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Identification and Quantification of *Rosmarinus officinalis* L. Leaf Extract Phytochemical Profiles Using Gas Chromatography-Mass Spectrometry

[1,2]*Hatem Amin Moh'd Hejaz and* [2]*Jummana Mohammad Ali Makhammra*

[1]*Department of Pharmacy, Faculty of Pharmacy, Arab American University, P.O. Box 240 Jenin, 13 Zababdeh, Jenin-Palestine*

[2]*Department of Pharmacy, College of Pharmacy and Medical Sciences, Hebron University, Palestine*

ABSTRACT

Background and Objectives: In Palestine, herbal medicine is widespread, with rosemary being one of the most commonly utilized herbs. However, its usage is more closely associated with traditional customs passed down through generations than scientific research. Rosemary contains secondary metabolites that have broad applications in folk medicine and the culinary sector. The research aim was the extraction of *Rosmarinus officinalis* L. leaves collected from different locations, followed by GC-MS analysis and phytochemical testing. **Materials and Methods:** Samples of rosemary leaves were gathered from various locations in the Southern Region of the West Bank, including Raqah, Khilt Al-Adrah, Umm Lasfah village, Hebron City and Bani Naim. The air-dried leaves were powdered and subjected to extraction using two different concentrations of methanol (80 and 90%). The evaluation of the methanol extracts of rosemary leaves was done using two extraction methods (room temperature and reflux) using GC-MS while the photochemistry of these extracts was studied using different tests. **Results:** The GC-MS revealed the presence of numerous volatile compounds, each showing varying levels across the samples. Common volatile compounds detected in all samples included 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate, bornyl acetate and caryophyllene. Phytochemical analysis of the rosemary leaves revealed the presence of compounds such as cardiac glycosides, phenolic groups, coumarin, saponins, steroids, tannins and terpenoids in all samples. Interestingly, alkaloids were found only in the leaves of the Raqaa and Bani Naim samples. **Conclusion:** The Palestinian rosemary is rich in phytochemicals; thus, rosemary leaves are recommended for medicinal purposes.

KEYWORDS

Rosemary, *Rosmarinus officinalis*, phytochemicals, GC-MS, eucalyptol, camphor

INTRODUCTION

Medicinal plants contain phytochemicals like flavonoids, alkaloids, tannins and terpenoids, known for their antibacterial and antioxidant qualities[1,2]. Rosemary extracts derived from dried leaves have garnered significant attention in the food and pharmaceutical sectors due to their wide-ranging health benefits, encompassing antioxidant, antibacterial, anti-inflammatory and anticancer properties[3-5].

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Rosmarinus officinalis L. consists of bioactive compounds known as phytocompounds, which play a role in carrying out diverse pharmacological functions, including anti-inflammatory, antioxidant, antimicrobial, antiproliferative, antitumor, protective, inhibitory and attenuating activities[6,7].

Limited research has been conducted on Palestinian herbs, leaving their composition, effectiveness and safety largely unexplored. With concerns surrounding the adverse effects of conventional synthetic drugs, including toxicity and carcinogenicity and the growing issue of microbial resistance to existing antimicrobial agents, there has been a notable surge in interest in identifying naturally occurring antioxidant and antimicrobial compounds suitable for food and medicinal applications. Although a few studies in Palestine have demonstrated the positive effects of *Rosmarinus officinalis* L. on overall health and therapeutic enhancement[8,9], an exploration of the presence and extent of antioxidants, minerals, antibacterial properties and other polyphenols still needs to be light of this, we propose to analyze *Rosmarinus officinalis* L. leaves using GC-MS to determine the active principles present and to assess and compare the phytochemicals, antioxidants, antimicrobial activity, nutritional composition and biological potential of rosemary leaves. The selection of *Rosmarinus officinalis* L. for this investigation is rooted in its established medicinal reputation among Palestinians and widespread use. The lack of comprehensive analysis of its volatile and semi-volatile phytochemical composition and mineral content, coupled with the scarcity of pharmacological studies like antioxidant and antimicrobial assessments, has driven the motivation behind this research. The concentration of phytochemicals in rosemary extract varies based on the extraction method employed[10]. Rosemary extract contains various chemical groups, including flavonoids, polyphenols, terpenoids and volatile oils[11]. The phytochemical composition of *R. officinalis* extracts encompasses compounds such as rosmarinic acid, caffeic acid, ursolic acid, betulinic acid, carnosic acid and carnosol[12]. The essential oils of rosemary consist of 1,8-cineole, α-pinene, verbenone, camphor and borneol, although their proportions can significantly differ[13]. This study was carried out to examine the phytochemical composition of *Rosmarinus officinalis* L. obtained from different regions of Palestine.

MATERIALS AND METHODS

Study area: This research was conducted at the Department of Pharmacy Laboratory, Faculty of Pharmacy and Medical Sciences, University of Hebron, Palestine, between January, 2021 and January, 2022.

Data and sample collection: During the 2021 season (from February to July, 2021), 5 samples of rosemary leaves were collected from different sites in the Southern Region of the West Bank, Hebron-Palestine, including Raqah, Khilt Al-Adrah, Umm Lasfah Village, Hebron City and Bani Naim. The leaves of *R. officinalis* were dried in the shade at room temperature. The dried samples were stored in airtight paper bags, protected from light and powdered before extraction. From each, 5 g of rosemary powder were extracted with 50 mL of methanol at two concentrations (80 and 90%) and two extraction methods (room temperature and reflux) and analyzed using GC-MS.

Gas chromatography-mass spectrometry (GC-MS) analysis: The GC-MS analysis was carried out using the GC-MS Perkin Elmer Auto System. Used as a DB-5ms capillary column (30 m, 0.25 µm film thickness, 0.25 µm capillary diameter), the injection volume was 1 µL as identified[7,14,15]. After being held at 80°C for 2 min, the oven's temperature was raised to 280°C at a rate of 6°C per min. The injector's temperature was set to 280°C. Helium is the chosen carrier gas and the gas flow and velocity were kept at 134.3 mL per min and 43.1 cm per min, respectively. The compounds' molecular masses (m/z), which were obtained at a rate of 70 mv, ranged from 50 to 500 m/z and the MS scan speed was 1000 amu s1.

