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Review on Anti-Oxidant Activity, Antimicrobial Activity and Chemical Composition of Rosemary Essential Oil by Gas Chromatography- Mass Spectroscopy

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ABSTRACT

This paper centers around portrayal of the bioactive compounds of rosemary volatile oil extracted by hydrodistilation by using gas chromatography–mass spectrometry (GC–MS). The investigation of GC–MS information coverd some active constituent. the foremost important constituents of the rosemary are 1,8-cineole (23.47%), α-pinene (21.74%), berbonone (7.57%), camphor (7.21%) and eucalyptol (4.49%). Antioxidant activity decided employing a quantitative DPPH (1,1-diphenyl2-picryl hydrazyl) assay. rosemary EOs exhibited effective radical scavenging capacity with 50% inhibitory concentration (IC50) of 189 ± 2.38 µg/mL respectively and thus acts as a natural antioxidant agent. Minimum inhibitory concentration (MICs), minimal bactericidal concentation (MBC) three Gram-positive microbes (Staphylococcus epidermidis, Staphylococcus aureus and Bacillus subtilis), three Gram-negative microscopic organisms (Proteus vulgaris, Pseudomonas aeruginosa and Escherichia coli) and two fungi (Candida albicans and Aspergillus niger) were decided for the oil, 1,8-Cineole and -Pinene. The oil indicated articulated antibacterial also, antifungal movement than 1,8-Cineole and -Pinene against everything of the tried organisms.

KEYWORDS: Rosemary Officinalis L.; 1,8-Cineole, α-Pinene, Camphor; Anti-microbial Activity; Antioxidant Activity; GC-MS

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1. INTRODUCTION

The volatile oil bio active constituent of rosemary officinalis using gas chromatography and spectroscopy and antimicrobial activity. Rosemary officinalis is woody, perennial evergreen herb belongs to the Lamiaceae family. ros name springs from thr latin word “ros” meaning dew and “marinus” meaning sea which together means dew of the ocean. R. offficinalis contain pin like leaves, which are the most crux of all the medicinal activity. Leaves are about 1.0-2.5 cm long and about 4 cm in breadth.[1] Rosemary is extremely high in iron, calcium, and vitamin B6. it's a really old reputation for improving memory, and has been used as a logo for remembrance in Europe Carnosic acid, found in rosemary, shields the brain from the free radicals.[2] Leaves are green from the side but appear to be grey from the lower surface thanks to numerous trichomes. volatile oil of r. officinalis, is an almost colorless to straw liquid and pleasant odor. Major constituents of volatile oil are α-pinene, myrcene, 1,8-cineole, camphor, camphene, α-terpineol, and borneol, terpenes and terpenoids.[3] due to remedy properties, rosemary ues to treat parkinsin’s, alzhimer, antidiabetogenic, antifungal, antimicrobial, anti – inflammatory, antiplatelet, and antioxidant effects.[1,2,5]

According to Napoli, Curcuruto, and Ruberto (2010), rosemary volatile oil are often classified into three chemotypes from a chemical point of view: cineoliferum (high content in 1,8-cineol); camphoriferum (camphor > 20%); and verbenoniferum (verbenone > 15%).[6,7]

The utilization of essentioal oils as a antimicrobial agent assume important role within the combat to the event of microbial resistance. The antimicrobial and antivirus activities of plant oils have formed the idea of application, as food preservation, pharmaceuticals, medicine and natural therapies.[8] The main compounds liable for the antimicrobial activity are α-pinene, bornyl acetate, camphor and 1,8-cineole.[9,10] Antimicrobial properties od herds and spices have recognized and since past for food preservatives and medicine . the utilization of antimicrobial compounds is vital mot only within the preservatives of food but also within the control of human and plant diseases of microbial origin. Rosemary oils possess useful antimicrobial and antioxidant properties which will be utilized within the food industry and as a dietary supplement.[11,12]

