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Research Article

ISOLATION, QUANTIFICATION, AND IDENTIFICATION OF ROSMARINIC ACID, GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF ESSENTIAL OIL, CYTOTOXIC EFFECT, AND ANTIMICROBIAL INVESTIGATION OF *ROSMARINUS OFFICINALIS* LEAVES.

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ABSTRACT

Objective: *Rosmarinus officinalis* L. is an aromatic perennial herb with fragrant evergreen needle-like leaves, and it is member species of Lamiaceae family raised from Mediterranean region. The aims of the study were isolation, quantification, and identification of rosmarinic acid of *R. officinalis* leaves and essential oil analysis using various chromatographic and spectroscopic methods, and also cytotoxic and antibacterial investigation against different species of bacteria.

Methods: It was isolated by preparative HPLC and preparative TLC, and then it was determined by HPTLC. The identification and the structural elucidation of isolated rosmarinic acid were performed by H-nuclear magnetic resonance, electrospray ionization mass spectrometry (MS), infrared, and ultraviolet. Essential oil was analyzed by Gas Chromatography/Mass.

Results: Results highlighted that rosmarinic acid content was 0.9% and the oil content was 1.8%, and *R. officinalis* chemotypes of Iraqi rosemary oil were camphor 23.04%, 1, 8-cineole 14.01%, and terpinen-4-ol 13.8%. The rosemary chemotype characterized as a high concentration of terpinen-4-ol and good inhibition effect of rosemary methanolic extract against different species bacteria: *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *Acinetobacter baumannii,* and *Proteus mirabilis*.

Conclusions: The plant has a good content of rosemary phytochemicals and antibacterial effect, so the plantation of rosemary in Iraq has been successes. These isolated compounds are a suitable candidate for further clinical and pharmacological study.

Keywords: Antimicrobial, *Rosmarinus officinalis*, Rosmarinic acid, Essential oil, Terpinen-4-ol, Isolation.

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INTRODUCTION

Ever since ancient times, the humankind was a turning point in the recognition, use, and development of methods for structural elucidation and isolation of plant-derived drugs that played an important role in traditional and modern drug [1]. *Rosmarinus officinalis* L. is an aromatic, perennial herb with fragrant evergreen needle-like leaves, and also, it is member species of Lamiaceae family raised from Mediterranean region [2,3]. *R. officinalis* L. be composed of monoterpenes, primarily phenolic acids, rosmarinic acid, flavonoids, diterpenoids, and triterpenes [4]. Rosmarinic acid ($C_{18}H_{16}O_8$) is α-O-caffeoyl-3,4-dihydroxy-phenyl lactic acid, and mainly it is presented in different species of Labiatae family [5,6].

According to a clinical study, examined the pharmacokinetic parameters of RA, after absorption to blood circulation, the compound was metabolized and degraded to give conjugated and/or methylated forms of caffeic acid (CA), m-coumaric acid, and ferulic acid before being eliminated or excreted gradually in the urine [7].

Literature review revealed that rosmarinic acid and its derivatives have been shown to have biological activities, which included: anti-inflammatory [8], anti hyperglycemic [9],anti allergic [10], anti bacterial activity [11], anti-oxidant [12], in addition those have beneficial effect in Parkinson's disease [13], neuroprotective effect[14], and also have nephroprotective activity belong to their antioxidant potency through enhancing the activity of antioxidant enzymes and enhancing the glutathione content [15]. *R. officinalis* contains a great quantity of essential oil that formed from chemical components; these are hydrocarbon monoterpenes (e.g., camphene, pinene, limonene, and myrcene), sesquiterpenes (e.g., caryophyllene), and oxygen deravatives such as alcohols (e.g., borneol, linalool, and terpineol), ketones (e.g., camphor and verbenone), oxide (e.g., 1, 8-cineole), esters (e.g., bornyl acetate), and others [16]. Monoterpene hydrocarbon ($C_{10}H_{16}$) chemically is ten carbon atoms (two isoprene units) with C=C double bonds joined head-to-head [17].

The resistant of pathogens to antimicrobial agent increased and spread, and also the efficiency of the antibiotic drugs are diminished; all these give rise to affect the human health. Hence, the use of traditional-alternative medicine as a source to treat infectious diseases has been accomplished since the origin of humankind [18]. Available information indicates that hydroalcoholic extract of rosemary was assayed against different strains of bacteria [19,20].