Peak identification: The identification of compounds was based mainly on matching their MS spectra with the National Institute of Standards and Technology (NIST) mass spectral library. Moreover, the Kovats Retention (KI) calculation was used to support the identification. The KI values were compared with NIST

values from the literature. Excellent agreement was obtained even using different chromatographic conditions. Quantitative analysis of the essential oils was performed once the MS knew the identities of the compounds.

Phytochemicals: Phytochemical tests of the samples were carried out according to the procedure described by Harborne[16] and Mujeeb *et al.*[17]:

- **Test for anthocyanin:** In a test tube, 2 mL of extract was added to 1 mL of (2 N) NaOH and heated for 5 min. The formation of a bluish-green color indicates a positive for anthocyanin
- **Test for coumarin:** In a test tube, 1 mL of extract was added to 1 mL of NaOH and kept in a boiling water bath for a few min. The presence of yellow color indicates positive coumarins
- **Test for saponins:** In a test tube, 5 mL of distilled water was shaken with 2 mL of extract and foam formation indicates positive saponins
- **Test for quinone:** As 1 mL of concentrated H_2SO_4 was added to 1 mL of extract in a test tube. The red coloration is a sign that quinines are present
- **Test for glycosides:** As 2 mL of the extract was mixed with 2 mL of 50% H_2SO_4 in a test tube. Fehling's solution is added and boiled in 10 mL after 5 min of water bath heating. A red prick precipitate suggests the presence of glycosides
- **Test for anthraquinones:** In a test tube, 2 mL of the extract and benzene were combined with 1 mL of a 10% NH_3 solution. A positive result for anthraquinones is indicated by a red, pink, or violet hue
- **Test for cardiac glycosides:** In a test tube, 2 mL of glacial acetic acid and 1 mL of conc. H_2SO_4 and a few drops of $FeCl_3$ were added to the 2 mL extract. The formation of the brown ring indicates a positive for glycosides
- **Test for steroids:** As 1 mL of extract was mixed with 2 mL of $CHCl_3$ and 1 mL of H_2SO_4 in a test tube, the presence of a reddish-brown ring confirms the presence of steroids
- **Test for flavonoids:** In a test tube, 2 mL of extract were combined with a few drops of 1% NH_3 solution. Yellow coloration is a sign that flavonoids are present
- **Test for phenolic groups:** As 1 mL of extract was placed in a test tube along with 2 mL of distilled water and a few drops of 10% $FeCl_3$. A positive for phenolic groups is the creation of a blue or black color
- **Test for tannins:** In a test tube, 1 mL of distilled water and 1-2 drops of $FeCl_3$ were added to a 2 mL extract, a green or blow black color indicates a positive for tannins
- **Test for terpenoids:** As 2 mL of $CHCL_3$ and 3 mL conc in a test tube. The H_2SO_4 was mixed with 2 mL of extract. The formation of a reddish-brown layer indicates a positive for terpenoids
- **Test for phlobatannins:** In a test tube, 1 mL of 10% NaOH was added to the 2 mL extract. The formation of a yellow color indicates a positive for phlobatannins
- **Test for alkaloids:** In a test tube, 1 mL of 1% HCl was added to a 2 mL extract and then a few drops of Meyer's reagent were added to the mixture. The presence of a white precipitate indicates a positive for alkaloids

RESULTS

Gas chromatography-mass spectrometry (GC-MS) analysis: Different concentrations of methanolic extracts from rosemary leaves were tested by GC-MS and identified by comparing them with the NIST library. The GC-MS analysis revealed the presence of many volatile compounds in each rosemary sample with different values. Despite their geographical location, all rosemary leaves were found to contain volatile compounds. Significant compounds were identified and their molecular formula, weight and retention time were summarized in Table 1-10. The GC-MS analysis of rosemary in the 80% methanol extract at room temperature (Table 1) showed the identification of many compounds. Major volatile compounds detected in all samples were 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl

Table 1: Compounds detected in methanol extracts 80 and 90% at RT. of rosemary leaves in the Raqaa region sample with their retention time (RT), molecular weight (MW) and molecular formula (MF)

Methanol extracts 80% at RT				Methanol extracts 90% at RT			
RT	Compound	MW	MF	RT	Compound	MW	MF
3.489	3-Carene	136	$C_{10}H_{16}$	3.489	3-Carene	136	$C_{10}H_{16}$
3.539	α-Pinene	136	$C_{10}H_{16}$	3.729	Camphene	136	$C_{10}H_{16}$
3.729	Camphene	136	$C_{10}H_{16}$	4.509	Alph-caryophyllene	136	$C_{10}H_{16}$
4.254	1.3 cyclopentadene	122	C_9H_{14}	5.020	Eucalyptol	154	$C_{10}H_{18}O$
4.825	Benzene, 1 methyl-3-(methylethyl)	134	$C_{10}H_{14}$	7.000	Camphor	152	$C_{10}H_{16}O$
4.905	D-Limonene	136	$C_{10}H_{16}$	7.436	Borneol	154	$C_{10}H_{18}O$
4.990	Eucalyptol	154	$C_{10}H_{18}O$	9.296	Isobornyl acetate	196	$C_{12}H_{20}O_2$
6.990	Camphor	152	$C_{10}H_{16}O$	11.592	Caryophyllene	204	$C_{15}H_{24}$
7.441	Borneol	154	$C_{10}H_{18}O$	12.152	Alpha-Caryophyllene	204	$C_{15}H_{24}$
7.556	Borneol chloride	172	$C_{10}H_{18}OCl$				
8.076	Bicycle (3,1,1 hepta-3-(-N-2-one)	150	$C_{10}H_{14}O$				
9.031	Cyclopropane carboxylic acid, 2,2-Dimethyl-3-(2-methyl-1-propen)	168	$C_{10}H_{16}O_2$				
9.301	Isobornyl acetate	196	$C_{12}H_{20}O_2$				
10.352	Cyclohexanmethanol	140	$C_9H_{16}O$				
11.337	Vitamin A aldehyde	284	$C_{20}H_{28}O$				
11.612	Humulen-(V1)	204	$C_{15}H_{24}$				
14.989	Naphthalene	204	$C_{15}H_{24}$				
15.324	Beta-Humulen	204	$C_{15}H_{24}$				
16.279	Thunbergol	290	$C_{20}H_{34}O$				
16.924	Aromadendrene oxide-(2)	220	$C_{15}H_{24}O$				
17.290	Caryophyllene	204	$C_{15}H_{24}$				
17.720	Thujopsene	204	$C_{15}H_{24}$				
18.735	Epicedrol	222	$C_{15}H_{26}O$				
20.086	3,4-Diethylphenol	150	$C_{10}H_{14}O$				
20.311	Lumisantonin	246	$C_{15}H_{18}O_3$				
20.501	Methoxsalen	216	$C_{12}H_8O_4$				
20.791	Menthol	156	$C_{10}H_{20}O$				
21.296	Ambrosin	246	$C_{15}H_{18}O_3$				
21.771	Isoparvifuran	254	$C_{16}H_{14}O_3$				
24.723	Furo[2,3B]Quinoline, 4, 6, 7-trimethoxy (Kokusaginine)	259	$C_{14}H_{13}O_4N$				
26.678	2(1H)-phenanthrenone, 3, 4, 4A, 9, 10, 10A-hexahydro-6-methoxy-1,1,4A-.	314	$C_{21}H_{30}O_2$				
27.674	Indolo[2,3-A] Quinalizine, 1, 2, 3, 4, 5, 6, 7, 12B-octahydro-12, 12B-Dimethyl	296	$C_{20}H_{28}ON_2$				

