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Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus oficinalis* leaves

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The aims of the study were to investigate the presence of phytochemical compounds from the leaves of *Rosmarinus oficinalis*, using methanolic extraction and report the main functional components by using FT-IR. The phytochemical compound screened by GC-MS method. Seventeen bioactive phytochemical compounds were identified in the methanolic extract of *R. oficinalis*. The identification of phytochemical compounds is based on the peak area, retention time molecular weight, molecular formula, chemical structure, MS Fragment ions and pharmacological actions. GC-MS analysis of *Rosmarinus oficinalis* revealed the existence of the α-pinene, Camphene, Eucalyptol, 2-Methoxy-4-vinylohenol, 1-Oxaspiro[4,5]deca-3,6-diene,2,6,10,10-tetramethyl, 1-Oxaspiro[4,5] deca-3,6-diene,2,6,10,10-tetramethyl, Neocurdione, Isoaromadendrene epoxide, 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9] cycloundec., Cis-Vaccenic acid, Phenanthrenol,4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trimethyl-1, Galanthamine, Dibenz[a,c]cyclohexane,2,4,7-trimethoxy, 2,4a,7-Trihydroxy-1-methyl-8-methyleneqibb-3-ene. 1,10-carboxylic acid, Retinoic acid, 7,8,12-Tri-O-acetyl-3-desoxy-ingol-3-one and 4,6-Androstadien-3β-ol-17-one,acetate. The FTIR analysis of *Rosmarinus oficinalis* leaves proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxlic acids, Esters, Nitro compounds, Alkanes, Aldehydes, Ketones compounds. It contain chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthamatic.

Key words: FT-IR, GC-MS analysis, Leaves, Methanol, *Rosmarinus oficinalis*.

INTRODUCTION

Rosmarinus officinalis , related to the lamiaceae family of plants. Rosemary is used as an antispasmodic in real colic and dysmenorrhoea, in relieving respiratory disorders and to stimulate growth of hair. Rosemary (Rosmarinus officinalis),which grows wild around Mediterranean area, is a species from Lamiaceae family. It grows as a shrub or herbaceous plant with about 0.8 to 2m height (Atik bekkara et al, 2007).This plant likes dry and arid regions, hills and low mountains, calcareous, shale, clay and rocky substrates. The potent antioxidant properties of rosemary extracts have been attributed to its phenolic compounds, mainly rosmarinic acid and

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diterpenes carnosic acid and carnosol (Sergi and Leonor, 2002; Troncoso et al., 2005; Ameera et al., 2015). Rosemary extract relaxes smooth muscles and has choleretic, hepatoprotective and antitumerogenic activity (Al-Sereiti et al., 1999). Recent research shows that rosemary extracts possess strong anticancer properties. In the last few years gas chromatography mass spectrometry has become firmly established as a key technological platform for metabolite profiling in plant (Fiehn et al., 2003; Sumner et al., 2003; Fernie et al., 2004; Kell et al., 2005; Robertson et al., 2005). GC-MS based metabolome analysis has profound applications in discovering the mode of action of drugs or herbicides and helps unravel the effect of altered gene expression on metabolism and organism performance in biotechnological applications. Infrared spectroscopy provides a useful method for herbal analysis and elucidate the compounds structures as well as for quantitative analysis of drugs Bunaciu et al., 2010; Cheng et al., 2010; Bunaciu et al., 2010; Huda et al., 2015). Fourier transform infrared spectrometry is a physico-chemical analytical technique and one of the most widely used methods to identify the structure of unknown composition or its chemical group, and the intensity of the absorption spectra associated with molecular composition or content of the chemical group (Surewicz et al., 1993; Imad et al., 2015). The present study involves an assessment using GC-MS and FT-IR spectroscopic techniques to investigate the authenticity of commercial sample of the herbal drug by analyzing their fingerprints.

MATERIALS AND METHODS

Collection and preparation of plant material

The leaves were purchased from local market in Hilla city, middle of Iraq. after thorough cleaning and removal foreign materials, the leaves were stored in airtight container to avoid the effect of humidity and then stored at room temperature until further use.

Preparation of sample

About 20 g of the plant sample powdered were soaked in 100 ml methanol for 16 h in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plant. The filtrates were used for further phytochemical analysis (Imad et al., 2015a). It was again filtered through sodium sulphate in order to remove the traces of moisture.

Gas chromatography mass spectrum analysis

The GC-MS analysis of the plant extract was made in a (QP 2010 Plus SHIMADZU) instrument under computer control at 70 eV (Imad et al., 2014; Imad et al., 2015b and Muhanned et al., 2015). About 1 µl of the methanol extract was injected into the GC-MS using a micro syringe and the scanning was done for 45 min. As the compounds were separated, they eluted from the column and entered a detector which was capable of creating an electronic signal whenever a compound was detected. The greater the concentration in the sample, bigger was the signal obtained which was then processed by a computer. The time from when the injection was made (Initial time) to when elution occurred is referred to as the Retention time (RT). While the instrument was run, the computer generated a graph from the signal called Chromatogram. Each of the peaks in the chromatogram represented the signal created when a compound eluted from the Gas chromatography column into the detector. The x-axis showed the RT and the y-axis measured the intensity of the signal to quantify the component in the sample injected. As individual compounds eluted from the Gas chromatographic column, they entered the electron ionization (mass spectroscopy) detector, where they were bombarded with a stream of electrons causing them to break apart into fragments. The fragments obtained were actually charged ions with a certain mass. The M/Z (Mass/Charge) ratio obtained was calibrated from the graph obtained, which was called as the Mass spectrum graph which is the fingerprint of a molecule. Before analyzing the extract using gas chromatography and mass spectroscopy, the temperature of the oven, the flow rate of the gas used and the electron gun were programmed initially. The temperature of the oven was maintained at 100°C. Helium gas was used as a carrier as well as an eluent. The flow rate of helium was set to 1ml per minute. The electron gun of mass detector liberated electrons having energy of about 70eV.The column employed here for the separation of components was Elite 1(100% dimethyl poly siloxane). The identity of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures (Imad et al., 2015c; Mohammed et al., 2015).

Fourier transform infrared spectrophotometer (FTIR)

The powdered sample of the plant specimen was treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). The sample was run at infrared region between 400 and 4000 nm (Ameera et al., 2015 and Huda et al., 2015).

RESULTS AND DISCUSSION

Gas Chromatography and Mass spectroscopy analysis of compounds was carried out in methanolic seed extract of *Rosmarinus oficinalis*, shown in Table 1. The GC-MS chromatogram of the 17 peaks of the compounds detected was shown in Figure 1. Chromatogram GC-MS analysis of the methanol extract of *Rosmarinus oficinalis* showed the presence of nine major peaks and the components corresponding to the peaks were determined as follows. The First set up peak were determined to be α-pinene Figure 2. The second peak indicated to be Camphene Figure 3. The next peaks considered to be Eucalyptol, 2-Methoxy-4-vinylohenol, 1-Oxaspiro[4,5]deca-3,6-diene,2,6,10,10-tetramethyl, 1-Oxaspiro[4,5]deca-3,6-diene,2,6,10,10-tetramethyl, Neocurdione, Isoaromadendrene epoxide, 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9] cycloundec., Cis-Vaccenic acid, Phenanthrenol,4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trimethyl-1, Galanthamine, Dibenz[a,c]cyclohexane,2,4,7-trimethoxy, 2,4a,7-Trihydroxy-1-methyl-8-methyleneqibb-3-ene. 1,10-carboxylic acid, Retinoic acid, 7,8,12-Tri-O-acetyl-3-