In this present study, we report essential oil yield content and gas chromatography-mass spectrometry (GC-MS) analysis of its constituents extracted from leaves of rosemary plant cultivated in Baghdad, Iraq, also we report identification, estimation of rosmarinic acid, and isolation, quantification, and the structure elucidation were done by nuclear magnetic resonance (NMR), infrared (IR), and MS spectroscopy.

To the best of our knowledge, This study is the first work studied the qualitative- quantitative analysis and isolation with structural elucidation of rosmarinic acid of *R. officinalis* recently grown in Iraq,

according to results of this study the cultivation of plant was success in Iraq.

METHODS

General procedures

Ultraviolet (UV) spectra were recorded in MEOH using CAMAG system, IR spectra in KBR disk on Fourier-transform IR (FTIR) (Jasco-6100), H-NMR spectrum was measured on BRUKER AVANCE II 400 MHz apparatus, LC/MS/MS spectra carried out on SHIMADZU (LCMS 8040) model triple-quadruple MS spectrometer apparatus, also GC-MS analysis was performed on Shimadazu-QP 2010 ultra, the capillary column (30 m×0.25 mm internal diameter, film thickness 0.25 µm) at a flow rate 1.53 mL/min, high-performance liquid chromatography (HPLC) analysis was performed using Shimadzu 10AV-LC equipped with binary delivery pump model LC-10A Shimadzu, the eluted peaks were monitored by UV-Vis 10A-SPD spectrophotometer, high-performance thin-layer chromatography (HPTLC) analysis was carried out using CAMAG system (Switzerland), pre-coated silica gel GF254(aluminum TLC) from Merck co., and the standard rosmarinic acid from Sigma-Aldrich chemicals Co. All the solvents were from Sigma-Aldrich chemicals Co and Merk chemical company.

Plant material

Leaves of *R. officinalis* plant were collected from the garden of medicinal plants, College of Pharmacy/Mustansiriyah University in Baghdad, Iraq. Authentication of plant carried out by the National Herbarium in Botany Directorate at Abu-Ghraib, Baghdad, Iraq.

Extraction of essential oil

The hydrodistillation of 200 g of air-dried rosemary leaves by Clevenger apparatus. The rosemary oil was kept at −4°C in an airtight container.

Preparation of aqueous methanolic extract

The test solution for an extract of rosemary leaves was prepared for detection of rosmarinic acid. The extracts prepare by mixing 1 g of powdered drug with 10 ml of 90% methanol and boiled in a water bath under reflux condenser for 30 min. Then, the sample was used for the determination or identification of rosmarinic acid in comparison with rosmarinic acid standard which is prepared by dissolving 1 mg/10 ml of methanol solvent and stored in freezer at −15°C until used as a reference solution of 0.1 mg/mL.

Chromatographic analysis for the detection of rosmarinic acid

TLC

TLC analysis of extract in comparison with rosmarinic acid standard was done by development with formic acid:acetone:methylene chloride (0.85:2.5:8.5) as a mobile phase [21].

HPTLC analysis was conducted to detect the presence of rosmarinic acid. HPTLC analysis was performed by using HPTLC silica gel 60 GF 254s (10x20 cm) , the layer thickness was 0.5 mm. The standard rosmarinic acid 2 µL and 3 µL from each extract (E_b and E_k) were applied automatically on the plate by CAMAG Linomat 5. The plate was automatically submerged into an automatic developing chamber (ADC2 CAMAG) using solvent system (hexane:ethyl acetate:formic acid, 20:19:1, v/v/v), with migration distance about 7.5 cm. The plates were air-dried after development and scanned under UV (366 and 245 nm) using CAMAG TLC scanner 4. The data were processed using win CATS software.

HPLC

HPLC analysis was performed for analysis and estimation of RA in hydroalcoholic rosemary extract. The HPLC analysis was carried out by prominence HPLC with a degasser (DGU-20A) and the separation was performed in reverse-phase Hypersil ODS-C18 column (250 mm×4.6 mm i.d); sample was filtered through 0.45 µm pore size membrane. Isocratic system was used with mobile phase consisting of 80% methanol as solvent (a) and 20% (water with 0.1% acetic acid) as solvent (b). A flow rate was set at 1 ml/min for 10 min, detected by UV at 320 nm [22].