acetate, bornyl acetate and caryophyllene. The Raqaa region sample showed the presence of more components compared with others (Table 1). In addition to the mentioned compounds, major volatile compounds were detected: Vitamin A, aldehyde, naphthalene, beta-humulen, epicedrol, isoparvifuran, kokusaginine, (2 (1H)-Phenanthrenone, 3, 4, 4A, 9, 10, 10A-Hexahydro-6-Methoxy-1,1,4A-) and (Indolo[2,3-A] Quinalizine, 1, 2, 3, 4, 5, 6, 7, 12B-Octahydro-12, 12B-Dimethyl. The other minor volatile compounds identified are shown in Table 1. Nine compounds were found in rosemary leaves with 90% methanol at room temperature in the Raqaa region (Table 1). The significant compounds identified were 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate and caryophyllene. The other minor volatile compounds identified are shown in Table 1. Table 2 shows 14 compounds found in rosemary leaves with 80% methanol in the Khilt Al-Adrah region sample and 15 compounds extracted from rosemary leaves with 90% methanol at room temperature. The additional significant compounds identified were isoparvifuran and 2 (1 hr)-pyridinone, 3, 4, 4A and 10A-hexahydro-6-methoxy-1,1,4A. The major compounds identified were 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate and caryophyllene. The other minor volatile compounds identified are shown in Table 2.

Table 2: Compounds detected in methanol extracts 80 and 90% at RT of rosemary leaves in the Khilt Al-Adrah Region sample with their retention time (RT), molecular weight (MW) and molecular formula (MF)

Methanol extracts 80% at RT				Methanol extracts 90% at RT			
RT	Compound	MW	MF	Rt	Compound	MW	MF
3.524	3-Carene	136	$C_{10}H_{16}$	3.534	3-Carene	136	$C_{10}H_{16}$
3.764	Camphene	136	$C_{10}H_{16}$	3.729	Camphene	136	$C_{10}H_{16}$
4.995	Eucalyptol	154	$C_{10}H_{18}O$	4.509	Alpha-phellandrene	136	$C_{10}H_{16}$
7.005	Camphor	152	$C_{10}H_{16}O$	5.015	Eucalyptol	154	$C_{10}H_{18}O$
7.451	Borneol	154	$C_{10}H_{18}O$	7.020	Camphor	152	$C_{10}H_{16}O$
9.296	Isobornyl acetate	196	$C_{12}H_{20}O_2$	7.446	Borneol	154	$C_{10}H_{18}O$
11.587	Caryophyllene	204	$C_{15}H_{24}$	7.811	Bornyl chloride	172	$C_{10}H_{17}Cl$
15.349	Beta-humulene	204	$C_{15}H_{24}$	8.076	D-Verbenone	150	$C_{10}H_{14}O$
16.914	Geraniol	430	$C_{10}H_{18}O$	9.306	Isobornyl acetate	196	$C_{12}H_{20}O_2$
18.760	Longifolenealdehyde	220	$C_{15}H_{24}O$	11.602	Caryophyllene	204	$C_{15}H_{24}$
21.761	Isoparvifuran	254	$C_{16}H_{14}O_3$	12.157	Alpha-caryophyllene	204	$C_{15}H_{24}$
24.752	Furo[2,3B]Quinoline, 4, 6, 7-trimethoxy (Kokusaginine)	259	$C_{14}H_{13}NO_4$	14.143	Vitamin A aldehyde	284	$C_{20}H_{28}O$
26.658	2(1H)-pyridinone, 3, 4, 4A, 10, 10A-hexahydro-6-methoxy-1, 1, 4A-	314	$C_{21}H_{30}O_2$	15.204	Methyl steviol	204	$C_{21}H_{32}O_3$
27.659	indolo[2,3-A]Quinalizine, 1, 2, 3, 4, 5, 6, 7, 12B-Octahydro-12, 12B-Dimethyl	296	$C_{20}H_{28}ON_2$	23.367	Ferruginol	286	$C_{20}H_{30}O$
				26.833	Alpha-amyrin	426	$C_{30}H_{50}O$

Table 3: Compounds detected in methanol extracts 80 and 90% at RT of rosemary leaves in the Umm Lasfah Village sample with their retention time (RT) (MW) and molecular formula (MF)