In sight of the very fact that there are some components in EOR, especially trace ones, the properties are very similar. Therefore, the phenomenon of overlapping chromatographic peaks is extremely common although chromatographic conditions were optimized. The low-content components often effuse alongside the high-content components, making it difficult to spot and quantify accurately. Gas

chromatography-tandem mass spectrometry (GC–MS/MS) performs huge advantages in quantification because it can give the actual peak area for every component through the study on precursor ion and merchandise ion and therefore the optimization on collision energy.[13]

Essential oils are produced using several techniques. Distillation uses water and steam to get rid of the oils from dried or fresh plants, and therefore the expression method uses machines to squeeze the oil out of the plants. Other techniques may use alcohol or solvents to get rid of essential oils from plant materials

2. PLANT MATERIAL AND ESSENTIAL OIL EXTRACTION

Rosemary L. plants were freshly collected in 2011 during the amount of full flowering on the mountain within the south of France (Mediterranean climate country and mountainous region). The specimens of collected plants were identified consistent with the forester flora of France. The seeds were dried at temperature. Air-dried leaves of thyme and rosemary were submitted to hydrodistillation (HD) for 3 h with 500 ml water employing a Clevenger-type apparatus consistent with the EU Pharmacopoeia (1975). The extracted oil was collected and dried over anhydrous sodium sulphate, then stored in sealed glass vials during a refrigerator at 4˚C before analysis. The quantities of the essential oils were determined gravimetrically. [8]

2.1 *Antioxidant activity*

2.1.1 DPPH(2,2-diphyenyl-2-picrylhydrazyl) free radical scavenging assay

The radical scavenging activity of Rosemarinus officinalis L. Eos were measured by 2,2-diphyenyl-2-picrylhydrazyl(DPPH) using the tactic described by Hanato et al. (1988). [15] One milliliter at volatile oil of known concentration was added to 0.25ml od a DPPH methanolic solution.[16] The mixture was shaken vigorously and left standing at temperature for 30min within the dark. The absorbance of resulting solution was the measured at 517nm and corresponded to the power of the volatile oil to scale back the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. [17] The antiradical activity was expressed as IC50(mg/ml), the extract dose required to cause a 50% inhibition. the power to scavenge the DPPH radical was calucalted using the subsequent equation.[18]

$$\text{Scavenging rate (\%)} = [\ (A_0 - A_1 / A_0)\ ] \times 100$$

Where

A_0 is the initial area of the antioxidant components in essential oil before reaction with free radical,
A_1 is the final area of the antioxidant components in essential oil after reaction with free radical.[19]

2.1.2 E-nose-ABTS radical cation scavenging assay

ABTS radical cation working solution are wont to conduct a scavenging assay. At temperature misunderstanding in aliquot of 100 µL volatile oil solution 910mg Ml-1) in 1900 µLABTS radical cation working solution for 10min. ABTS radical cation working solution was replaced by ethanol within the blank. The ABTS radical cation scavenging capacity of the volatile oil was described by the scavenging rate and was also calculated consistent with Eqn 1.[19,20]

2.1.3 E-nose-OH radical scavenging assay

An aliquot of 250 µL FeSO4 solution (10 mg Ml-1) and 500µL volatile oil solution (0.1 mg ML-1) were added into a 10mL volumetric tube and mixed well. Subsequently, 250µL 15% H2O2 solution was added to the volumetric tube to start out the reaction within the water bathe at 370c for 30min. FeSO4 and H2O2 solutions replaced by degassed ultrapure water within the blank. The OH radical scavenging capacity of the volatile oil was described by the scavenging rate and was also calculated consistent with eqn 1.[19,20]