Isolation and purification of rosmarinic acid

The powdered leaves of rosemary were defatted, then after extracted twice with 2 l of water at 80°C for 50 min with stirring**,** acidifying with 25% HCl, partitioning (3 times) of each 100 ml of resulting solution with 35 ml of diethyl ether was performed to get rosmarinic acid extract.

The rosmarinic acid extract was dissolved in methanol and subjected to preparative HPLC using RP-C_{18} column with a mobile phase (methanol: 0.1% acetic acid) as isocratic mixture (80:20), flow rate was 10 ml/min, detected at 320 nm, and run time was 10 min. Rosmarinic acid peak was collected off the column in comparison with a retention time of rosmarinic acid standard.

Furthermore, preparative TLC was carried out for further purification technique and eluted with formic acid:acetone:methylene chloride (0.85:2.5:8.5) as a mobile phase, and the separated bands were visualized under UV light at 254 nm. The band at R_f=0.51 was scrapped off in comparison with the standard, then eluted with methanol and recrystallization from water to gets purified compound, and then weighted the resulted powder.

Identification of isolated rosmarinic acid

Identification by HPTLC

HPTLC analysis of isolated rosmarinic acid was performed, by application of standard rosmarinic acid (5 µL), and isolated compound by preparative chromatography (5 µL) was applied automatically on the plate by CAMAG Linomat 5 and developed with toluene: ethyl acetate:formic acid 5:4:1 as a solvent system, scanned under UV (254 and 366 nm) using CAMAG TLC scanner 4 [23]. R_f value was 0.25.

Spectrometric analysis

Chemical structure elucidation was obtained by IR, UV, NMR, and electrospray ionization MS (ESI-MS), liquid mass detection was equipped with an ESI source working in negative ion mode, and LC separation was carried out on phenomenex C18 guard column (150 mm ×4.6 mm i.d, 5 µ).

GC-MS

GC-MS analysis was carried out to identify the essential oil from leaves of Bagdad plants with GC-MS Shimadzu-QP-2010 ULTRA. The capillary column (30 m ×0.25 mm internal diameter and film thickness 0.25 µm) at a flow rate 1.53 mL/min, helium carrier gas was used, injection mode was split, and injection temperature was 240°C; the oven temperature was programmed at 70°C for (3 min), then raised to 150°C with an hold time 2.00 min, and then increased to 240°C, and the ionization mode was electronic impact mode (SEI) at 70e. Identification of the components of the oil was performed by comparing their mass spectra with database library of the National Institute of Standards and Technology (NIST08) and also comparing with the available references**.**

Cytotoxicity

The toxic effect of the tested compounds on mammalian cells was done *in vitro* on human red blood cells using well plate method; blood agar media were prepared according to the instructions of the manufacturing companies and sterilized in autoclave. Then, 100 µl of extract was poured in a well, plates were incubated for 24 h at 37°C, and positive result was indicated by inhibition zone around the wells.

Investigation of the antibacterial activity

Preliminary antibacterial was performed for methanolic extract of rosemary leaves.

Preparation of the extract

Crude methanolic extract of rosemary leaves was prepared by the extraction of 20 g from powdered leaves with 150 ml of methanol in Soxhlet apparatus. The yielded methanolic extract was dried by rotary

evaporator. The powdered extracts were kept in sterilized Petri-dishes at a refrigerator, and they were wrapped with parafilm and aluminum covers. The stock solution 100 mg/mL was prepared by dissolving 100 mg from alcoholic extract of plant in 1mL of dimethyl sulfoxide (DMSO). The dilution serials of 50.0, 25.0, and 12.5 ml were prepared for antibacterial assay.

Antibacterial Assay

Four species of bacteria were used to assay the antibacterial activity of crude extract in this study, two of them are Gram-positive (*Enterococcus faecalis* and *Staphylococcus saprophyticus*) and the other two were Gram-negative (*Acinetobacter baumannii* and *Proteus mirabilis*).

The antibacterial activity of alcoholic extract was determined by agar/ well diffusion assay and carried out using pure culture for all species of bacteria. Inoculum of bacteria was first subcultured in Brain Heart Infusion Broth and incubated at 37°C for 18–24 h. After incubation, a loopful of each species was transferred to tube containing 3 ml of normal saline and vortex well. A concentration of 1.5×10^8 colony-forming unit/ mL was obtained using McFarland turbidity standard of each bacteria inoculated using glass spreader on the surface of Mueller-Hinton Agar (MHA) plates previously prepared. The plate was allowed to dry and punched wells (five) in diameter of 6 mm into agar. Subsequently, in each agar plate of tested bacteria, five wells were made and 100 µl of dilutions of the extracts (100, 50.0, 25.0, and 12.5 mg/mL) introduced into wells on MHA plate. DMSO was used as the negative controller, and then, the plates were kept at 37°C for 24 h.