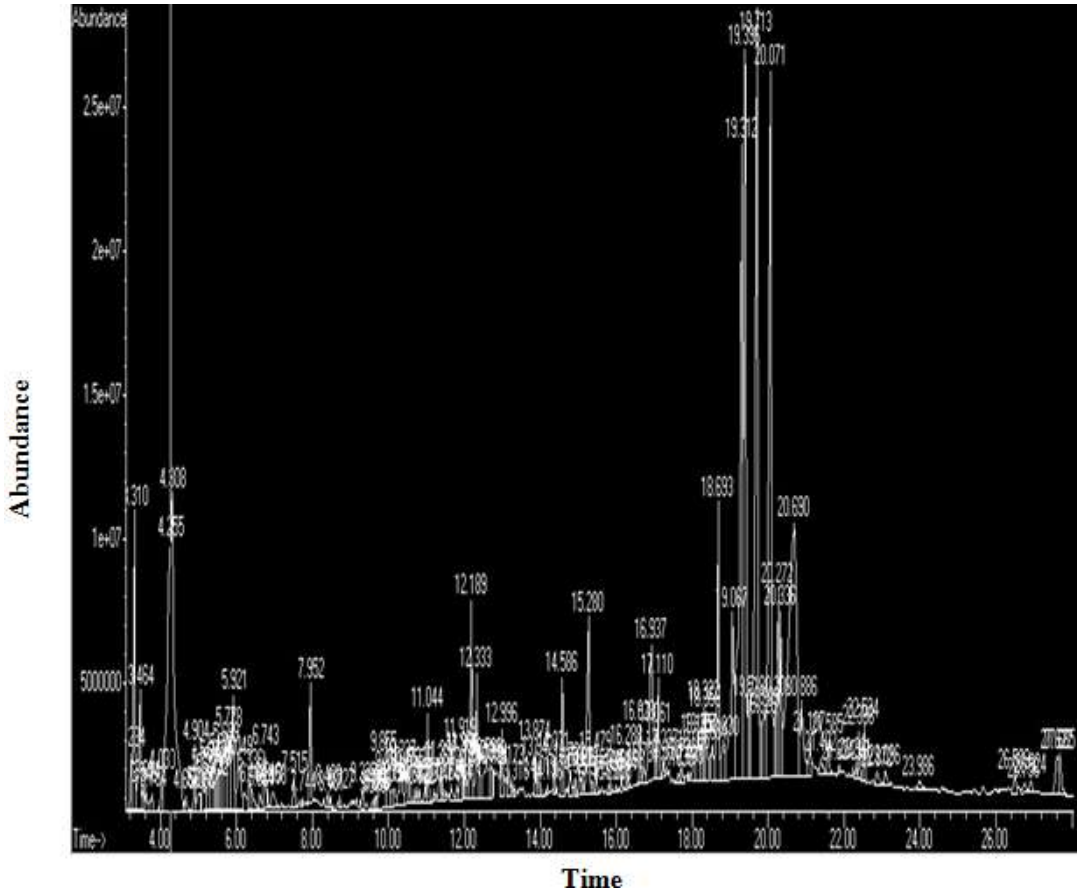


Figure 1. GC-MS profile of leaf extract of *Rosmarinus officinal.*

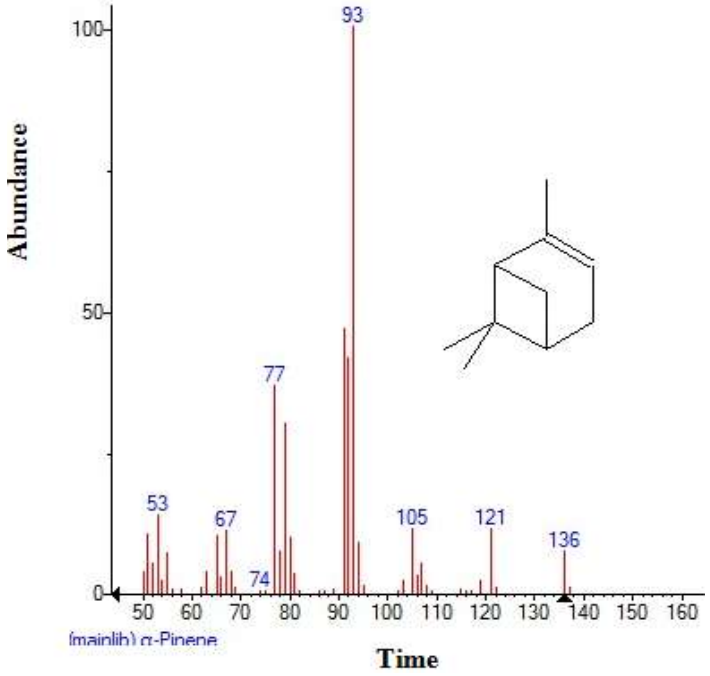


Figure 2. Structure of α-pinene present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

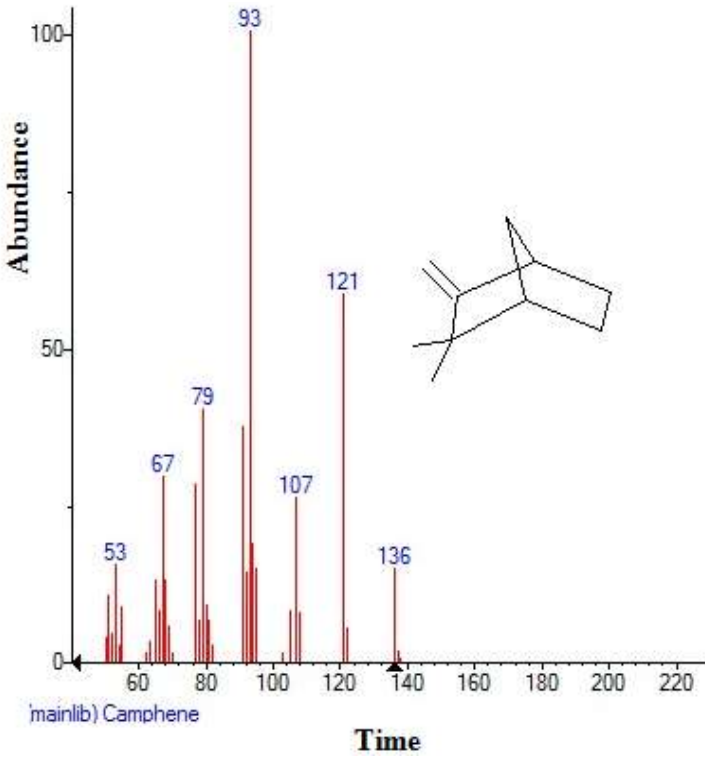


Figure 3. Structure of camphene present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