The entire antioxidant activity was also determined using the tactic described by Benzie and strain with some modification. Each extract was diluted with water or ethanol in 1000 ppm solution.[21, 22] Then, the FRAP reagent was prepared with 20ML 300 mmol/ L acetate buffer Ph = 3.6, 2Ml 20mmol/L FeCl3.6h2o and 2ML 10mmol/L TPTZ (2,4,6-tripyridyl-s-triazine) in 40mmol/L HCL. Then, 1mL of the FRAP reagent was added to 100µL of sample and a typical solution of 500µM Trolox. After incubation for 4min, the absorbance was measured was 593nm. The antioxidant power was expressed as µM Trolox Equivalents (TE) per g extract.[23,24]

2.2 *Antimicrobial activity*

Antimicrobial screening was performed consistent with the eneral qualitative assay described by Bacillus subtilis (ATCC 6633), Staphylococcus aureus (ATCC 6538), Escherichia coli (ATCC 10536), Pseudomonas ueruginosa (ATCC 15422), Mycobacterium intracellularae (ATCC 23068), Candida albicans (NIH B 31 l), baker's yeast (ATCC 9763), Aspergillus flavus (ATCC 9170), A. fumigatus (ATCC 26934), Cryptococcus neoformans (ATCC 32264) and Trichophyton mentagrophytes (ATCC 9972).[25]

The oil was tested as a dimethyl sulphoxide solution (20 mg/ml). The antimicrobial activity was represented by the width in millimetres of the zone of inhibition measured from the sting of the agar

well to the sting of the zone after 24 h and 48 h incubation for bacteria and Candida, 48 h and 72 h for fungi and 72 h and 96 h for Mycoba\cterium. Streptomycin sulphate, rifamycin and amphotericin B, were utilized in a degree of 1 mg/ml in each assay as antibacterial and antifungal controls. Results are listed in Table no. 1.[26]

Table 1. Antifungal activity of essential oil of Rosmarinus officinalis L.

	Candida albicans 24 h148 h	Cryptococcus neoformans 48 h172 h	Mycobacterium intracellularae 72 h/% h
Essential oil	++/+ +	++/+ +	++/+ +
Amphotericin B	+++/+++	+++/+++	+++/+++
Rifamycin	-	-	-

2.2.1 Minimum inhibitory concentration(MIC) and minimum bactericidal concentration determination(MBC)

The antimicrobial activities of the volatile oil even as 1,8-Cineole and - Pinene were assessed against the test microorganisms as indicated by the National Committee of Clinical Laboratory Standards (CLSI, 2007). The samples were independently broke down in cleaned physiological saline arrangement (0.9% w/v) enhanced with Tween 80 at a final grouping of 0.5% (v/v). seial doubling dilution of the oils were found out during a 96-well microtiter plate within the range 4.0–0.2%.[(27)] Overnight broth culture of every strain were readied and therefore the last fixation in each all around was acclimated to 105–106 CFU/mL for microscopic organisms(bacteria) and contagious(fungi) strains. 96-Well microtiter plates were brooded (incubated) at 37 ◦C for twenty-four h apart from A. niger which was incubated at 25 ◦C for five days.[28] The MIC is characterized because the lowes concentarion of samples at which the microorganism doesn't exhibit visible growth.[29] The MBC which is characterized because the lowest concentration of tests samples at which incubated bacterial and fungal strains were completely killed was affirmed by reinoculating on agar plates with 10µL of every medium from the microplates.[30] All conclusions were acted in duplicate. Penicillin (Sigma) served as a positive control.[31]

2.2.2 Fractional inhibitory concentration(FIC) testing

The fractional inhibitory concentration (FIC) was derived from rock bottom concentration of antibiotic and extract combination permitting no visible growth of the test organisms on the plates. The FIC value for each agent was calculated using the formula.[32]

FIC(antibiotic) = MIC of antibiotic in combination / MIC of antibiotic alone

FIC (extract) = MIC of extract in combination / MIC of extract alone [33]

Combinations were classified as synergistic, if the FIC indices were <1, additive if the FIC indices were = 1, indifferent if the FIC indices were between 1 and 2 and antagonistic if the FIC indices were >2[34].

