

## ***Determination of the Chemical Compounds in Rosemary***

***(Rosmarinus officinalis L)***

***by HPLC and Study the Effect of its Volatile Oil on Some Types of Bacteria***

**تقدير المركبات الكيميائية من نبات ( اكليل الجبل ) بواسطة**

**كروماتوغرافيا السائل عالي الاداء ودراسة تأثير زيت الطيار**

**على بعض انواع البكتريا**

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### **ABSTRACT**

The results of the study were based on the extract of *Rosmarinus officinalis L* and its oil, The chemical compounds separated by the High Performance Liquid Chromatography technique were extracted for the extract and the volatile oil. Some of them were identical with the standard chemical compounds in the analysis (1-8 Cinole, Camphor, Rosmarinc acid , Bronel, Bronyl acetate, Olenolic acid). The volatile oil inhibitor was studied for inhibition of three strains of *Pseudomonasaeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, using the spread method around the

tablets. Each type of bacteria studied Bacteria from the center of the steel plant Mueller Henton and then added different concentrations of rosemary oil . The study of the inhibitory by measuring the diameter of the transparent field caused by inhibition of bacterial growth and the results showed a significant inhibitory effect against the bacterial under study. The effectiveness has increased with more Concentration gave focus (100 mg / ml) had the highest inhibitory capacity and *Staphylococcus* was the most affected by the volatile oil concentrations. *Pseudomonas* were the least affected among the bacteria used.

## INTRODUCTION

Recently, the world has turned its attention to medicinal plants in the treatment of many diseases and even incurable ones. Due to the harmful effects of many drugs on human health, volatile oils have been used as medical drugs for a long time. Their sterilized properties have been used against bacteria, viruses and fungi<sup>(1)</sup>.

*Salvia* is considered an important species of the Labiatae family, with more than a thousand species such as *S. triloba*, *S. officinalis* and *S. miltiorrhizae* spread throughout the world<sup>(2,3)</sup>. *Salvia* contains many active substances such as some volatile oils and compounds with anti-microbial activity such as Terpenes, Saponins, Tanins<sup>(4)</sup>.

*Rosemarinus* belongs to the Labiatae family, often called Mint family<sup>(1)</sup>. The rosemary plant is rich in volatile oils that have a distinctive taste and smell and have many medicinal and non-medicinal uses<sup>(5)</sup>. The oils in this plant are used to inhibit pathogenic microorganisms<sup>(6,7)</sup>. Show<sup>(4)</sup> that is essential oil of the *S. tomentosa* aerobic extract has the potential to inhibit the growth of Gram stain positive and negative bacteria except *Ps. aeruginosa*, and<sup>(8,9)</sup>

) show that the aromatic oil of rosemary has a slowing effect on the growth of Gram stainpositive bacteria such as *S.aureus*, *B.subtilis*, and the Cram stainnegative bacteria of such as *E.col* and *P.aeruginosa*. Therefore, our study aimed to know the inhibitory role of the volatile oil extracted from the rosemary plant for some types of bacteria after separation and evaluation of the compounds of the rosemary plant.

### Experimental

The rosemary plant was obtained from the local markets of Samarra and after they were confirmed to be classified in herbarium the College of Science University of Tikrit took 533,30 gm were grinded in the mill and then immersed in normal alcohol (96% ethanol) for 48 hours, During Nathal Cloth and then through the Watman No.3 eight layers filtration paper and then rotary evaporator to separate the solvent used for active ingredients and when the mixture reached 20 ml Centrifuged at 3000 cycles for 15 minutes, showing no precipitation confirming the accuracy of the extracted obtained , and to obtain the volatile oil was abandoned from solvent extracted at room temperature of a four-week. the Volatile Oil was obtained.<sup>(10)</sup>

The preliminary qualitative data on the extract were carried out, such as the Trim Hill detection of, Lieberman Burghard's detection of turbens<sup>(11)</sup> and the ferric chloride detection of tannins and phenols<sup>(12)</sup>. The quantitative determination of the extract of volatile oils by HPLC technique using the Geneve method <sup>(13)</sup> Column (Phenomenex C-18) Particle size 3 Micromel (fixed phase) The mobile phase of the conveyor consists of: methanol, deionized water, phosphoric acid (88: 12: 0.1) respectively and flow rate of 1.2 ml / min. 25 µg / ml and dilution factor 20 µg / ml measured by JH G UV at a wavelength of 285 nanometers.