Methanol extracts 80% at RT				Methanol extracts 90% at RT			
RT	Compound	MW	MF	RT	Compound	MW	MF
3.479	3-Carene	136	$C_{10}H_{16}$	3.434	3-Carene	136	$C_{10}H_{16}$
3.754	Camphene	136	$C_{10}H_{16}$	3.729	Camphene	136	$C_{10}H_{16}$
4.995	Eucalyptol	154	$C_{10}H_{18}O$	4.509	Alpha-phellandrene	136	$C_{10}H_{16}$
7.005	Camphor	152	$C_{10}H_{16}O$	5.005	Eucalyptol	154	$C_{10}H_{18}O$
7.436	Borneol	154	$C_{10}H_{18}O$	7.010	Camphor	152	$C_{10}H_{16}O$
8.076	D-Verbenone	150	$C_{10}H_{14}O$	7.441	Borneol	154	$C_{10}H_{18}O$
9.301	Isobornyl acetate	196	$C_{12}H_{20}O_2$	7.801	Bornyl chloride	172	$C_{10}H_{17}Cl$
11.582	Caryophyllene	204	$C_{15}H_{24}$	8.066	D-Verbenone	150	$C_{10}H_{14}O$
15.199	Humulen-(V1)	204	$C_{15}H_{24}$	9.306	Isobornyl acetate	196	$C_{12}H_{20}O_2$
20.791	Menthol	156	$C_{10}H_{20}O$	11.597	Caryophyllene	204	$C_{15}H_{24}$
21.751	Isoparvifuran	254	$C_{16}H_{14}O_3$	12.152	Alpha-caryophyllene	204	$C_{15}H_{24}$
26.653	2 (1H)-phenanthrenone, 3, 4, 4A, 10, 10A-hexahydro-6-methoxy-1, 1, 4A-	314	$C_{21}H_{30}O_2$	15.194	Methyl steviol	204	$C_{21}H_{32}O_3$
				23.362	Ferruginol	286	$C_{20}H_{30}O$

Table 3 presents the 12 compounds extracted from rosemary leaves in 80% methanol. The 13 compounds found in rosemary leaves in 90% methanol at room temperature in the Umm Lasfah Village Region. The significant compounds identified were 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate, bornyl acetate and caryophyllene and 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate and caryophyllene in 80 and 90% methanol, respectively. The other minor volatile compounds identified are shown in Table 3. Table 4 shows the 13 compounds in rosemary leaves in 80% methanol. The nine compounds found in rosemary leaves in 90% methanol at room temperature in the Hebron region. The significant compounds identified were 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate, bornyl acetate and caryophyllene for 80% methanol and 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate and caryophyllene for 90% methanol. The other minor volatile compounds identified are shown in Table 4.

Table 4: Compounds detected in methanol extracts 80 and 90% at RT of rosemary leaves in the Hebron sample with their retention time (Rt) (MW) and molecular formula (MF)

Methanol extracts 80% at RT				Methanol extracts 90% at RT			
RT	Compound	MW	MF	RT	Compound	MW	MF
3.474	3-Carene	136	$C_{10}H_{16}$	3.489	3-Carene	136	$C_{10}H_{16}$
3.719	Camphene	136	$C_{10}H_{16}$	3.734	Camphene	136	$C_{10}H_{16}$
5.005	Eucalyptol	154	$C_{10}H_{18}O$	4.509	Alpha-phellandrene	136	$C_{10}H_{16}$
7.005	Camphor	152	$C_{10}H_{16}O$	5.025	Eucalyptol	154	$C_{10}H_{18}O$
7.446	Borneol	154	$C_{10}H_{18}O$	7.015	Camphor	152	$C_{10}H_{16}O$
7.806	Bornyl chloride	172	$C_{10}H_{17}Cl$	9.301	Isobornyl acetate	196	$C_{12}H_{20}O_2$
8.076	D-Verbenone	150	$C_{10}H_{14}O$	11.597	Caryophyllene	204	$C_{15}H_{24}$
9.301	Bornyl acetate	196	$C_{10}H_{20}O_2$	12.157	Alpha-caryophyllene	204	$C_{15}H_{24}$
11.587	Caryophyllene	204	$C_{15}H_{24}$	23.362	Ferruginol	286	$C_{20}H_{30}O$
15.209	Beta-humulen	204	$C_{15}H_{24}$				
20.801	Menthol	156	$C_{10}H_{20}O$				
21.766	Isoparvifuran	254	$C_{16}H_{14}O_3$				
24.552	2-Bromo-5,8-dimethoxy-3-methyl-1-naphthol	296	$C_{13}H_{13}O_3Br$				

Table 5: Compounds detected in methanol extracts 80 and 90% at RT. of rosemary leaves in the Bani Naim sample with their retention time (RT) (MW) and molecular formula (MF)

Methanol extracts 80% at RT				Methanol extracts 90% at RT			
RT	Compound	MW	MF	RT	Compound	MW	MF
3.474	3-Carene	136	$C_{10}H_{16}$	3.494	3-Carene	136	$C_{10}H_{16}$
3.759	Camphene	136	$C_{10}H_{16}$	3.734	Camphene	136	$C_{10}H_{16}$
4.990	Eucalyptol	154	$C_{10}H_{18}O$	4.179	Alpha-pinene	136	$C_{10}H_{16}$
7.005	Camphor	152	$C_{10}H_{16}O$	4.514	Alpha-phellandrene	136	$C_{10}H^{165}$
7.446	Borneol	154	$C_{10}H_{18}O$	5.015	Eucalyptol	154	$C_{10}H_{18}O$
7.806	Bornyl chloride	172	$C_{10}H_{17}Cl$	7.015	Camphor	152	$C_{10}H_{16}O$
8.081	D-Verbenone	150	$C_{10}H_{14}O$	7.446	Borneol	154	$C_{10}H_{18}O$
9.301	Isobornyl acetate	196	$C_{12}H_{20}O_2$	8.116	D-Verbenone	150	$C_{10}H_{14}O$
11.582	Caryophyllene	204	$C_{15}H_{24}$	9.306	Isobornyl acetate	196	$C_{12}H_{20}O_2$
15.204	Beta-humulen	204	$C_{15}H_{24}$	11.597	Caryophyllene	204	$C_{15}H_{24}$
20.796	Menthol	156	$C_{10}H_{20}O$	15.204	Vitamin A aldehyde	284	$C_{20}H_{28}O$
21.766	Isoparvifuran	254	$C_{16}H_{14}O_3$	23.367	Ferruginol	286	$C_{20}H_{30}O$
24.552	2-Bromo-5, 8-dimethoxy-3-methyl-1-naphthol	296	$C_{13}H_{13}O_3Br$				

Table 5 shows 13 compounds found in rosemary leaves in 80% methanol and 12 compounds extracted from rosemary leaves in 90% methanol at room temperature in the Bani Naim region sample. The significant compounds identified were 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate, bornyl acetate and caryophyllene. The 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate and caryophyllene for 80 and 90% methanol, respectively. The other minor volatile compounds identified are shown in Table 5. Table 6 indicates that 11 components were found in the methanol extracts at 80 and 13 were found in the methanol extract at 90% reflux of rosemary leaves in the Raqaa region sample. The major volatile compounds identified were 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate, borneol acetate and caryophyllene for 80% methanol and 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate and caryophyllene for 90% methanol reflux method. The other minor volatile compounds identified are shown in Table 6.