4. RESULTS AND DISCUSSION

4.1 *Antioxidant activity*

The antioxidant activity of rosemary L. EOs was assessed by DPPH assay, evaluating the H-donating or radical scavenging ability of the oils using the stable radical 2,2-diphenyl-1- picrylhydrazyl (DPPH) as a reagent. The concentrations that led to 50% inhibition (IC50) for thyme and rosemary oil is 189 ± 2.38 µg/mL.[35] Ruberto and Barratta (2000)[36] who tested the antioxidant activity of about 100 pure components of essential oils, acknowledged that the phenolic compounds like thymol, carvacrol and camphor showed the very best activity. Thus, many aromatic plants are today considered because the most vital sources for the extraction of compounds with strong antioxidant activity. Rosemary (R. officinalis L.) is merely spices widely utilized in folk medicine, cosmetics, phytopharmacy, and therefore the flavoring of food products.[37] Furthermore, rosemary is that the only spice commercially available to be used as an antioxidant in Europe and therefore the us. [17,38]

4.1.1 Screening of ABTS (2,2-azino-bis[3-etillbenzolin]-6-sufonoc acid) radical cation scavenging activity

The chromatograms of EOR before and after the reaction with ABTS radical cation are shown in Fig. 1 and therefore the scavenging activity of every component within the volatile oil is shown in Table 3. It are often seen that verbenone, camphor, and bornyl acetate showed strong activity in scavenging DPPH radical but poor activity in scavenging ABTS radical cation because different sorts of free radicals and therefore the way antioxidants interact with free radicals both affect the scavenging effect of antioxidants. The cyclic ether group is especially important for ABTS radical cation scavenging.[20]

Table 2. chemical composition of essential oil from rosemary analyzed by gas chromatography-mass spectroscopy

Author	Material/ Product	Method	Chromatographic parameters			Compound	Retention Time/ Retention index	References
			Oven temperature	Detector	Column			
Mehdi jala-heravi	R.officinalis from north of Tehran	Agilent technologies 6890 Gc system	40ºc for1min, 3ºc/min, 250ºc for 20min	Mass selective detector	Capillary fused silica column	Eucalyptol Berbonone	28.01 37.57	Jalali-Heravi M, Moazeni RS, Sereshti H. Analysis of Iranian rosemary essential oil: Application of gas chromatography–mass spectrometry combined with chemometrics. Journal of chromatography A. 2011 May 6;1218(18):2569-76.
Marcelo rossato	R.officinalis from Rio grande do sil state	GC Hewlett Packard 6890 series	180ºc-3ºc/min, 180º-230ºc at 20min	Mass selective detector	Capillary column	Bornyl acetate, geraniol	1.3 2.6	Atti-Santos AC, Rossato M, Pauletti GF, et al. Physico-chemical evaluation of Rosmarinus officinalis L. essential oils. Brazilian Archives of Biology and Technology. 2005 Nov;48(6):1035-9.
Iram Ayoob	R.officinalis from kasmir	GC Perkin Elmer autosystem	60º-220ºc at 5ºc/min	FID	Capillary fused silica column	Verbenene, β-pinene	1.39 5.97	Ayoob I, Rahman-ur M, Rehman-u S. Essential oil composition of Rosmarinus officinalis L. from Kashmir (India). EC Microbiol. 2018;14(2):29-32.
Mehmet Musa Ozcan	Flower and leaves of rosemary from Mersin	HP 5890 GC	50ºc for 5min, 220º at rate 3ºc/min	FID	Fused silica WCOT column	Linalool, thymol	14.52 20.62	Özcan MM, Chalchat JC. Chemical composition and antifungal activity of rosemary (Rosmarinus officinalis L.) oil from Turkey. International journal of food sciences and nutrition. 2008 Jan 1;59(7-8):691-8.
MariaJ. Jordan	R.officinalis	A6890 N GC	60ºc, 2.5ºc/min to 155ºc, 250ºc at rate 10ºc/min	Mass selective detector	HP-5 column	α-pinene, 3-octanol	965(RI), 1004(RI)	Jordán MJ, Lax V, Rota MC, Lorán S, Sotomayor JA. Effect of bioclimatic area on the essential oil composition and antibacterial activity of Rosmarinus officinalis L. Food Control. 2013 Apr 1;30(2):463-8.