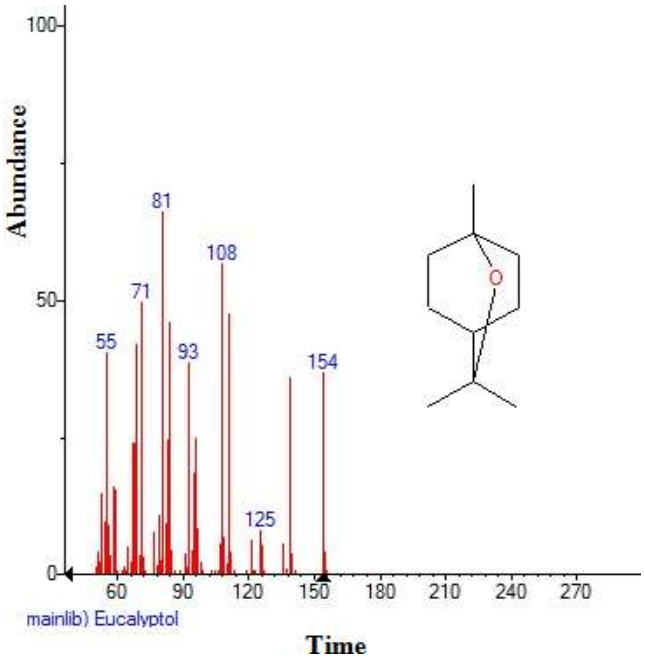


Figure 4. Structure of eucalyptol present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

desoxy-ingol-3-one, 4, 6- Androstadien-3β-ol-17- one, acetate (Figures 4 to 17). FTIR analysis of dry

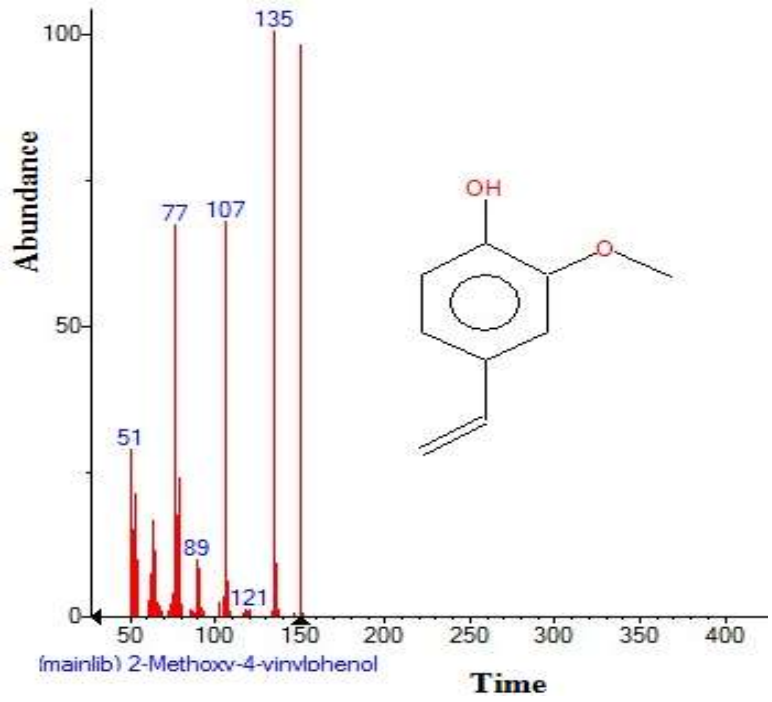


Figure 5. Structure of 2-Methoxy-4-vinylohenol present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

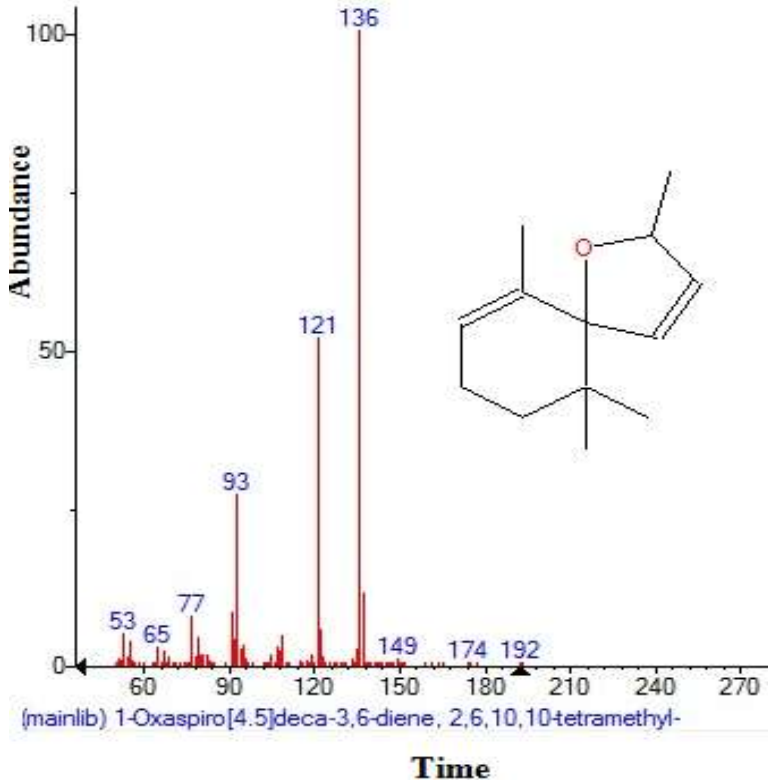


Figure 7. Structure of 1-Oxaspiro[4,5]deca-3,6-diene,2,6,10,10-tetramethyl present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

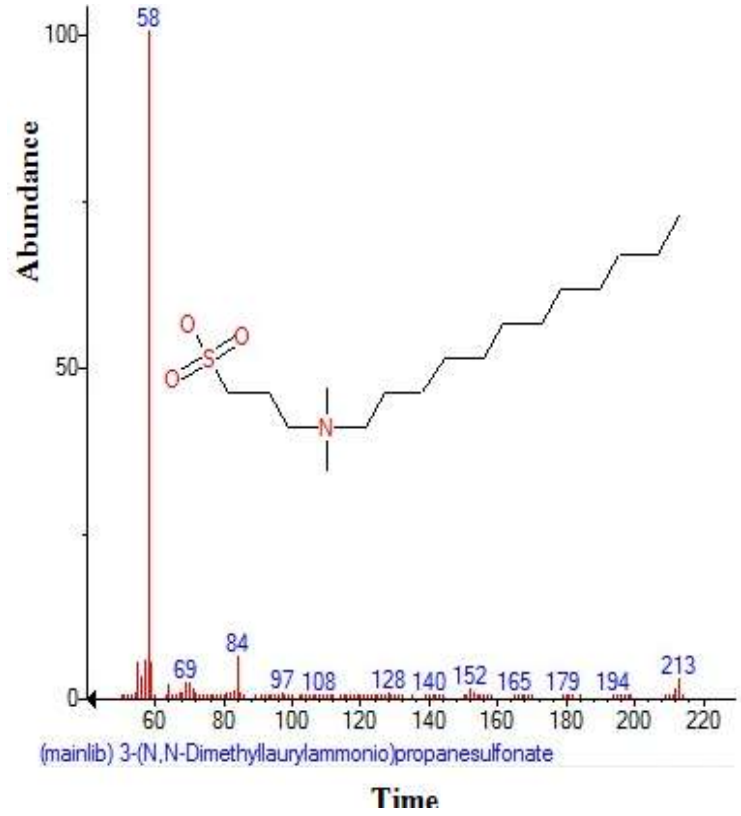


Figure 8. Structure of 3-(N,N-Dimethyllaurylammonio)propanesulfate present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

