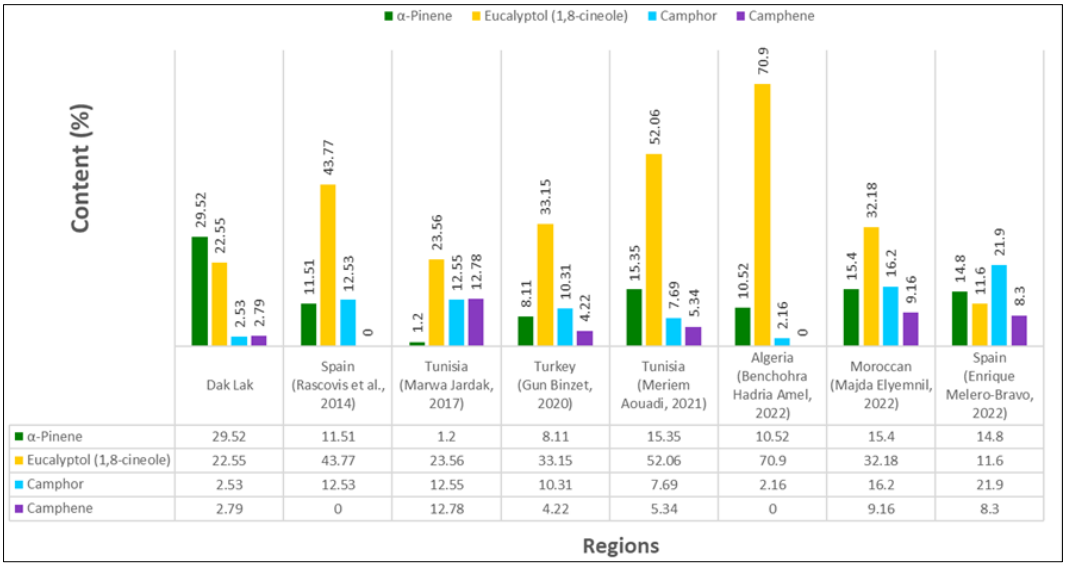


Fig 3: The main components of Rosemary essential oil in Dak Lak and Da Lat

When comparing the chemical composition of rosemary essential oils from Dak Lak with those from Spain as studied by Rascovis *et al* (2014), from Algeria as researched by Benchohra Hadria Amel *et al*. (2022), and from Tunisia as studied by Meriem Aouadi *et al*. (2021), along with other regions, the differences are described in Figure 4 [10, 13].



	Dak Lak	Spain (Rascovis et al., 2014)	Tunisia (Marwa Jardak, 2017)	Turkey (Gun Binzet, 2020)	Tunisia (Meriem Aouadi, 2021)	Algeria (Benchohra Hadria Amel, 2022)	Moroccan (Majda Elyemnil, 2022)	Spain (Enrique Melero-Bravo, 2022)
α-Pinene	29.52	11.51	1.2	8.11	15.35	10.52	15.4	14.8
Eucalyptol (1,8-cineole)	22.55	43.77	23.56	33.15	52.06	70.9	32.18	11.6
Camphor	2.53	12.53	12.55	10.31	7.69	2.16	16.2	21.9
Camphene	2.79	0	12.78	4.22	5.34	0	9.16	8.3

Fig 4: The main components of Rosemary essential oil in different regions

The α-pinene content (29.52%) in rosemary essential oil from Dak Lak is higher compared to regions such as Spain, Tunisia, Turkey, Algeria, and Morocco [8, 9,1 3]. Meanwhile, the Eucalyptol/1,8-cineole content (70.9%) in rosemary essential oil according to Benchohra Hadria Amel's study (2022) in Algeria is the highest among the regions compared. In contrast, the Eucalyptol content in rosemary essential oil from Dak Lak is 22.5% [13].

The reasons for these differences could be attributed to varying climatic conditions, soil nutrients, or experimental conditions, which can affect the composition of essential oils.

Conclusion

The chemical composition of rosemary essential oil (*Rosmarinus officinalis* L.) has been identified using GC-MS, revealing 60 compounds. The major chemical constituents include α-pinene (29.52%), Eucalyptol (22.55%), cis-Verbenone (9.98%), and Geraniol (5.62%).

The rosemary essential oil has demonstrated strong antioxidant activity using the DPPH method, with an IC_{50} value of 12.44 ± 0.07 µg/mL, which is lower than that of the positive control ascorbic acid (IC_{50} = 44.12 ± 1.08 µg/mL). Regarding its antibacterial activity compared to the positive control chloramphenicol, rosemary essential oil showed weak antibacterial activity against *E. coli*.

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