The unknown concentrations were calculated according to the following

$$\text{The Concentration of volatile oils } \mu/\text{ml} = \frac{\text{area of sample}}{\text{area of standard}} \times \text{Conc. Of standard} \times \text{dilution factor}$$

The bacterial study was obtained from the Department of Pathological Analysis, Faculty of Applied Sciences, University of Samarra, *Pseudomonas*, *E.coli*, *Staphylococcus*, and the preparation of the Muller hinton agar, and for the purpose of preparing the efficacy test plates, the 5 mm metal cutter and 5 mm diameter was used to drill the Muller hinton agar, ( $1.5 \times 10^8$ ) cell / ml and using McFarland for calibration purpose. The extracts were then drilled with the following concentrations (100 mg / ml, 75 mg / ml, 50 mg / ml , 25 mg / ml ). 96% ethanol was used for dilution and incubated at 37 ° C for 48 hours. The results were measured by measuring the diameter of inhibition with the inserted control (germ-free zone) <sup>(14)</sup>.

### Results and discussion

the initial data for the detection of turbines was obtained. The Trimm Hill detection with the extract showed a green color indicative of the presence of single turbines, while Lieberman Burgad's detector did not respond and the ferric chloride detection showed a green color indicating the presence of tannin or phenolic compounds.

The results of the high-performance liquid chromatography analysis of the plant extract indicated that seven bands eight compounds, six of which were identical to the standard chromatographic compounds except  $\alpha$ - Pinene, as shown in Fig. (1) which shows the peaks and time of the RT per minute.

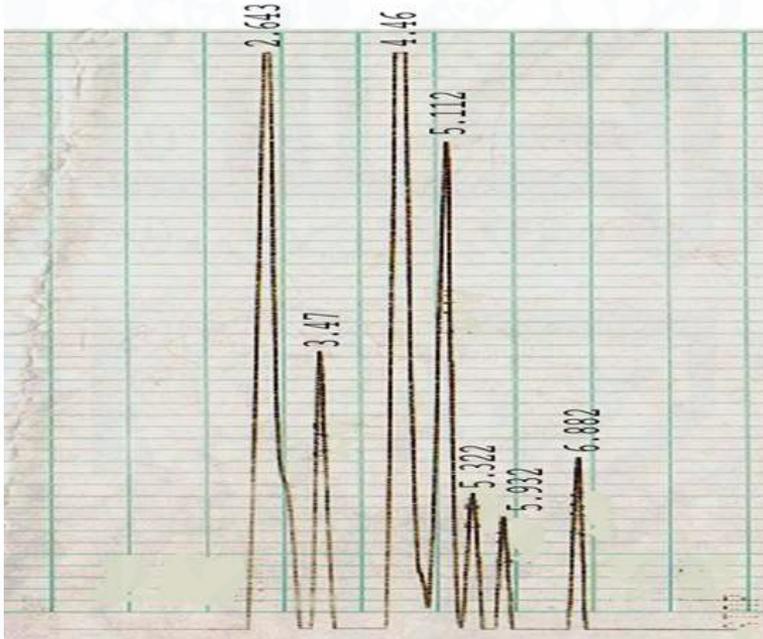


Fig (1) HPLC For separated compounds from the rosemary extract

Table (1) Retention time and peak area for compounds separated from the rosemary extract

Concentration	Separated Compounds	Peak area	Retention time
---	$\alpha$ - Pinene	-----	-----
0.10929	1-8 Cinol	230127	2.643
0.030103	Camphor	69393	3.47
0.054577	Rosmarinic acid	149920	4.46
0.039467	Bronel	73189	5.112
0.026115	Bronyl acetate	46591	5.932
0.014624	Olenolic acid	39286	6.882

The standard compounds of the rosemary extract used in chromatography are  $\alpha$ -Pinene (1-8 Cinol, Camphor, Rosmarinic acid, Bronel, Bronyl acetate, Olenolic acid), as shown in Fig. 2 which shows the peaks and time of the standard extracted rosemary compounds.