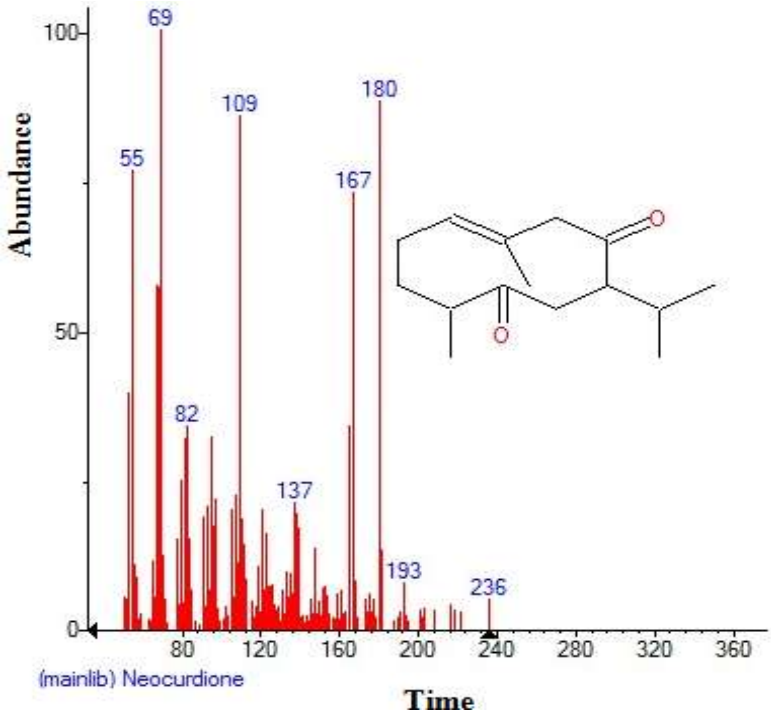


Figure 9. Structure of Neocurdione present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

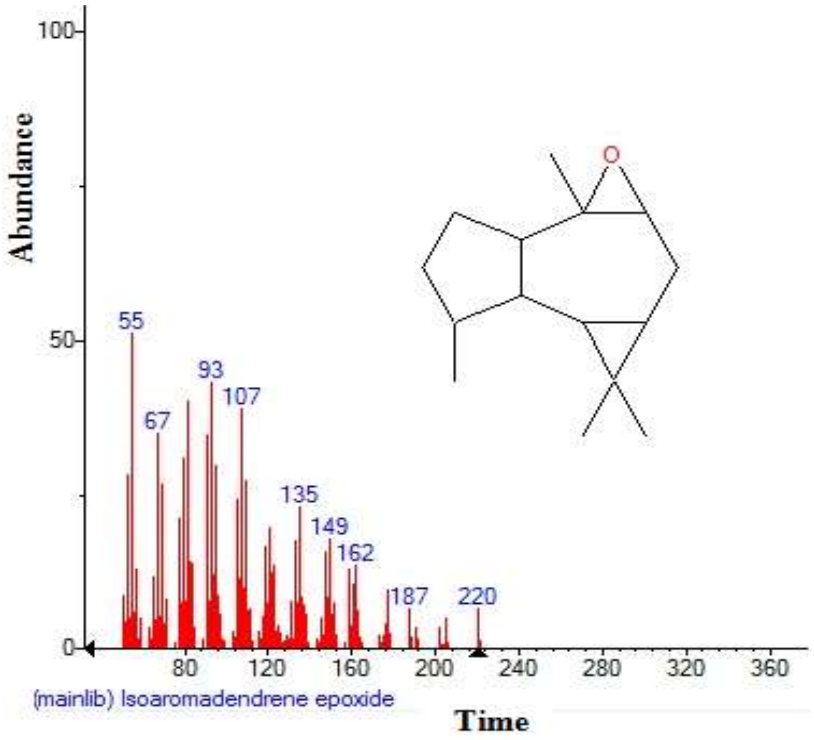


Figure 10. Structure of Isoaromadendrene epoxide present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

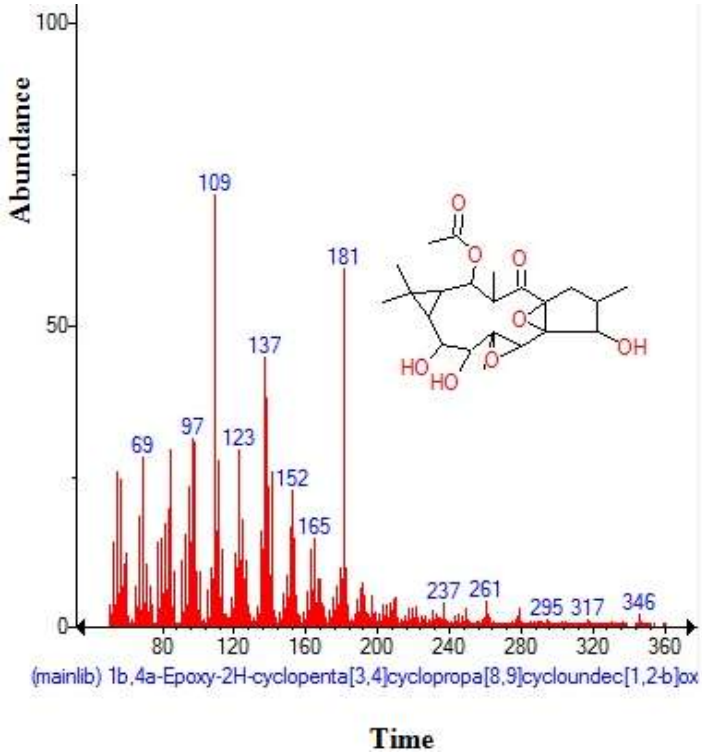


Figure 11. Structure of 1b,4a-Epoxy-2H-cyclopenta[3,4]cyclopropa[8,9]cycloundec. present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

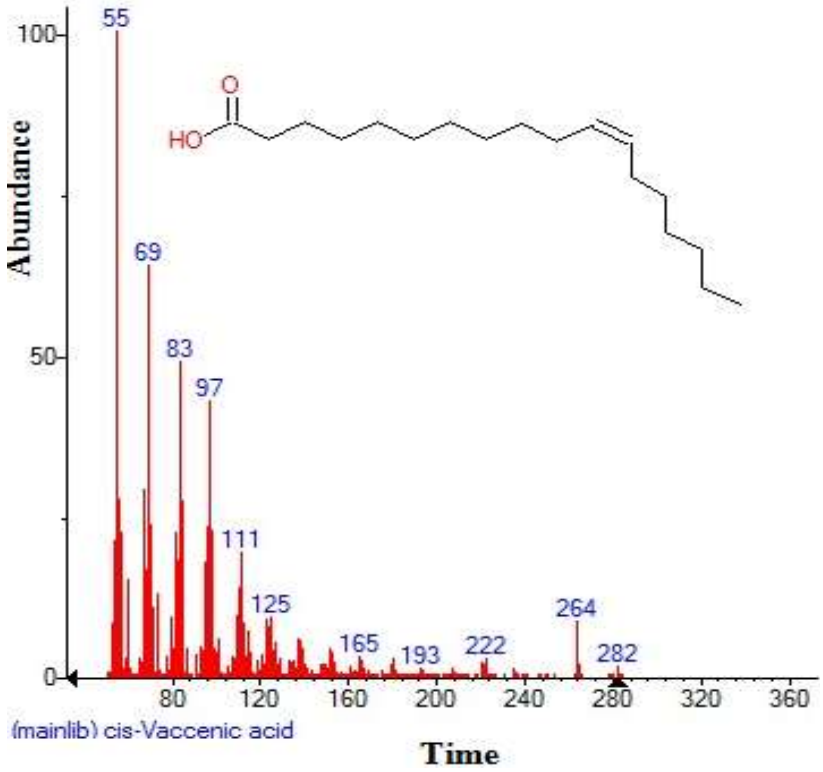


Figure 12. Structure of Cis-Vaccenic acid present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