RESULTS

Chromatographic analysis for detection of rosmarinic acid

The extract of rosemary leaves was analyzed by HPLC and HPTLC.

TLC

The result showed the best separation of compounds in the aqueous methanolic extract and a fluorescent blue spot of rosmarinic acid was showed under 366 nm of UV light with R_f value 0.5 in comparison with standard rosmarinic acid.

HPTLC chromatogram of the rosemary extract showed well-defined peak number 6 at maximum Rf of 0.30 that represents 37.60% of the total extract compositions as shown in Fig. 1. With reference to R_f value of standard rosmarinic acid, the observed peak identified as rosmarinic acid.

HPLC

HPLC analysis showed rosmarinic acid peak at R_t 3.057 min representing 46.607% of the total compositions of rosemary extract compared with a retention time of rosmarinic acid standard (2.99 min) as shown in Fig. 2.

Total rosmarinic acid content

The amount of RA was isolated and quantified about 0.9% w/v in rosemary leaves extract, (Fig. 3) shows the separation bands of rosmarinic acid extract by prep. TLC the amount of RA was 0.9% w/v in rosemary leaves.

Spectrometric analysis

The isolated compound obtained as off-white powder with R_f=0.5**,** chemical investigation of this compound indicated the structure of rosmarinic acid (Fig. 4), and ESI-MS under negative ion mode together with IR, ^{1}H-NMR, and spectral data confirmed the molecular formula to be $C_{18}H_{16}O_8$. The maximum UV absorption of characteristics of rosmarinic acid was 328 nm in comparison with standard 329 nm, and ESI-MS showed the molecular ion peak $[M-1]^-$ at m/z=359 (Fig. 5) and base peak at m/z=161, Also appear other peaks at m/z 197 and 179 of two main constituents of RA: 2-hydroxyl derivative of hydro CA and CA, respectively, this pattern of fragmentation compared with those data reported previously [24].

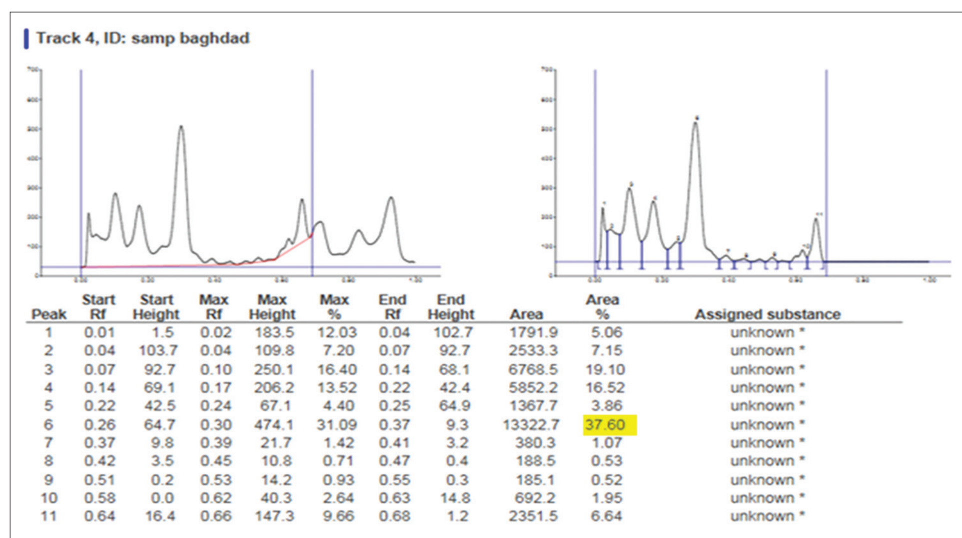
The IR spectrum (KBr) showed broad absorption band at 3181/cm (OH of carboxylic acid, broad), in addition to other bands at 1718/cm (C=O of carboxylic acid), 1683/cm (C=O of conjugated with double bond), 1514 and 1481/cm (stretching of aromatic ring), and 1400–1350/cm C-O-C stretching as shown in Fig. 6. ^{1}H-NMR spectral data (DMSO-d_6, 400 MHz) are listed in Table 1.