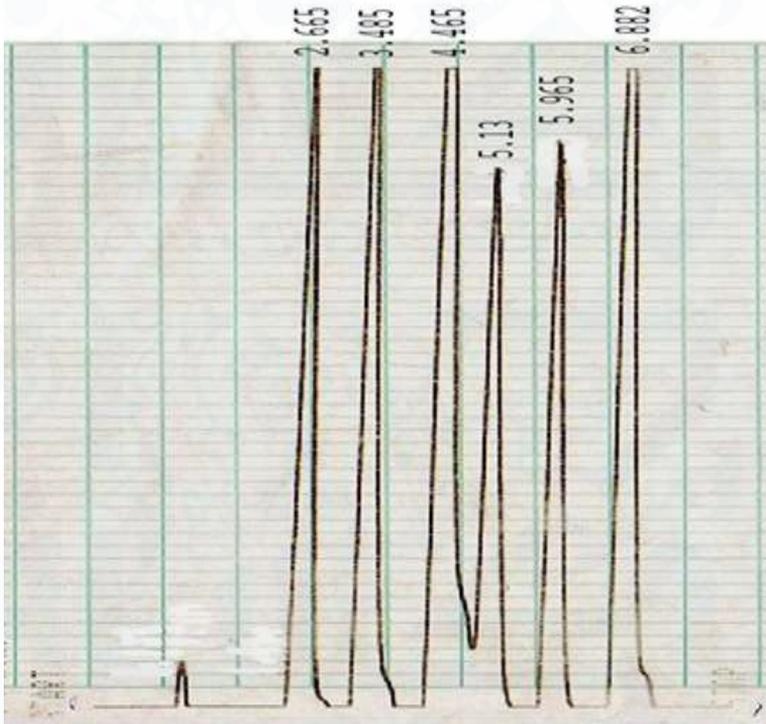


Fig (2) Analysis of HPLC for standard separated compounds

Table (2) Retention time and peak area for standard compounds curves (abstract)

Peak area	retention time	Standard Compounds
70376	1.35	$\alpha$ -Pinene
105275	2.655	1-8Cinol
113596	3.485	Camphor
135119	4.465	Rosmarinic acid
92720	5.13	Bronel
89203	5.965	Bronyl acetate
134274	6.882	Olenolic acid

As for the volatile oils extracted from the rosemary plant, eight bands were shown on the presence of eight compounds, seven of which were identical to the standard compounds approved by the chromatographic analysis, as

in Fig. 3 which shows the peaks and time of the Rt of the compounds separated from the volatile oils of the rosemary plant.

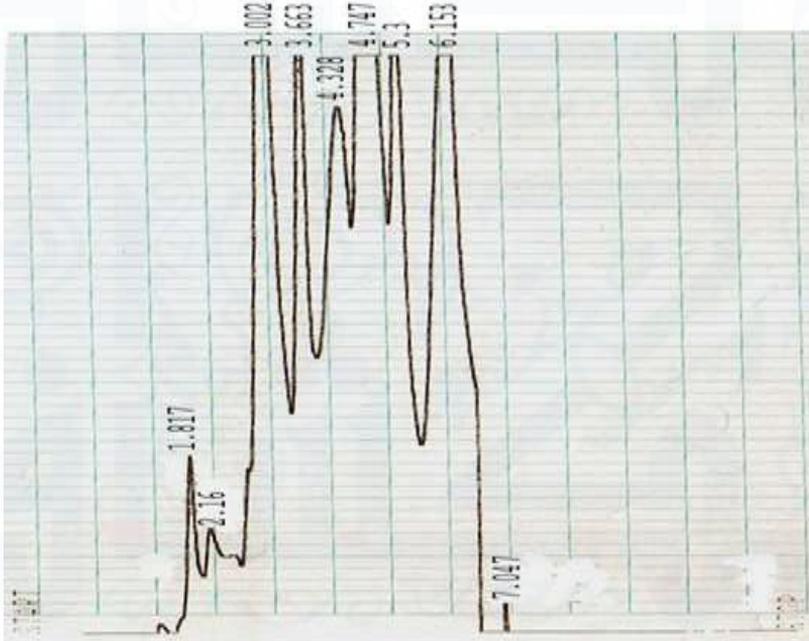


Fig (3) Analysis of HPLC of compounds separated from the volatile oil of rosemary

Table (3) Retention time and peak area for the extract of volatile oils separated compounds from the rosemary plant

Concentration	separated compounds	Peak area	Retention time
---	$\alpha$ - Pinen	---	---
0.74134	1-8 Cinol	213286	3.002
0.05545	Camphor	104118	3.663
0.06287	Terpnine -4- Ole	128411	4.328
0.06325	Rosmarinic acid	268351	4.747
0.03773	Bronel	115114	5.3
0.03294	Bronyl acetate	227361	6.153
0.00181	Olenolic acid	8316	7.047

Standard compounds for volatile oils from the rosemary plant used in chromatography ( $\alpha$  -Pinen, 1-8 Cinol, Camphor, Terpnine -4- Ole, Rosmarinic acid, Bronel, Bronyl

acetate, Olenolic acid. Which shows the peaks and time of Rt for volatile oils extracted from the standard rosemary plant.