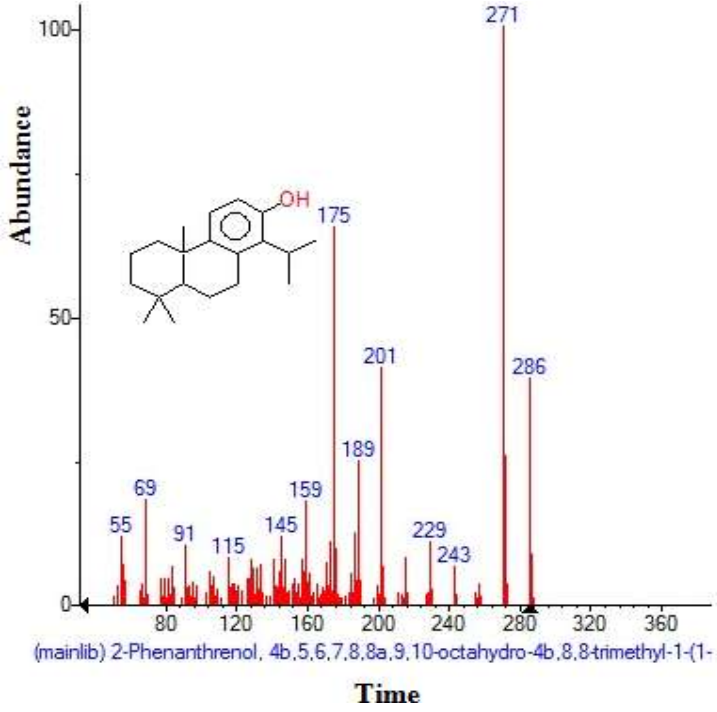


Figure 13. Structure of 2-Phenanthrenol,4b,5,6,7,8,8a,9,10-octahydro-4b,8,8 trimethyl-1 present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

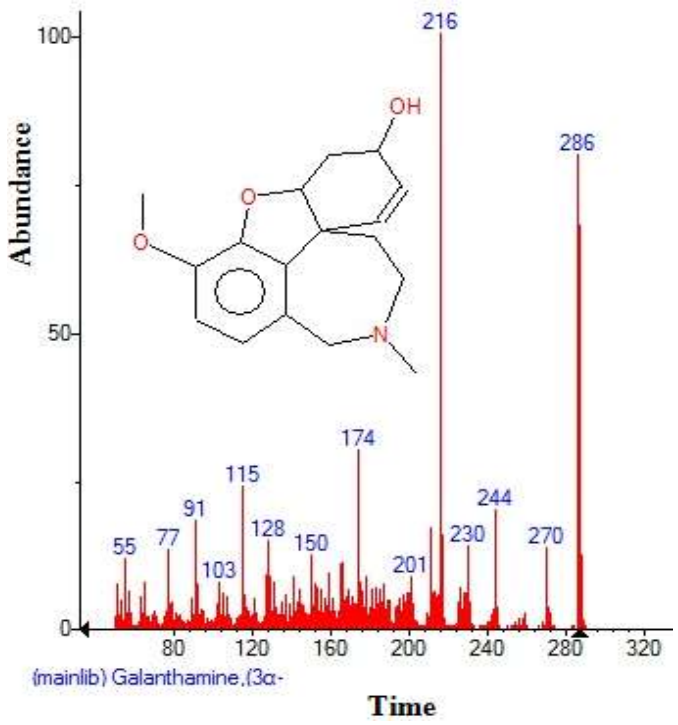


Figure 14. Structure of 2 Galanthamine present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

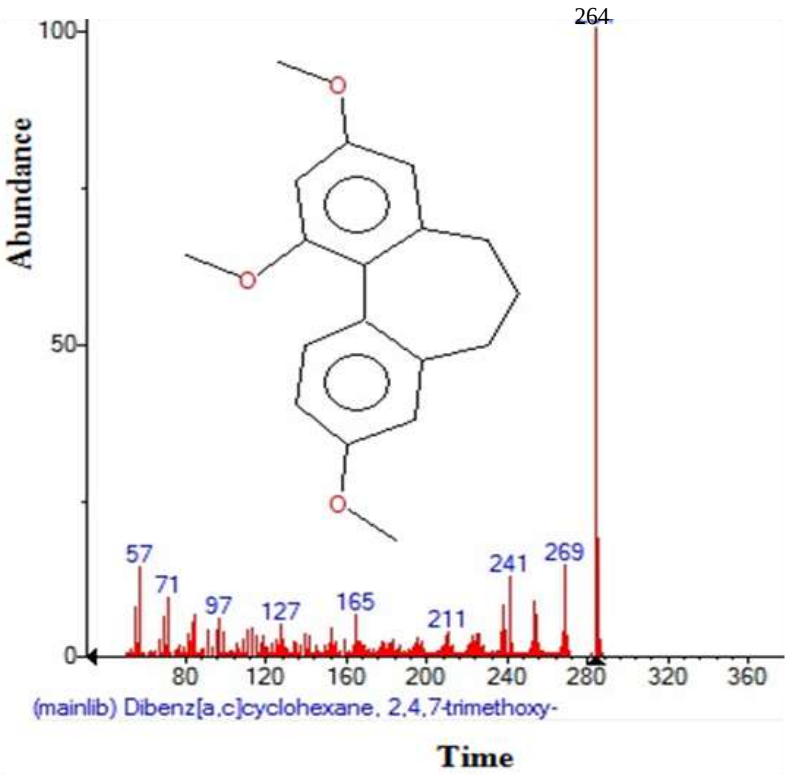


Figure 15. Structure of Dibenz[a,c]cyclohexane,2,4,7-trimethoxy present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

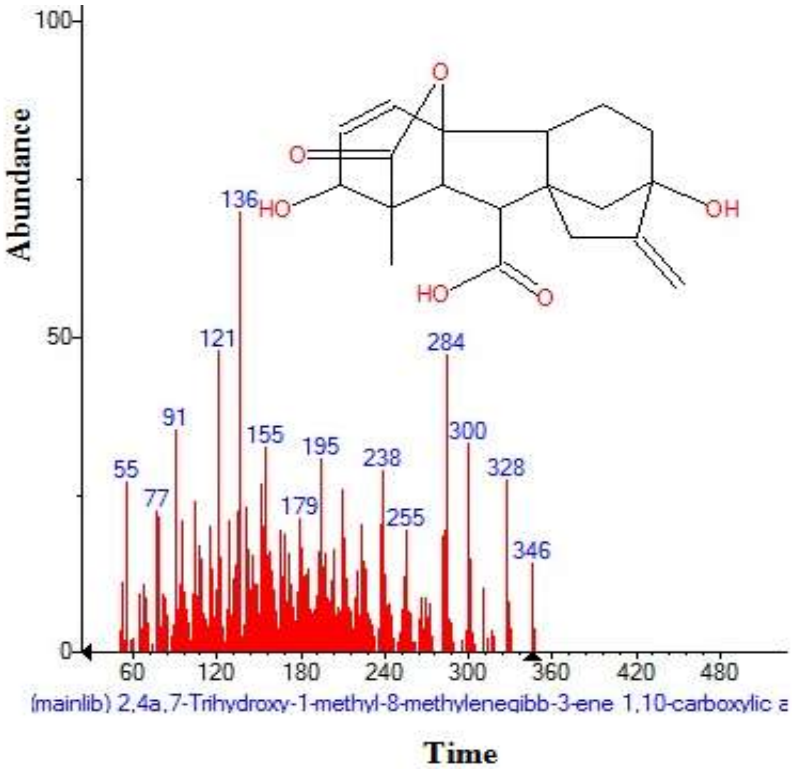


Figure 16. Structure of 2,4a,7-Trihydroxy-1-methyl-8-methyleneqibb-3-ene. 1,10-carboxylic acid. present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