GC-MS analysis of essential oil

The pale-yellow oil isolated from rosemary leaves was 1.8% w/v. The GC-MS analysis has been shown 12 identified components from 19 components representing 91.36% of the total oil. Oxygenated monoterpenes were found to be the major group of these components. Furthermore, the dominant constituents were camphor (23.04%), 1, 8-cineole (14.01%), and terpinen-4-ol (13.8 %) respectively, and also the minor common components of volatile oil are verbenone (11.47%), β-terpineol (10.8%), bornyl acetate (6.96%), and borneol (3.78%). The D-limonene and camphene have low percentage about 0.42% and 0.36%, respectively, as shown in Table 2. The UN identified compounds represent the remaining compound.

Anti-bacterial assay

Minimum inhibitory concentration values determined by the well diffusion method showed values ranging from 12.5 to 100 mg/ml for *R. officinalis* extract according to different species of bacteria (Table 3).



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	1.5	0.02	183.5	12.03	0.04	102.7	1791.9	5.06	unknown *
2	0.04	103.7	0.04	109.8	7.20	0.07	92.7	2533.3	7.15	unknown *
3	0.07	92.7	0.10	250.1	16.40	0.14	68.1	6768.5	19.10	unknown *
4	0.14	69.1	0.17	206.2	13.52	0.22	42.4	5852.2	16.52	unknown *
5	0.22	42.5	0.24	67.1	4.40	0.25	64.9	1367.7	3.86	unknown *
6	0.26	64.7	0.30	474.1	31.09	0.37	9.3	13322.7	37.60	unknown *
7	0.37	9.8	0.39	21.7	1.42	0.41	3.2	380.3	1.07	unknown *
8	0.42	3.5	0.45	10.8	0.71	0.47	0.4	188.5	0.53	unknown *
9	0.51	0.2	0.53	14.2	0.93	0.55	0.3	185.1	0.52	unknown *
10	0.58	0.0	0.62	40.3	2.64	0.63	14.8	692.2	1.95	unknown *
11	0.64	16.4	0.66	147.3	9.66	0.68	1.2	2351.5	6.64	unknown *

Fig. 1: High-performance thin-layer chromatography chromatogram of extract from leaves of *Rosmarinus officinalis* cultivated in Iraq