Table 7 presents the 12 compounds extracted from rosemary leaves in the methanol (80% reflux method) and the 12 compounds extracted from the methanol extract (90% reflux method) in the Khilt Al-Adrah region sample. Reflux method in the Khilt Al-Adrah region sample. The major volatile compounds identified were 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate, bornyl acetate and caryophyllene for 80% methanol and 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate

Table 6: Compounds detected in methanol extracts 80 and 90% by reflux method of rosemary leaves in the Raqaa region sample with their retention time (RT) (MW) and molecular formula (MF)

Methanol extracts 80% at RT				Methanol extracts 90% at RT			
RT	Compound	MW	MF	RT	Compound	MW	MF
3.479	3-Carene	136	$C_{10}H_{16}$	3.489	3-Carene	136	$C_{10}H_{16}$
3.754	Camphene	136	$C_{10}H_{16}$	3.734	Camphene	136	$C_{10}H_{16}$
4.990	Eucalyptol	154	$C_{10}H_{18}O$	4.179	Alpha-pinene	136	$C_{10}H_{16}$
7.000	Camphor	152	$C_{10}H_{16}O$	4.509	Alpha-phellandrene	136	$C_{10}H_{16}$
7.441	Borneol	154	$C_{10}H_{18}$	5.020	Eucalyptol	154	$C_{10}H_{18}O$
7.806	Bornyl chloride	172	$C_{10}H_{17}Cl$	7.015	Camphor	152	$C_{10}H_{16}O$
8.076	D-Verbenone	150	$C_{10}H_{14}O$	7.451	Borneol	154	$C_{10}H_{18}O$
9.296	Borneol acetate	196	$C_{12}H_{20}O_2$	8.076	D-Verbenone	150	$C_{10}H_{14}O$
11.582	Caryophyllene	204	$C_{15}H_{24}$	9.296	Isobornyl acetate	196	$C_{12}H_{20}O_2$
12.152	Alpha-caryophyllene	204	$C_{15}H_{24}$	11.597	Caryophyllene	204	$C_{15}H_{24}$
15.209	Humulen-(V1)	204	$C_{15}H_{24}$	12.152	Alpha-caryophyllene	204	$C_{15}H_{24}$
				15.204	Vitamin A aldehyde	284	$C_{20}H_{28}O$
				23.362	Ferruginol	286	$C_{20}H_{30}O$

Table 7: Compounds detected in methanol extracts 80 and 90% by reflux method of rosemary leaves in the Khilt Al-Adrah sample with their retention time (RT) (MW) and molecular formula (MF)

Methanol extracts 80% at RT				Methanol extracts 90% at RT			
RT	Compound	MW	MF	RT	Compound	MW	MF
3.529	3-Carene	136	$C_{10}H_{16}$	3.479	3-Carene	136	$C_{10}H_{16}$
3.764	Camphene	136	$C_{10}H_{16}$	3.764	Camphene	136	$C_{10}H_{16}$
4.995	Eucalyptol	154	$C_{10}H_{18}O$	4.179	Alpha-pinene	136	$C_{10}H_{16}$
7.000	Camphor	152	$C_{10}H_{16}O$	4.499	Alpha-phellandrene	136	$C_{10}H_{16}$
7.441	Borneol	154	$C_{10}H_{18}O$	5.005	Eucalyptol	154	$C_{10}H_{18}O$
7.806	Bornyl chloride	172	$C_{10}H_{17}O_2$	7.015	Camphor	152	$C_{10}H_{16}O$
8.076	D-Verbenone	150	$C_{10}H_{14}O$	8.076	D-Verbenone	150	$C_{10}H_{14}O$
9.301	Borneol acetate	196	$C_{12}H_{20}O_2$	9.296	Isobornyl acetate	196	$C_{12}H_{20}O_2$
11.587	Caryophyllene	204	$C_{15}H_{24}$	11.597	Caryophyllene	204	$C_{15}H_{24}$
12.152	Alph-caryophyllene	204	$C_{15}H_{24}$	12.152	Alpha-caryophyllene	204	$C_{15}H_{24}$
15.209	Humulen-(V1)	204	$C_{15}H_{24}$	15.199	Vitamin A aldehyde	284	$C_{20}H_{28}O$
23.362	Ferruginol	286	$C_{20}H_{30}O$	23.362	Ferruginol	286	$C_{20}H_{30}O$

Table 8: Compounds detected in methanol extracts 80 and 90% by reflux method of rosemary leaves in the Umm Lasfah village sample with their retention time (RT) (MW) and molecular formula (MF)

Methanol extracts 80% at RT				Methanol extracts 90% at RT			
RT	Compound	MW	MF	RT	Compound	MW	MF
3.524	3-Carene	136	$C_{10}H_{16}$	3.479	3-Carene	136	$C_{10}H_{16}$
3.764	Camphene	136	$C_{10}H_{16}$	3.769	Camphene	136	$C_{10}H_{16}$
4.990	Eucalyptol	154	$C_{10}H_{18}O$	4.544	Alpha-phellandrene	136	$C_{10}H_{16}$
5.365	Alpha-pinene	136	$C_{10}H_{16}$	5.000	Eucalyptol	154	$C_{10}H_{18}O$
7.015	Camphor	152	$C_{10}H_{16}O$	7.010	Camphor	152	$C_{10}H_{16}O$
7.441	Borneol	154	$C_{10}H_{18}O$	7.446	Borneol	154	$C_{10}H_{18}O$
7.801	Bornyl chloride	172	$C_{10}H_{17}O_2$	7.806	Bornyl chloride	172	$C_{10}H_{17}Cl$
8.071	D-Verbenone	150	$C_{10}H_{14}O$	8.076	D-Verbenone	150	$C_{10}H_{14}O$
9.301	Isobornyl acetate	196	$C_{12}H_{20}O_2$	9.311	Isobornyl acetate	196	$C_{12}H_{20}O_2$
11.587	Caryophyllene	204	$C_{15}H_{24}$	11.592	Caryophyllene	204	$C_{15}H_{24}$
12.147	Alph-caryophyllene	204	$C_{15}H_{24}$	12.152	Alpha-caryophyllene	204	$C_{15}H_{24}$
				15.209	Humulen-(V1)	204	$C_{15}H_{24}$
				23.367	Ferruginol	286	$C_{20}H_{30}O$