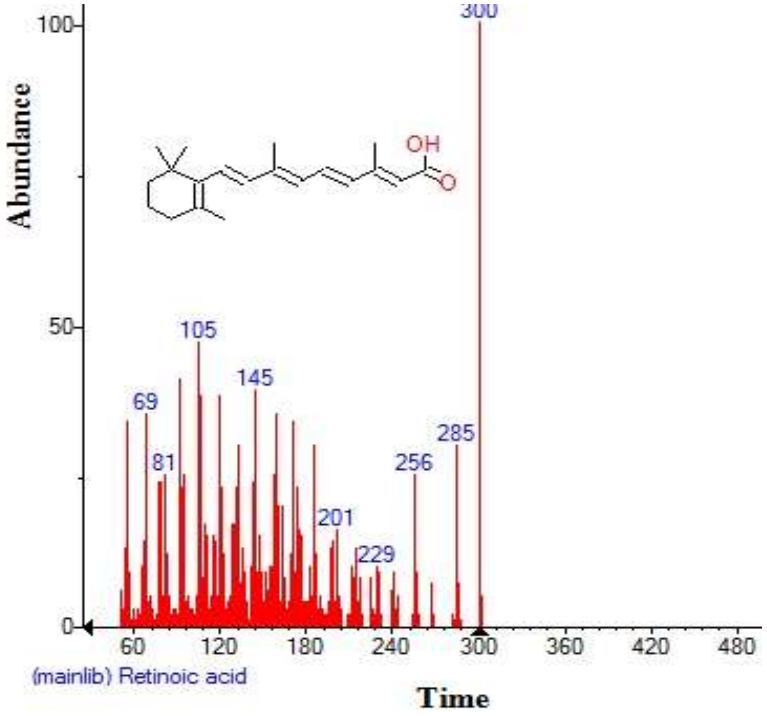
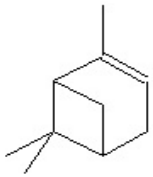
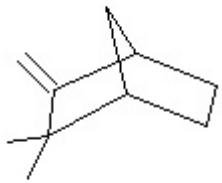
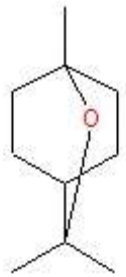


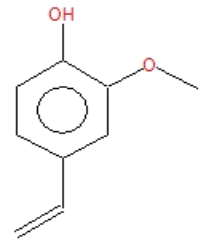
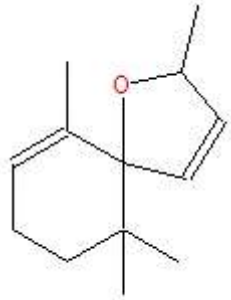
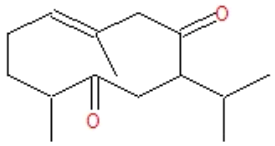
Figure 17. Structure of Retinoic acid present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

Table 1. Major phytochemical compounds identified in methanolic extract of *Rosmarinus oficinalis* leaves.

Serial No.	Phytochemical compound	RT(min)	Formula	Molecular weight	Exact mass	Chemical structure	MS Fragment ions	Pharmacological actions
1.	α-pinene	3.298	$C_{10}H_{16}$	136	136.1252		53,67,74,77,93,105,121,136	Antimicrobial against bacterial and fungal cells activities
2.	Camphene	3.476	$C_{10}H_{16}$	136	136.1252		53,67,79,93,107,121,136	Antimicrobial against Escherichia coli, Staphylococcus aureus and Candida albicans, but was not active against Clostridium perfringens up to the concentration of 100 g/ml. The significant antimicrobial and antioxidant activities of R. minima oil suggests that it could serve as a source for compounds with therapeutic potential.
3	Eucalyptol	4.317	$C_{10}H_{18}O$	154	154.13576		55,71,81,93,108,125,154	Eucalyptol, 1,8 cineole, is an essential oil present in large amounts in a variety of plants which is frequently used in the manufacture of cosmetics, to increase percutaneous penetration of drugs, as a nasal decongestant and anticough agent, in aromatherapy, and in dentistry (1-4). Eucalyptol has been used to treat bronchitis, sinusitis and chronic rhinitis and also for the treatment of asthma

methanolic extract of *Rosmarinus oficinalis* leaves proved the presence of Alkenes, Aliphatic fluoro compounds, Alcohols, Ethers, Carboxlic acids, Esters, Nitro compounds, Alkanes, Aldehydes, Ketones compounds which shows major peaks at 918.12, 1028.06, 1101.35, 1141.15, 1313.52, 1361.74, 1732.08 (Table 2; Figure 18). Among the identified phytocompounds have the property of antioxidant and antimicrobial activities (He, 2010 and Deus-de-Oliveira, 2011). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with

Table 1. Cont'd.

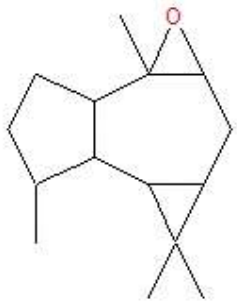
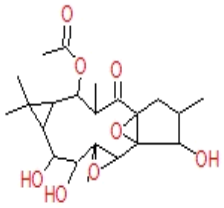
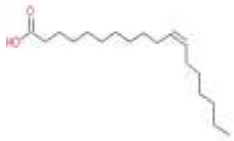
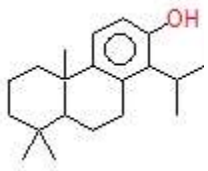
4.	2-Methoxy-4-vinylohenol	7.928	$C_9H_{10}O_2$	150	150.06808		51,77,89,107,121,135	Anti-Oxidant and Anti-inflammatory
5.	1-Oxaspiro[4,5]deca-3,6-diene,2,6,10,10-tetramethyl	9.862	$C_{13}H_{20}O$	192	192.151415		53,65,77,93,121,136,149,174,192	New chemical compound
6.	3-(N,N-Dimethyllauryla mmonio)propan esulfate	10.348	$C_{17}H_{37}N_5O_3$	335	335.249414		69,84,97,108,128,140,152,165,179,194,213	New chemical compound
7	Neocurdione	11.063	$C_{15}H_{24}O_2$	236	236.17763		55,69,82,109,137,167,180,193,236	Anti-viral, Anti-bacteria and Anti-Tumor activity

lesser side effects. Continued further exploration of plant derived antimicrobials is needed today.

Boutekedjiret et al., (2003) studied the constituents of rosemary essential oil from Algeria

They reported 1, 8-cineole, camphor, β-pinene,and α-Pinene as the major constituents in

Table 1. Cont'd.