F. M. Soliman	R.officinails From Egypt	GC model 3300	60-180ºc at 3ºc/min	Ion trap detector	Capillary fused silica column	Camphene, Terpinen-4-ol	0.51 1.15	Soliman FM, El-Kashoury EA, Fathy MM, Gonaid MH. Analysis and biological activity of the essential oil of Rosmarinus officinalis L. from Egypt. Flavour and Fragrance Journal. 1994 Jan;9(1):29-33.
Yang Jiang	Leaves of R.officinails	GC-Ms	50ºc for 2min, 160ºc at 5ºc/min, 280ºc at 5ºc/min	Mass selective detector	DB-5Ms capillary column	1,8cineole, Isopulegol	15.13, 15.41	Jiang Y, Wang W, Zhao CJ, et al. Chemical composition and antimicrobial activity of the essential oil of Rosemary. Environmental toxicology and pharmacology. 2011 Jul 1;32(1):63-8.
R. Jamshidi	Rosemary from iran	Thermoquest-finningan	60ºc to 250ºc at rate 5ºc/min	FID	DB-1 fused silica column	Tricyclene, camphor	926(RI), 1136(RI)	Jamshidi R, Afzali Z, Afzali D. Chemical composition of hydrodistillation essential oil of rosemary in different origins in Iran and comparison with other countries. American-Eurasian Journal of Agricultural & Environmental Sciences. 2009;5(1):78-81.
Hanene miladi	R.officinalis L. from south france	Agilent technologies 6890N GC	50ºc for 1min, 7ºc/min to 250ºc for 5min	FID	Capillary column	β-Myrcene Borneol	992(RI), 1170(RI)	Miladi H, Slama RB, Mili D, et al. Essential oil of Thymus vulgaris L. and Rosmarinus officinalis L.: Gas chromatography-mass spectrometry analysis, cytotoxicity and antioxidant properties and antibacterial activities against foodborne pathogens.
Maria lo presrti	Rosmaru plant from messina	Shimadzu GC	50ºc for 2min, 250ºc for 10min	FID	MDN-5S column	Sabinene β - pinene	9 9.3	Lo Presti M, Ragusa S, Trozzi A, Dugo et al. A comparison between different techniques for the isolation of rosemary essential oil. Journal of separation science. 2005 Feb;28(3):273-80.
Aziza kamal Genena	Rosemary leaves	GC Varia cp 3800 coupled with Saturn 2000	60ºc for 3min to 220ºc at rate 5ºc/min, 220ºc for 5min	Mass selective detector	Capillary column	Phyto, Caryophyllene	0.69, 4.39	Genena AK, Hense H, Smânia Junior A, Souza SM. Rosemary (Rosmarinus officinalis): a study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. Food Science and Technology. 2008 Jun;28(2):463-9.

Among the compounds given in Supporting Information Table S1, only eucalyptol features a cyclic ether group, which makes it show the strongest activity in scavenging ABTS radical cation and therefore the scavenging rate was 39.5%. Meanwhile, ABTS radical cation performs poor selectivity in reaction with hydrogen donors, unlike the highly selective reactions between DPPH radicals and hydrogen donors. it's also the rationale that o-cymene, exo-fenchol, camphene and ʊ-pinene all have an identical scavenging rate about 25% in ABTS radical cation.