and caryophyllene for 90% methanol reflux. The other minor volatile compounds identified are shown in Table 7. Table 8 shows the 11 compounds found in the methanol extracts at 80%. The 13 compounds found in the methanol (90% reflux) of rosemary leaves in the Umm Lasfah village region sample. The major volatile compounds identified were 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate, bornyl acetate and caryophyllene for 80% methanol and 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate and caryophyllene for 90% methanol reflux method. The other minor volatile compounds identified are shown in Table 8.

Table 9: Compounds detected in methanol extracts 80 and 90% by reflux method of rosemary leaves in the Hebron sample with their retention time (RT) (MW) and molecular formula (MF)

Methanol extracts 80% at RT				Methanol extracts 90% at RT			
RT	Compound	MW	MF	RT	Compound	MW	MF
3.534	3-Carene	136	$C_{10}H_{16}$	3.494	3-Carene	136	$C_{10}H_{16}$
3.769	Camphene	136	$C_{10}H_{16}$	3.734	Camphene	136	$C_{10}H_{16}$
4.995	Eucalyptol	154	$C_{10}H_{18}O$	4.509	Alpha-phellandrene	136	$C_{10}H_{16}$
7.005	Camphor	152	$C_{10}H_{16}O$	5.010	Eucalyptol	154	$C_{10}H_{18}O$
7.441	Borneol	154	$C_{10}H_{18}O$	7.005	Camphor	152	$C_{10}H_{16}O$
7.556	Bornyl chloride	172	$C_{10}H_{17}O_2$	7.446	Borneol, heptafluorobutyrate (ester)	350	$C_{14}H_{17}O_2F_7$
7.806	*P*-Menth-1-en-8-ol	154	$C_{10}H_{18}O$	8.081	D-Verbenone	150	$C_{10}H_{14}O$
8.076	D-Verbenone	150	$C_{10}H_{14}O_2$	9.301	Isobornyl acetate	196	$C_{12}H_{20}O_2$
9.296	Bornyl acetate	196	$C_{12}H_{20}O_2$	11.592	Caryophyllene	204	$C_{15}H_{24}$
11.587	Caryophyllene	204	$C_{15}H_{24}$	12.162	Alpha-caryophyllene	204	$C_{15}H_{24}$
15.214	Humulen-(V1)	204	$C_{15}H_{24}$	15.204	Vitamin A aldehyde	284	$C_{20}H_{28}O$
				23.367	Ferruginol	286	$C_{20}H_{30}O$

Table 10: Compounds detected in methanol extracts 80 and 90% by reflux method of rosemary leaves in the Bani Naim sample with their retention time (RT) (MW) and molecular formula (MF)

Methanol extracts 80% at RT				Methanol extracts 90% at RT			
RT	Compound	MW	MF	RT	Compound	MW	MF
3.524	3-Carene	136	$C_{10}H_{16}$	3.494	3-Carene	136	$C_{10}H_{16}$
3.764	Camphene	136	$C_{10}H_{16}$	3.739	Camphene	136	$C_{10}H_{16}$
4.529	Alpha-phellandrene	136	$C_{10}H_{16}$	4.129	Alph-pinene	136	$C_{10}H_{16}$
5.005	Eucalyptol	154	$C_{10}H_{18}O$	4.514	Alpha-phellandrene	136	$C_{10}H_{16}$
7.000	Camphor	152	$C_{10}H_{16}O$	5.020	Eucalyptol	154	$C_{10}H_{18}O$
7.446	Borneol	154	$C_{10}H_{18}O$	7.005	Camphor	152	$C_{10}H_{16}O$
7.801	Bornyl chloride	172	$C_{10}H_{17}O_2$	7.476	Borneol, heptafluorobutyrate (ester)	350	$C_{14}H_{17}O_2F_7$
8.081	D-Verbenone	150	$C_{10}H_{14}O_2$	8.126	D-Verbenone	150	$C_{10}H_{14}O$
9.321	Bornyl acetate	196	$C_{12}H_{20}O_2$	9.316	Isobornyl acetate	196	$C_{12}H_{20}O_2$
11.592	Caryophyllene	204	$C_{15}H_{24}$	11.597	Caryophyllene	204	$C_{15}H_{24}$
12.177	Alph-caryophyllene	136	$C_{10}H_{16}$	12.162	Alpha-caryophyllene	204	$C_{15}H_{24}$
				15.204	Vitamin A aldehyde	284	$C_{20}H_{28}O$
				23.367	Ferruginol	286	$C_{20}H_{30}O$

Table 9 indicates that 11 components were found in the methanol extracts at 80% and 12 were found in the methanol at 90% reflux method of rosemary leaves in the Hebron region sample. The major volatile compounds identified were 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate, borneol acetate and caryophyllene for 80% methanol and 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate and caryophyllene for 90% methanol reflux method. The other minor volatile compounds identified are shown in Table 9. Table 10 presented the 11 compounds extracted from rosemary leaves in the methanol extracts with 80 and 12 compounds extracted from rosemary leaves in the methanol with 90% reflux method in the Bani Naim region sample. The major volatile compounds identified were 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate, bornyl acetate and caryophyllene for 80% methanol and 3-carene, camphene, Eucalyptol, camphor, borneol, isobornyl acetate and caryophyllene for 90% methanol reflux method. The other minor volatile compounds identified are shown in Table 10.