8.	Isoaromadendrene epoxide	12.179	$C_{15}H_{24}O$	220	220.182715		55,67,93,107,135,149,162,187,220	Antibacterial activity and antioxidant activity
9.	1b,4a-Epoxy-2H-cyclopenta[3,4] cyclopropa[8,9] cycloundec.	12.356	$C_{22}H_{32}O_8$	424	424.209419		69,97,109,123,137,152,165,181,237,261,295,317,346	New chemical compound
10.	Cis-Vaccenic acid	16.940	$C_{18}H_{34}O_2$	282	282.25588		55,69,83,97,111,125,165,193,222,264,282	anti-inflammatory
11.	2-Phenanthrenol, 4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trimethyl-1	18.696	$C_{20}H_{30}O$	286	286.226999		55,69,91,115,145,159,175,189,201,229,243,271,286	Antimicrobial activity

the oil. Viuda-Martos et al., (2007) investigated chemical composition of the essential oil of anther sample of rosemary leaves from Spain. The major constituents identified were α-pinene, camphor, 1,8-cineole and camphene. Chemical composition of essential oils of rosemary from various geographic origins in Iran were determined by GC-MS. The main components detected in the oils were: α-pinene, 1, 8-cineole, camphene, camphor,

Table 1. Cont'd.

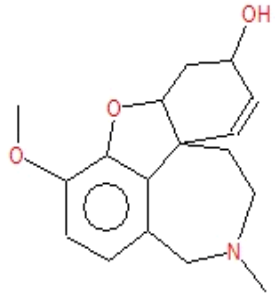
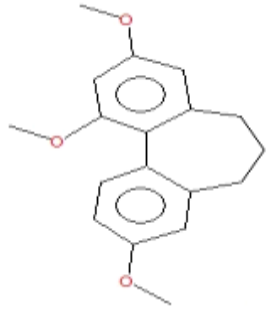
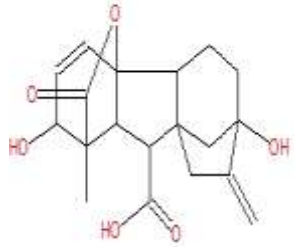
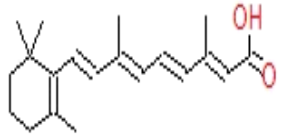
12.	Galanthamine	20.047	$C_{17}H_{21}NO_3$	287	287.152143		55,77,91,103,115,128,150,174,201,216,230,244,270,286	Galantamine hydrobromide is a tertiary alkaloid drug that has been developed and approved in a number of countries including the USA and several countries in Europe as a treatment for mild-to-moderate Alzheimer's disease (AD).
13.	Dibenz[a,c]cyclohexane,2,4,7-trimethoxy	20.682	$C_{18}H_{20}O_3$	284	284.141245		57,71,97,127,165,211,241,269,284	New chemical compound
14.	2,4a,7-Trihydroxy-1-methyl-8-methyleneqibb-3-ene. 1,10-carboxylic acid.	20.911	$C_{19}H_{22}O_6$	346	346.141623		55,77,91,121,136,155,179,195,238,255,284,300,328,346	New chemical compound
15.	Retinoic acid	22.387	$C_{20}H_{22}O_2$	300	300.208931		69,81,105,145,201,229,256,285,300	Antibacterial activity and antioxidant activity

Table 1. Cont'd.

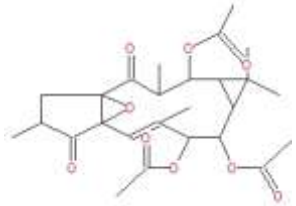
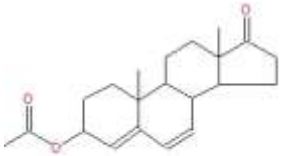
16.	7,8,12-Tri-O-acetyl-3-desoxy-ingol-3-one	26.512	$C_{26}H_{34}O_9$	490	490.220284		60,91,137,163,181,208,243,311,328,371,431,448,490	Antibacterial activity and antioxidant activity
17.	4,6-Androstadien-3β-ol-17-one,acetate	27.680	$C_{21}H_{28}O_3$	328	328.203844		55,79,119,149,197,232,268,286,328	antioxidant activity and antibacterial activity

Table 2. FT-IR peak values of *Rosmarinus oficinalis* methanolic leaves extract.

No.	Peak (Wave number cm-ˡ)	Intensity	Bond	Functional group assignment	Group frequency
1.	918.12	79.224	C-H	Alkenes	675-995
2.	1028.06	65.553	C-F stretch	Aliphatic fluoro compounds	1000-10150
3.	1101.35	71.963	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
4.	1141.15	75.676	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
5.	1240.23	76.745	C-O	Alcohols, Ethers, Carboxlic acids, Esters	1050-1300
6.	1313.52	80.282	NO2	Nitro Compounds	1300-1370
7.	1361.74	79.555	C-H	Alkanes	1340-1470
8.	1606.70	82.791	-	Unknown	-
9.	1732.08	81.440	C=O	Aldehydes, Ketones, Carboxylic acids, Esters , Alkenes	1690-1760
10.	2848.86	83.071	-	Unknown	-
11.	2918.30	78.923	C-H	Alkanes	2850-2970

myrcene and broneol (Jamshidi et al., 2009). GC and GC-MS analysis of oils from rosemary leave samples from India revealed the presence of