4.1.2 Screening of OH radical scavenging activity.

The chromatograms of EOR before and after the reaction with OH radical are shown in Fig. 2 and therefore the scavenging activity of every component within the volatile oil is shown in Table 3. The OH radical tends to attack the groups with high electron cloud density,[37] and therefore the presence of a covalent bond can accelerate the formation into a secondary group of OH radical, 24 which showed good activity in scavenging OH. Among the compounds given in Table S1, the structures of o-cymene and p-cymenene contain benzene rings. The conjugated bond structures within the benzene formula enable them to possess higher electron cloud density and therefore the addition into a secondary group of OH radicals, which ultimately gave them the very best scavenging activity in OH radicals, with the scavenging rate up to 69.9% and 68.09% respectively. exo-Fenchol showed weaker scavenging activity than the primary two due to the shortage of a covalent bond group. However, thanks to the lone pair electrons of oxygen atoms in its hydroxyl, it can chelate some transition metal ions (such as Fe2+ and Cu2+), which are essential within the production of OH radicals, thus playing an antioxidant role[40,41] with the scavenging rate of 59.3%. Among the opposite compounds, ʊ-pinene, ʉ-pinene and ʊ-bisabolene had a way higher scavenging activity than camphor, linalool oxide acetate and camphene, which can be due to the various positions of the double bonds they need. Moreover, the CH3 group attached to the covalent bond in ʊ-pinene and ʊ-bisabolene makes them show higher scavenging activity.

Table 3. 2,2-diphyenyl-2-picrylhydrazyl (DPPH), 2,2-azino-bis[3-etillbenzolin]-6-sufonoc acid (ABTS) and (hydroxide) OH radical scavenging abilities active ingredients of essential oil from rosemary

Sl no.	Compounds	Scavenging rate (5%)		
		Scavenging DPPH.	Scavenging ABTS.	Scavenging OH.
1	Tricyclene	32.7 ± 0.18	-	-
2	A-pinene	6.9 ± 0.08	24.5 ± 0.08	57.0 ± 0.10
3	Camphene	8.6 ± 0.06	25.7 ± 0.03	16.4 ± 0.08
4	2,4(10)-hujadiene	50.1 ± 0.23	-	-
5	B-Pinene	14.7 ± 0.09	-	47.1 ± 0.07
6	3-Carene	23.9 ± 0.32	-	-
7	O-Cymene	16.2 ± 0.56	28.6 ± 0.09	69.9± 0.05
8	P-Cymene	-	-	-
9	Eucalyptol	41.9 ± 0.75	-	16.7 ± 0.05
10	Benzeneacetaldehyde	-	-	-
11	Linalool oxide acetate	7.1 ± 0.04	-	16.7 ± 0.05
12	P-Cymenene	47.9 ± 1.11	-	66.7 ± 0.12
13	Exo-Fenchol	1.4 ± 0.08	26.0 ± 0.04	59.3 ± 0.05
14	Camphor	66.1 ± 0.5	14.3 ± 0.09	21.1 ± 0.05
15	Pinocamphone	-	-	-
16	Terpinen-4-ol	13.4 ± 0.03	-	-
17	A-Terpineol	-	-	-
18	Myrtenol	-	-	-
19	Verbenone	67.9 ± 0.07	12.5 ± 0.07	-
20	Bornyl acetate	65.0 ± 0.09	9.7 ± 0.06	-
21	Thymol	-	-	-
22	Eugenol	-	38 ± 0.06	-
23	Methyleugenol	-	7.3 ± 0.09	-
24	A-Bisabolene	6.2 ± 0.04	4.7 ± 0.03	45.7 ± 0.21

Figure 1. Chromatogram of essential oil from rosemary scavenging ABTS radical cation