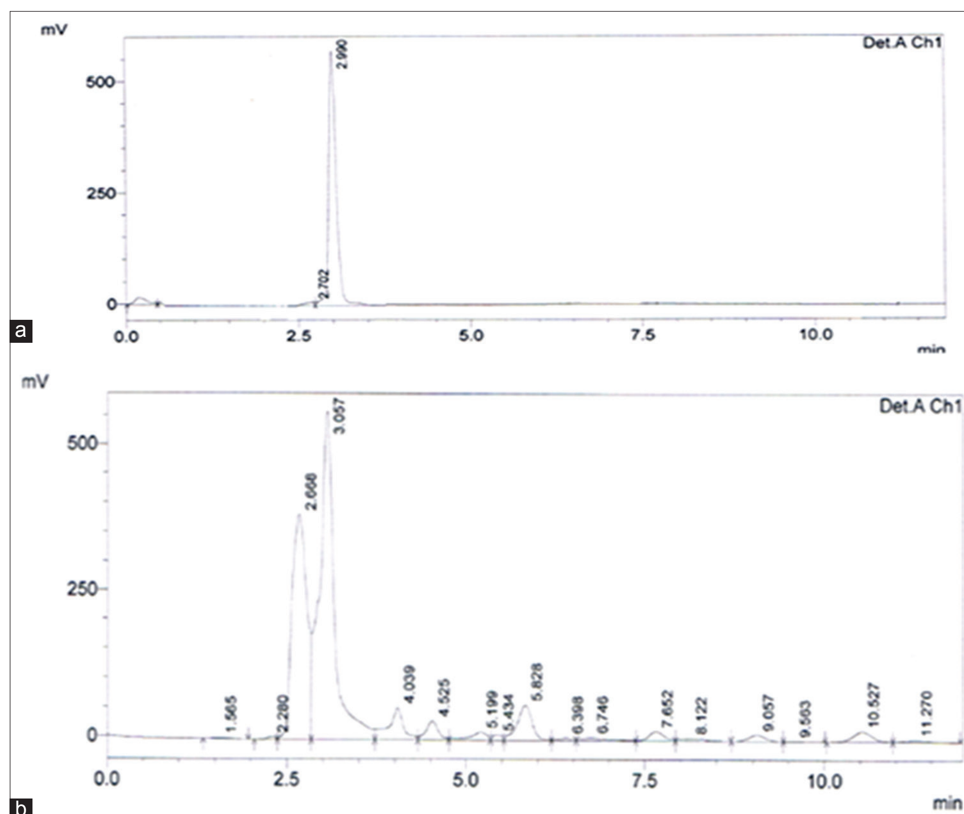


Fig. 2: The high-performance liquid chromatography analysis of 90% methanolic extract of rosemary leaves, (a) peak of standard rosmarinic acid was detected at 2.99 min (b) rosmarinic acid in hydrolcholic extract was detected at 2.66 min

Table 1: H-NMR data and their interpretation for rosmarinic acid compound

No. of group	Chemical Shift ppm	No. of proton	Interpretation
A	2.8	1 H	Multiplate of methylene
a’	3.02	1 H	Group
B	5.05	1H	Triplet of proton at chiral center
C	6.28	1H	Doublet of olefinic proton
D	6.52–6.65	3H	Doublet of protons at aromatic ring B
E	6.76–6.78	2H	Doublet of protons ortho to protons of –OH group at aromatic ring A
F	7.05	1H	Doublet of aromatic ring A
G	7.45–7.49	1H	Doublet of vicinal proton
h, h’	8.73–8.79	2H	Broad singlet protons of aromatic ring A
i, i’	9.51–9.64	2H	Broad singlet protons of aromatic ring B
J	13.033	1H	Singlet of carboxylic acid proton

NMR: Nuclear magnetic resonance

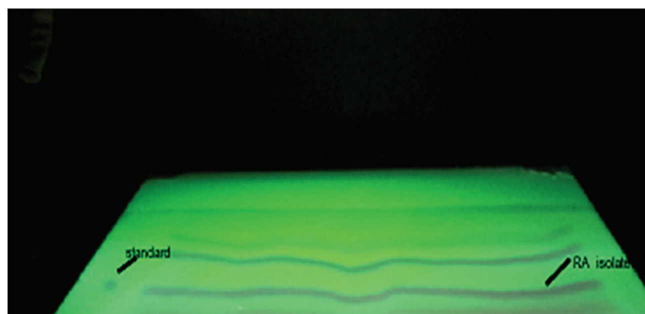


Fig. 3: Preparative thin-layer chromatography for isolation of rosmarinic acid extract under ultraviolet light at 254 nm

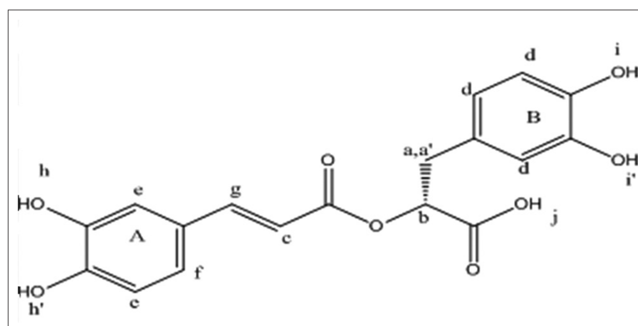


Fig. 4: Chemical structure of rosmarinic acid

DISCUSSION

From results highlights, the high amount of rosmarinic acid was 0.9% in leaves of Iraqi *R. officinalis* and this quantity of RA is considered higher than RA in some other different geographical location such as Romania, Turkey, and Iran (0.133%, 0.31%, and 0.72%, respectively) and <1.1% of rosmarinic acid of rosemary plant from trade of UK [25- 27]. The variability in RA content of a plant is known to depend on any environmental factors including harvested time, soil, climatic conditions, and other factors [28].