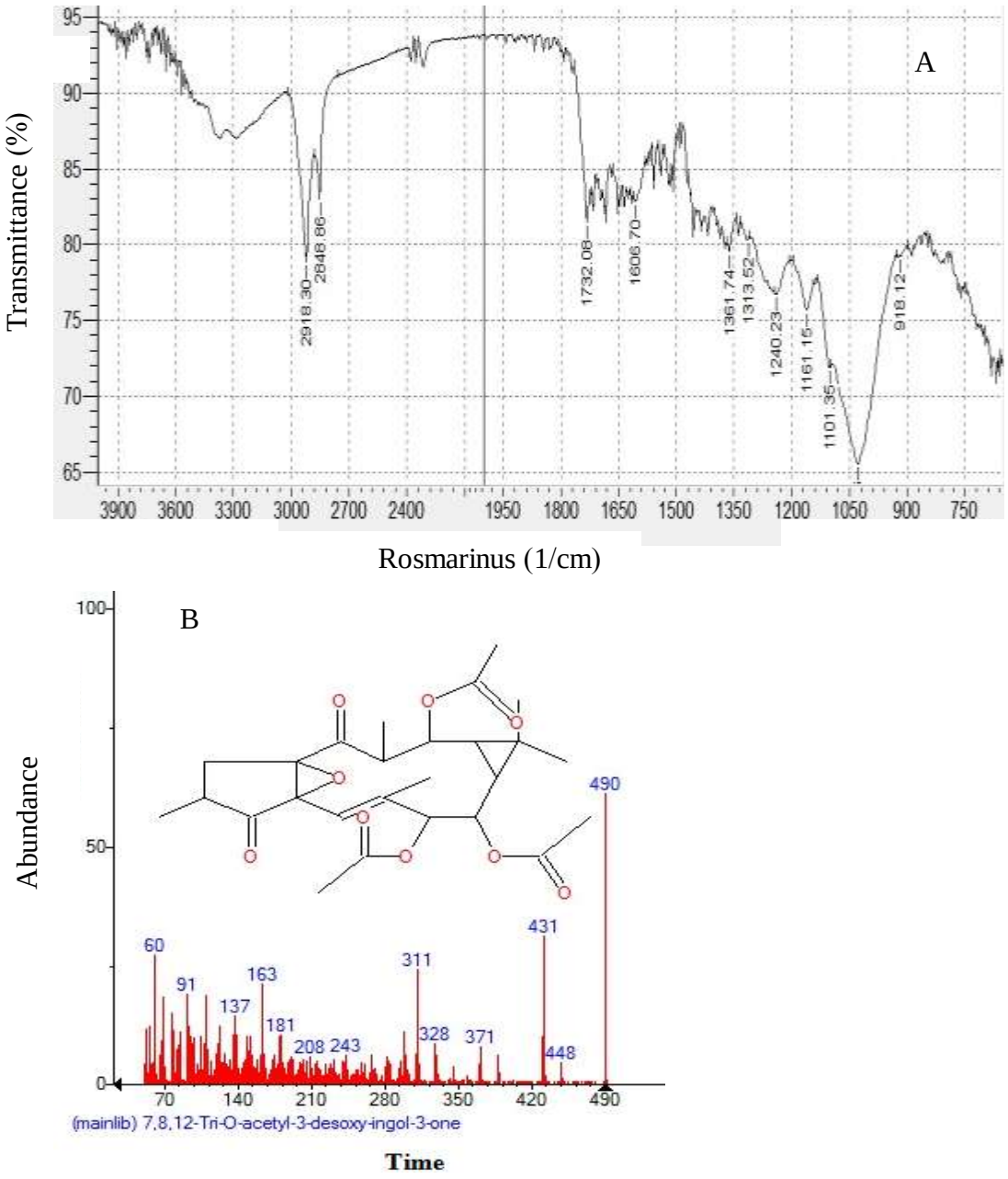


Figure 18. (A) FT-IR profile of leaf extract of *Rosmarinus oficinalis*. (**B)** Structure of 7,8,12-Tri-O-acetyl-3-desoxy-ingol-3-one present in the leaf extract of *Rosmarinus oficinalis* using GC-MS analysis.

camphor, 1,8-cineole and α-pinene as major constituents in the oils (Ram et al., 2011)

Conclusion

Rosmarinus oficinalis is native plant of Iraq. It contains chemical constitutions which may be useful for various herbal formulation as anti-inflammatory, analgesic, antipyretic, cardiac tonic and antiasthamatic.

Conflict of Interest

The authors have not declared any conflict of interest**.**

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REFERENCES

Al-Sereiti MR, Abu-Amer KM, Sen P (1999). Pharmacology of rosemary (Rosmarinus officinalis) and its therapeutic potentials. Indian. J. Exp. Biol. 37(2):124-30.

Ameera OH, Imad HH, Huda J, Muhanned AK (2015). Determination of Alkaloid Compounds of Ricinus communis by gas chromatography-mass spectroscopy (GC-MS). J. Med. Plants Res. 9(10):349-359.

Atik BF, Bousmaha L, Taleb BS, Boti JB, Casanova J (2007). Composition chimique de l'huile essentielle de Rosmarinus officinalis L poussant à l'état spontané et cultivé de la région de Tlemcen. Biologie Santé. 7:6-11.

Boutekedjiret C, Bentahar F, Belabbes R, Bessiere J (2003). Extraction of rosemary essential oil by steam distillation and hydrodistillation. Flavour Fragr. J. 18:481–484.

Bunaciu AA, Aboul-Enein HY, Fleschin S (2010). Application of Fourier transform infrared spectrophotometry in pharmaceutical drugs analysis. Appl. Spectros. Rev. 45(3):206-219.

Bunaciu AA, Aboul-Enein HY, Fleschin S (2011). Recent applications of Fourier transform infrared spectrophotometry in herbal medicine analysis. Appl. Spectros. Rev. 46(3):251-260.

Cheng CG, Liu J, Wang H, Xiong W (2010). Infrared spectroscopic studies of Chinese medicines. Appl. Spectros. Rev. 45:165-178.

Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L, 2004. Innovation – Metabolite profiling: from diagnostics to systems biology. Nat. Rev. Mol. Cell Biol. 5:763-769.

Fiehn O (2002). Metabolomics – the link between genotypes and phenotypes. Plant Mol. Biol. 48:155-171.

Huda J, Ameera OH, Imad HH, Muhanned AK (2015) Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* by using (GC-MS). J. Pharmacogn. Phytother. 7(4):56-72.

Imad H, Muhanned A, Aamera J, Cheah Y (2014). Analysis of eleven Y-chromosomal STR markers in middle and south of Iraq. African J. Biotechnol. 13(38):3860-3871.

Imad HH, Huda J, Muhanned AK, Ameera OH (2015a) Alkaloid constitution of Nerium oleander by using gas chromatography- mass specroscopy (GC-MS). J. Med. Plants Res. 9(9):326-334.

Imad HH, Mohammed AJ, Muhanned AK (2015b). Forensic analysis of mitochondrial DNA hypervariable region HVII (encompassing nucleotide positions 37 to 340) and HVIII (encompassing nucleotide positions 438-574) and evaluate the importance of these variable positions for forensic genetic purposes. Afr. J. Biotechnol. 14(5):365-375.

Imad HH, Muhanned AK, Rafid HH (2015c). X-chromosome short tandem repeat, advantages and typing technology review. Afr. J. Biotechnol. 14(7):535-541.

Jamshidi R, Afzali Z, Afzali D (2009). Chemical Composition of Hydrodistillation Essential Oil of Rosemary in Different Origins in Iran and Comparison with Other Countries. American-Eurasian J. Agric. Environ. Sci. 5(1):78-81.

Kell DB, Brown M, Davey HM, Dunn WB, Spasic I, Oliver SG (2005). Metabolic footprinting and systems biology: The medium is the message. Nat. Rev. Microbiol. 3:557-565.

Mohammed AJ, Imad HH, Muhanned AK (2015). Detection of New Variant "Off-ladder" at the (D12S391, D19S433 and D1S1656 loci) and Tri-allelic Pattern at the D16S539 Locus in a 21 Locus Autosomal Short Tandem Repeat Database of 400 Iraqi Individuals. Afr. J. Biotechnol. 14(5):375-399.

Muhanned AK, Ameer IA, Imad HH, Mohammed AJ (2015). A New Polymorphic Positions Discovered in Mitochondrial DNA Hypervariable Region HVIII From Central and North-Central of Iraq. Mitochondrial DNA. pp. 1–5.

Ram SV, Ur Rahman L, Sunita M, Rajesh K V Amit C, Anand S (2011). Changes in essential oil content and composition of leaf and leaf powder of *Rosmarinus officinalis*. CIM-Hariyali during storage. Maejo Int. J. Sci. Technol. 5(02):181-19.

Robertson DG (2005). Metabonomics in toxicology: A review. Toxicol. Sci. 85:809-822.

Sergi M, Leonor A (2002). Subcellular Compartmentation of the Diterpene Carnosic Acid and Its Derivatives in the Leaves of Rosemary. Plant Physiol. 125:1094-1102.

Sumner LW, Mendes P. Dixon RA (2003). Plant metabolomics: largescale phytochemistry in the functional genomics era. Phytochemistry 62(6):817-836.

Surewicz WK, Mantsch HH, Chapman D (1993). Determination of protein secondary structure by fourier transform infrared spectroscopy: A Critical Assessment. Biochemistry 32(2):389-393.

Troncoso N, Sierra H, Carvajal L, Delpiano P, Gunther G (2005). Fast high performance liqiuid chromatography and ultravioletvisible quantification of principle phenolic antioxidants in fresh rosemary. J. Chromat. 1100:20-50.

Viuda-Martos M, Yolanda R, Juana F, José P (2007). Chemical Composition of the Essential Oils Obtained From Some Spices Widely Used in Mediterranean Region. Acta Chem. Slov. 54:921-926.