Qualitative phytochemical screening: The results of the rosemary leaf qualitative phytochemical screening tests were carried out and showed that the methanol extract of the samples contains a wide range of phytochemical groups such as cardiac glycosides, phenolic groups, alkaloids, coumarin, saponins, steroids, tannins and terpenoids in all regions. Interestingly, alkaloids were found only in the leaves of the Raqaa and Bani Naim samples. However, other groups, such as anthocyanins, anthraquinone, flavonoids, glycosides and phlobatnnins, were absent. The qualitative results of the phytochemical compounds in rosemary samples were expressed in terms of their presence and (-) absence, as presented in Table 11.

Table 11: Phytochemical screening for the methanol extracts from rosemary leaf samples

Rosemary sample location	Parts	Phytochemical screening tests													
		Cardiac glycosides	Phenolic groups	Alkaloids	Anthocyanin	Couarin	Saponins	Anthraquinone	Quinones	Steroids	Tannins	Terpenoids	Flavonoids	Glycosides	Phlobatnnins
Raqaa	Leaves	+	+	+	-	+	+	-	-	+	+	+	-	-	-
Khilt Al-Adrah		+	+	-	-	+	+	-	-	+	+	+	-	-	-
Umm Lasfah Village		+	+	-	-	+	+	-	-	+	+	+	-	-	-
Hebron		+	+	-	-	+	+	-	-	+	+	+	-	-	-
Bani Naim		+	+	+	-	+	+	-	-	+	+	+	-	-	-

-:Absence and +: Presence

Table 12: Major compounds detected in all samples by GC-MS analysis of rosemary leaves

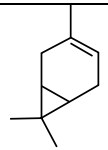
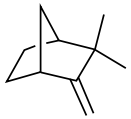
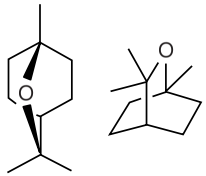
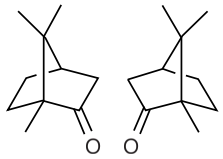
Name of the compound	Nature of the compound	Compound structure	Activity
3-Carene	Monoterpene		Antimicrobial, antioxidant, anticancer, semiochemical and fumigant properties[18]
Camphene	Monoterpene (terpenoid)		Antibacterial, antifungal, anticancer, antioxidant, antiparasitic, antidiabetic, antiinflammatory, hypolipidemic, anti-leishmanial, hepatoprotective, antiviral and anti-acetylcholinesterase[19]
Eucalyptol	Monoterpenoid		Antiinflammatory and antioxidant[20]
Camphor	Monoterpene (terpenoid)	 (+)- and (−)-camphor	Antiviral, antimicrobial, antitussive and analgesic agent[21]

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Table 12: Continued

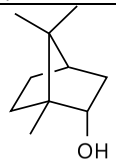
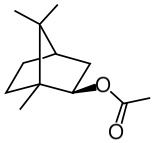
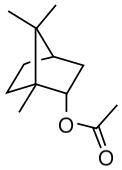
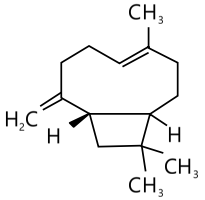
Name of the compound	Nature of the compound	Compound structure	Activity
Borneol	Monoterpenoid (terpene)		Antiinflammatory and cell penetration enhancing effect[22]
Isobornyl acetate	Monoterpene (terpenoid)		Antimicrobial[23]
Bornyl acetate (ester of borneol)	Monoterpenoid (terpene)		Antimicrobial[24]
Caryophyllene	Sesquiterpene		Antimicrobial, Anticarcinogenic, Antiinflammatory, Antioxidant and Anxiolytic, Local anesthetic[25]

Table 12 shows the chemical structures, nature of the compounds and biological activities of the main constituents in all studied samples of rosemary.

DISCUSSION

Rosemary plant samples from all regions tested for phytochemicals were found to have cardiac glycosides, phenolic groups, alkaloids, coumarin, saponins, steroids, tannins and terpenoids. The volatile components of 80 and 90% methanol at room temperature and reflux for rosemary leaf extracts were detected using GC-MS with the Electron Impact (EI) mode and compared to the NIST database. The main components of the rosemary plant samples are Eucalyptol, camphor, 3-carene, camphene, borneol, isobornyl acetate, bornyl acetate and caryophyllene. These results were also compatible with the results obtained by Verma *et al.*[26] and Masumoto *et al.*[27]. Borneol, bornyl acetate, camphor, 1,8-cineole (Eucalyptol) and verbenone are the main volatile components that contribute to rosemary extracts' distinctive flavor and aroma[28]. Rosemary contains several phytochemicals that could be used to treat diseases or disorders. It was found that the significant chemotypes of Mediterranean-grown rosemary are Eucalyptol and campho [29]. Eucalyptol has several drug properties that are gaining medical attention and evidence of its anti-inflammatory and antioxidant modes of action[30]. Eucalyptol (1,8-cineole) is also a potent cytokine inhibitor with a significant improvement in anti-inflammatory activity, according to research by Juergens *et al.*[31]. Volatile components like Eucalyptol and α-pinene have an anti-hyperglycemic impact because they lower plasma glucose levels, raise insulin levels and aid in glucose utilization by cells[32]. Camphor is also widely used on the skin for its antipruritic, analgesic and anti-irritant properties[33]. Phytochemicals can vary greatly depending on plant parts (stems, leaves, flowers and roots), extraction circumstances (time, solvents and extraction method), processing procedures and environmental conditions in which plants grow[34-36].

There is variation in the phytochemicals in the different samples tested and analyzed, this might be due to varying extraction concentrations and methods. In addition to the various locations of the samples collected, which seems to be an essential factor for the variation of the components in rosemary leaves used in this study, the concentration of the extraction solvent and the method or conditions of the extraction method play a significant role in the components that are present in the plant, i.e., 80% methanol and RT extraction are optimum for enhancing the quality and the quantity of the phytochemicals presents in the plant. For example, ferruginol was only present in the sample tested when 90% methanol was used as the solvent of extraction and with the reflux method, only the exceptions were in the Khilt Al-Adrah sample, it was present in both solvent concentrations (Table 7) and it is not present in the Raqaa region sample (Table 1). However, if ferruginol is required to be obtained, the hot extraction method with 90% methanol was used. Ferruginol is a diterpene phenol with antibacterial, antitumor, antimalarial and cardioprotective properties[37]. The differences in the composition between the samples analyzed were given in Table 1-10, the blue-colored text in the tables indicates the standard components in common between each sample at different extraction conditions.