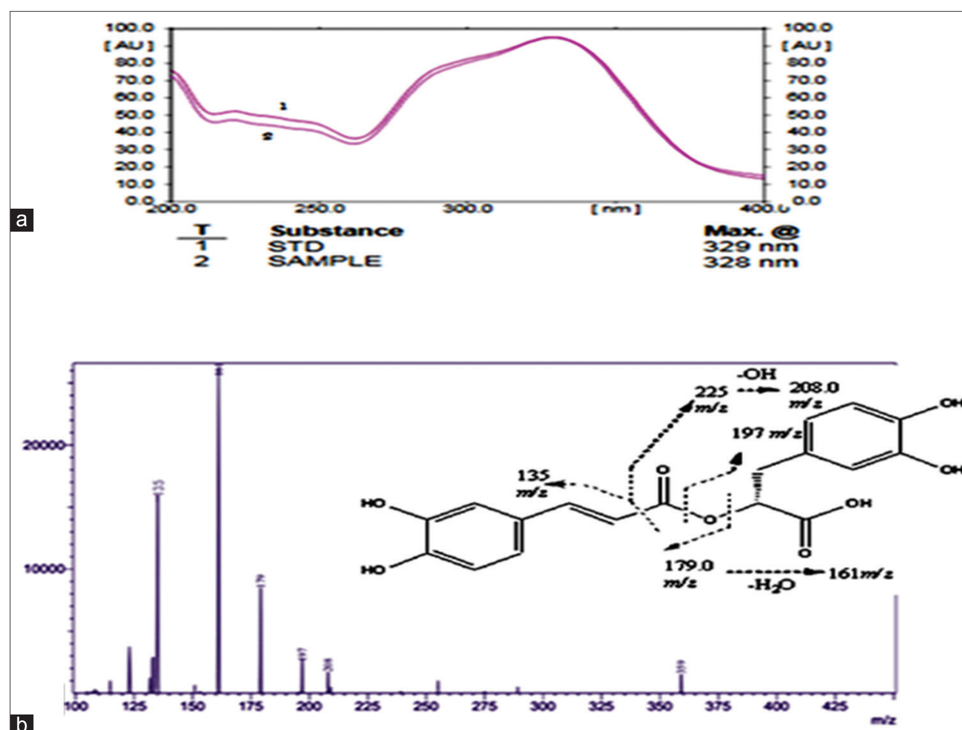


Fig. 5: Spectral analysis of rosmarinic acid (a) ultraviolat spectra of rosmarinic acid detected at 328 nm in comparison with standard was detected at 329 nm. (b) Mass spectrum obtained after mass spectrometry/mass spectrometry fragmentation of deprotonated molecular ion at M/Z 359 (M-1)

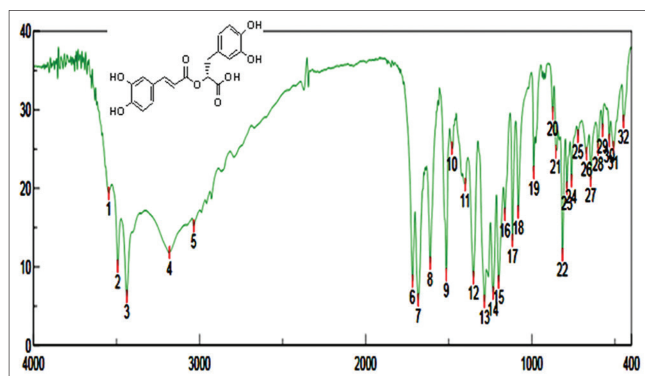


Fig. 6: Fourier-transform-infrared spectroscopy for rosmarinic acid

Spectral analysis of RA compound indicates that compound is rosmarinic acid,^{1}H-NMR spectrum showed the signals for rosmarinic acid (isolated compound), the aromatic protons showed at 7.063–6.52 ppm, and The chemical shift of two diasteriotopic protons of methylene group presence at 3.02, 2.8, it showed doublet of doublet splitting. The spectral data were agreement with previously reported literature for the same compounds [29,30].

From the results mentioned above, that essential oil content was 1.8% isolated from leaves of Iraqi *R. officinalis.* Furthermore, the leaves of Iraqi *R. officinalis* chemotypes were camphor 23.04%, 1, 8-cineole 14.01%, and terpinen-4-ol 13.8 %. This chemotype characterized with good concentrations of components and high concentration of terpinen- 4-ol than some other countries in comparison with chemical point view from the previous study of oil in varying geographical environments (Table 4) [31-37].