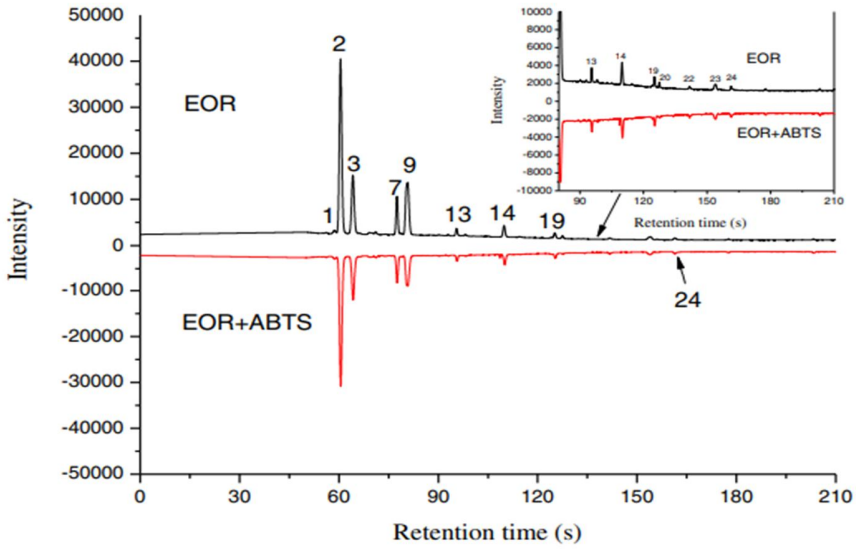
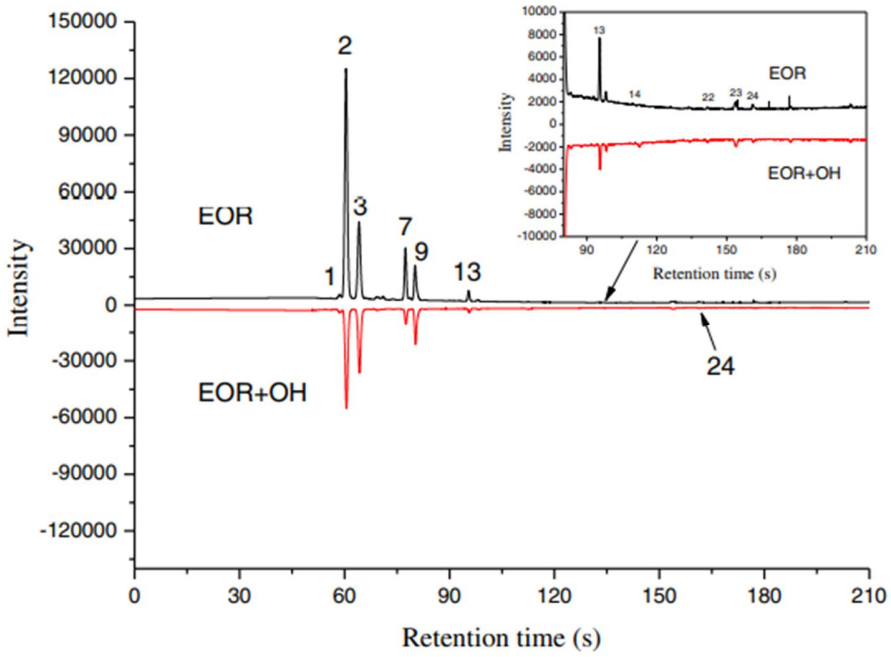


Figure 2. Chromatogram of essential oil from rosemary scavenging OH radical



4.2 *Antimicrobial activity*

The oil prepared from plants collected from the ESMP showed an honest inhibitory activity against C. ulbicans, C. neoformans and M. intracellularae. It showed no activity against the opposite microorganisms tested. The relatively high antifungal activity of the oil suggested its potential use in treatment of meningitis and pneumonia caused by C. neoformans in AIDS patients, [42] for treatment of skin infections, diaper dermatitis also as for diarrhoea caused by C. albicans.[43, 26] the use of the oil for treatment of systemic infection caused by M. intracellular in patients affected by AIDS has also been recrnmended.[26]

The vermicidal screening of both oils showed a marked activity against the earthworms (Aflolobophora caliginosa). 50 pl of every oil caused slow movements followed by death of the worms after 50 min for sample I and 30 min for sample 11. While 100 pl of every oil caused death after 25 and 15 min for both oils respectively. 200 ~l of every oil caused paralysis and death of the worms after 15 and 10 min for both oils respectively. No mortality was noticed among the controls. The oil is thus recommended for further studies to prove its anthelminthic activity also as its safety.