The present study concluded that the plants contained many cardiac glycosides, phenolic groups, alkaloids, coumarin, saponins, steroids, tannins and terpenoids (Table 12). According to a study by Tabassum *et al.*[38], the leaves of rosemary plants present tannins, flavonoids, terpenoids, alkaloids, cardiac glycosides, phenols and saponins. In addition, Johar *et al.*[39] showed that rosemary has terpenoids, flavonoids and saponins but not tannins. Phenolic compounds may help prevent several chronic illnesses, including diabetes, cancer, cardiovascular disease and infections caused by bacteria and parasites. The rosemary leaf extracts tested in this current study show that rosemary also contains tannins, which have antioxidant properties, enhance wound healing and are beneficial against peptic ulcers. The presence of terpenoids in rosemary leaf extracts may also have cardioprotective and antioxidant properties[40]. Another secondary metabolite detected in rosemary leaf extracts was steroids, which aid in reducing

cholesterol and improving airway inflammation in asthma[41]. Saponins have historically been used as natural detergents, too. Their physicochemical and biological properties are exploited in food, cosmetics and medicine[42]. It was reported that alkaloids have pharmacological activities like antimicrobial, analgesic, antioxidant and anti-inflammatory[43]. Rosemary has been demonstrated to reduce iron absorption and utilization. Thus, it should be used cautiously in patients at risk of iron deficiency because the extract is rich in phenol. According Samman *et al.*[44], the biological activities and the nutritional composition of the methanol-extracted rosemary leaves, which include antibacterials, antioxidants, minerals, etc., should be determined.

The implications and application of the phytochemical analysis of *Rosmarinus officinalis* L. (rosemary) leaves using GC-MS can be multifaceted and significant for many aspects such as, the identification of bioactive compounds in rosemary leaves. This knowledge can help researchers and industries understand the plant's chemical composition, which can have implications for its medicinal, culinary, or cosmetic uses. Besides, by identifying the phytochemicals in rosemary, researchers can explore its potential medicinal properties. Rosemary has been traditionally used for its antioxidant, anti-inflammatory and antimicrobial properties. Understanding the specific compounds responsible for these effects can lead to the development of new herbal medicines or supplements. In addition, phytochemical analysis can provide information about the nutritional value of rosemary leaves. This data can be used to incorporate rosemary into diets for potential health benefits or to enhance the flavor of various food products. The findings can be valuable for the food industry. Rosemary extracts and essential oils are used as natural flavorings and preservatives. A detailed analysis can help improve the quality and consistency of such products. The study can inform agricultural practices by highlighting the compounds responsible for rosemary's resistance to pests or diseases. This information can be used to develop more robust and sustainable cultivation methods. The identification of bioactive compounds can inspire the development of pharmaceuticals or nutraceuticals. For example, if a specific compound shows potent antioxidant properties, it might be incorporated into pharmaceuticals or dietary supplements. Understanding the phytochemical profile of *Rosmarinus officinalis* can also have implications for its conservation. If certain compounds are found to be unique or important, it may influence conservation efforts to protect this species and its natural habitat. The analysis contributes to the broader scientific understanding of plant chemistry and biochemistry. It can serve as a reference for future research on similar plant species and their potential applications. In summary, the phytochemical analysis of *Rosmarinus officinalis* L. leaves using GC-MS can have wide-ranging implications and applications, spanning from medicine and nutrition to agriculture and industry as mentioned. There are general recommendations that might apply to the study such as optimizing extraction methods to obtain a more comprehensive and representative sample of phytochemicals from rosemary leaves. This could involve experimenting with different solvents, temperatures and extraction times. Identify and characterize the most abundant and bioactive compounds found in rosemary leaves. Provide detailed information on their chemical structures, concentrations and potential health benefits. It is also recommended to focus on specific compounds that exhibit noteworthy properties, such as antioxidants or antimicrobial agents. The development of quality control standards to ensure consistent and safe product formulations. It is also recommended further investigations into the development of herbal medicines or dietary supplements containing rosemary extracts. Dosage recommendations and potential therapeutic applications may be explored too. Using phytochemical analysis data to enhance the flavor, shelf life, or nutritional content of food products. This might involve incorporating rosemary extracts into recipes or using them as natural preservatives. Guiding using rosemary leaves in cooking to maximize their flavor and potential health benefits. Safety assessments and regulatory considerations may be necessary. Additionally, collaboration with experts in related fields such as pharmacology, nutrition, or culinary arts may be necessary to translate the findings into practical applications. The GC-MS is a valuable technique, but it has some limitations and challenges such as limited identification as GC-MS can identify compounds based on their mass spectra, but it may not provide

unequivocal identification of all compounds. Some compounds may have similar mass spectra, making it challenging to distinguish them accurately. The quantification of the phytochemicals can be challenging. Plant extracts can contain a complex mixture of compounds and GC-MS may not be able to separate and identify all of them. Co-elution of compounds can occur, making it challenging to distinguish individual peaks. The GC-MS is best suited for the analysis of volatile and semi-volatile compounds. It may not effectively analyze non-volatile or high molecular weight compounds, limiting the scope of the analysis. Despite these limitations, GC-MS remains a powerful tool for phytochemical analysis when used appropriately and in conjunction with other analytical techniques.

CONCLUSION

Two extraction methods with different solvent concentrations were used to detect and identify the phytochemicals in rosemary leaves. The GC-MS technique was utilized and found to be accurate and reliable in separating and identifying components of the methanol extracts of *Rosmarinus officinalis* L. The study established the chemical composition of the rosemary leaves from different locations and compared them. The main phenolic and volatile compounds in a rosemary plant in Palestine have been identified. A comparable study has been conducted between the samples using different extraction methods. The major volatile compounds detected were Eucalyptol and camphor in all samples. Palestinian rosemary is rich in phytochemicals, thus, rosemary leaves are recommended for medicinal purposes.

SIGNIFICANCE STATEMENT

The study established and compared the chemical composition of the rosemary leaves from different locations. The main phenolic and volatile compounds in a rosemary plant in Palestine have been identified. A comparable study has been conducted between the samples using different extraction methods. Palestinian rosemary is rich in phytochemicals, thus, rosemary leaves are recommended for medicinal purposes.

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