In general, the diversity in quantity of oil contents, percentage of components, and chemotypes of *R. officinalis* in different locations have been ascribed to many factors, including geographical environment, genetic heritance, population density of plant in addition to physical

Table 2: The chemical compositions of essential oil of *R. officinalis* leaves, analyzed by GC-MS

Peak no.	*R.T	Area%	Molecular ion (m/z)	Name of compound
1	4.82	0.42	136	D-limonene
2	5.19	14.01	154	1,8-cineole
5	9.37	23.04	152	Camphor
6	9.66	10.8	136	β- terpineol
7	10.18	6.96	154	Bornyl acetate
8	10.43	3.78	139	Borneol
9	11.12	0.45	137	Cis-verbenol
11	11.81	13.80	154	Terpinen-4-ol
12	11.98	11.47	150	Verbenone
14	12.29	0.36	136	Camphene
15	12.73	0.48	152	Camphenol

Total identified compounds: 91.36%. *RT: Retention time. *R. officinalis: Rosmarinus officinalis*, GC-MS: Gas chromatography-mass spectrometry

Table 3: Antibacterial activity of *R. officinalis* extracts with different bacterial species measured in millimeter

Bacterial species	Concentrations of extracts mg/mL				MIC mg/mL
	100	50	25	12.5	
Inhibition zone mm					
P. mirabilis	20	32	21	34	100
A. baumannii	40	36	35	34	12.5
S. saprophyticus	25	22	18	13	12.5
E. faecalis	22	20	17	18	25

MIC: Minimum inhibitory concentration. *R. officinalis: Rosmarinus officinalis, P. mirabilis: Proteus mirabilis, A. baumannii: Acinetobacter baumannii, S. saprophyticus: Staphylococcus saprophyticus, E. faecalis: Enterococcus faecalis*

Table 4: The results of several studies of essential oil yields and chemotypes of different parts *of R. officinalis*

Geographical origins	Percentage of yield oil (v/w) (%)	*R. officinalis* chemotypes (major constituents) and % of terpinen-4-ol	References
Sudan	3	Bornyl acetate (20.27%), caryophyllene (13.61%), and eucalyptol (12.84%)	[31]
Lalehzar, Iran	2.6	α-pinene (43.9%), 1,8-cineole (11.1%), and camphene (8.6%). Also, has 0.1% of terpinen-4-ol as a minor compound	[32]
Konya, Turkey	1.9	P-cymene (44.02%), linalool (20.5%), and terpinene (16.62%)	[33]
Chtouka Ait Baha, Morocco	1.9	α-pinene (34.83%), 1.8-cineole (28.30%), and other components at relatively low levels: Camphor (10.54%) and camphene (6.21%)	[34]
Vienna (Austria)	1.84	1,8-cineole (41.6%), camphor (17.0%), and α-pinene (9.9%)	[35]
Giza, Egypt	0.41	Camphor (14.9%), α-pinene (14.9%), and 1, 8-cineole (9%)	[36]
Different locations in India	-	Camphor (23.1–35.8%), 1,8-cineole (21.4–31.6%), and α-pinene (6.7–15.6%)	[37]

Adash (-) indicate not reported. *R. officinalis: Rosmarinus officinalis*

and chemical characteristics of soil, growing media, and time of harvest [38,39].

The crude extracts *of R. officinalis* leaves cultivated in Baghdad, Iraq, were evaluated for their cytotoxic effect, and the results showed no hemolysis of blood cells after 24 h incubation at 37°C. However, no cytotoxic effects have been shown with maximum concentration test of rosemary extracts on human erythrocytes by using well plate method.

The antibacterial effect of rosemary leaves against different species showed dose-dependent activity that is increased when the dose of extract increased on bacteria except the *P. mirabilis* had a minimum inhibition by extract effect at high concentration (100 mg/ml) and high inhibition effect at a low concentration 12.5 mg/ml. The most sensitive bacteria was *A. baumannii* with inhibition zone 40 mm at high concentration.

Therefore, according to our obtained findings, the rosemary plant has good antibacterial activity against some Gram-negative and Gram-positive pathogenic bacteria. This antibacterial effect belongs to the presence of diterpeneoids and phenolic acid compounds. Therefore, it is recommended to use in an alternative medicine as a source of natural antibiotics.

CONCLUSION

Our results highlight the successful grown of rosemary in Iraq with a good quantity of rosmarinic acid and good essential oil percentage. This study also introduces Iraqi *R. officinalis* as a plant rich with camphor, terpinen-4-ol, and 1,8-cineole as major compounds of monoterpene in this plant. No cytotoxic effect of rosemary on human cell and good inhibition effect of alcoholic extract of rosemary leaves against *E. faecalis*, *S. saprophyticus*, *A. baumannii,* and *P. mirabilis* bacteria. We suggest that these isolated compounds are a suitable candidate for further clinical and pharmacological study.

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