4.2.1 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assay

The volatile oil, -Pinene and 1,8-Cineole were evaluated for antimicrobial activity against Gram positive (S. epidermidis, S. aureus and B. subtilis), Gram negative (P. vulgaris, P. aeruginosa and E. coli) bacteria and fungi (C. albicans, A. niger). Rosemary volatile oil was found to be the foremost active against all of the bacterial strains. It also showed a marked antifungal activity against Candida albicans. The MICs for the rosemary volatile oil ranged from 0.03% (v/v) to 1.0% (v/v) for all test microorganisms, while MICs for -Pinene ranged from 0.3% (v/v) to 4.0% (v/v). We couldn't detect the MIC value of 1,8-Cineole against E. coli, P. aeruginosa and A. niger within the range from 0.2% to 4.0%. MBC values of the three samples were similar or maybe above the corresponding MIC values. These differences within the susceptibility of the test microorganisms to the test samples might be attributed to variation within the rate of samples' penetration through the cell membrane and cell wall structures (Cox et al., 2000). generally, the oil showed greater antimicrobial activity than -Pinene and 1,8-Cineole (see Table 4).

Table 4- Minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) of rosemary oil, α-pinene and 1,8-cineole against microorganism.

Sl no.	Microoragnism	Positive control Penicillin (µg/ml)		Rosemary essential oil (%v/v)		A-pinene (%v/v)		1,8-cineole (%v/v)	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	Se	3.1	12.5	0.1	0.1	0.5	1.0	0.5	1.0
2	Sa	3.1	6.3	0.03	0.1	0.3	1.0	0.3	1.0
3	Bs	6.3	25.0	0.1	0.1	0.5	0.5	0.5	>4.0
4	Es	6.3	50.0	0.3	0.5	1.0	2.0	>4.0	>4.0
5	Pv	3.1	12.5	0.1	0.5	0.3	1.0	0.5	1.0
6	Pa	100.0	>200.0	0.1	0.5	2.00	>4.0	>4.0	>4.0
7	Ca	6.3	12.5	0.1	0.5	0.5	2.0	0.5	1.0
8	An	200.0	>200.0	1.0	>4.0	4.0	>4.0	>4.0	>4.0

Se: Staphylococcus Epidermidis, **Sa:** Staphylococcus Aureus, **Bs:** Bacillus Subtilis, **Ec:** Escherichia Coli, **Pv:** Proteus Vulgaris, **Pa:** Pseudomonas Aeruginosa, **Ca:** Candida Albicans, **An:** Aspergillus Niger.

4.2.2 FIC

The interpretations of the activity of rosemary extract combined with cefuroxime produced an interesting synergistic activity against the tested 5 MRSA isolates (Table 5). The MICs of rosemary extracts for the MRSAs were decreased from the range of (0.78-0.049) to (0.49- 0.39) mg/mL when these extracts were combined with cefuroxime at a degree like 1/2 MIC.

Table:5 Fractinal inhibitory concentration (FIC) values for the combinations between Cefuroxime and Rosemary officinalis extract

Sl no.	Test isolate	FIC (Cefuroxime)	FIC (Extract)	FIC (Index)	Interaction
1	MRSA-1	0.500	0.063	0.563	Synergy
2	MRSA-2	0.500	0.063	0.563	Synergy
3	MRSA-3	0.500	0.125	0.625	Synergy
4	MRSA-4	0.500	0.246	0.749	Synergy
5	MRSA-5	0.500	0.501	1.00	Synergy

MRSA: Methicillin-Resistant Staphylococcus Aureus

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