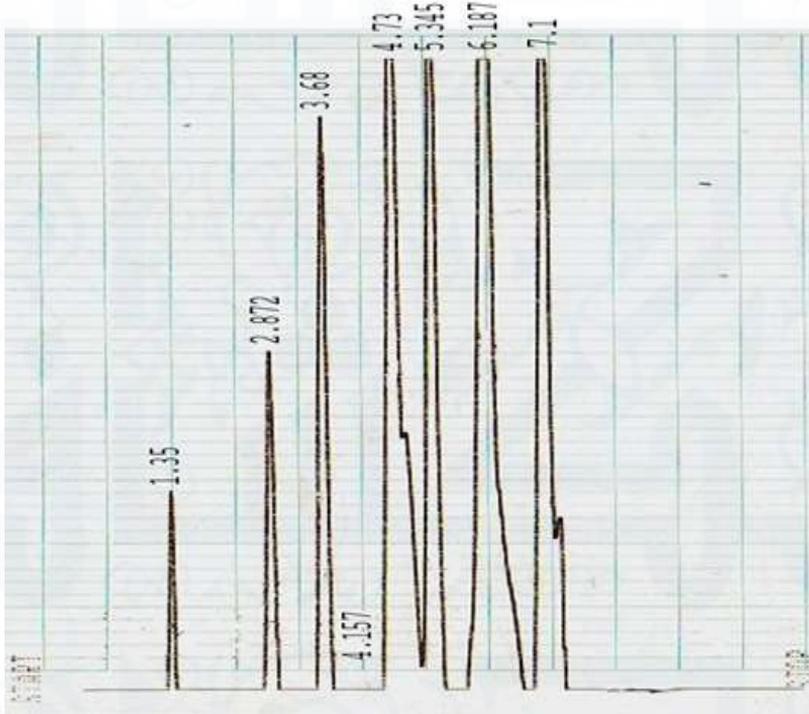


Fig (4) Analysis of HPLC for seven separated compounds

Table (4) Shows the peak area and summaries of the standard compounds for volatile oil

Peak area	Retention time	Standard Compounds
70376	1.35	α- Pinen
143850	2.872	1-8Cinol
93878	3.68	Camphor
102129	4.157	Terpnine -4- Ole
212133	4.732	Rosmarinic acid
152539	5.345	Bronel
345088	6.187	Bronyl acetate
229536	7.10	Olenolic acid

In comparison with the standard compounds six compounds were found in the extract and in the volatile oil of the rosemary plant, before and after the oil, the  $\alpha$ -Pinene compound did not appear, and the compound Terpinene -4- Ole appeared in the volatile oil of rosemary, show tables (1),(3) and figures (3),(4). The results of our study agree with <sup>(15)</sup> that some phenolic compounds and terpenes such as Oleonic acid (Rosmarinic acid) of the rosemary plant were evaluated, as well as <sup>(16)</sup>, which evaluated compounds including Rosmarinic acid from rosemary by HPLC.

In the bacterial study, three types of negative and positive bacteria were selected for Kram isolated from UTIs. Bacterial isolates were identified by laboratory methods known in the Department of Pathological Analysis- Department of Applied Sciences –University of Samarra.



*Staphylococcus*

*E. coli*

*Pseudomonas*

**Table (5) Shows the rate of inhibition of growth of the studied bacteria for the volatile oil extracted from the coriander plant Concentrate mg / ml**

Concentration mlg/m	<i>Pseudomonas aeruginosa</i>	<i>Escherichiacoli</i>	<i>Staphylococcus</i>
25	Cc 5.00± 2.00	Db 7.66 ± 1.52	Da 10.66 ± 0.57
50	Bb 14.66 ± 1.52	Cc 11.00 ± 2.00	Ca 21.00± 3.21
75	Bb 17.00 ± 3.46	Bc 14.33 ± 1.15	Ba 24.33 ± 3.05
100	Aa 26.66± 6.11	Ab 19.00 ± 1.73	Aa 28.66 ± 0.56

Similar characters indicate no significant differences (probability > 0.05) between totals.

The different letters indicate significant differences (probability 00.05) between the totals.

Large characters refer to the comparison of single-row totals (horizontal comparison).

Small characters indicate a comparison between single column totals (vertical comparison).

The results showed that there were significant differences and good bio-effectiveness against bacterial isolates. It was observed that the volatile oil had a inhibitory effect for all concentrations. The concentration of 100 mg / mL gave the highest inhibitory for all types of negative and positive bacteria. The diameter of the inhibition was 28 mm for *Staphylococcus* bacteria, The concentration of 25 mg / ml was 5 mm inhibition for *Pseudomonas* bacteria, and the results of our study agree with <sup>(17)</sup> which indicated the effect of the extract Alcoholic of the rosemary plant on the positive and negative bacteria Gram stain as well as of the effect of the alcoholic extract of the rosemary has an inhibitory effect on the positive bacteria *S.aureus* and *B.cereus* rather than on the negative *E.col* and *P.aeruginosa*<sup>(18)</sup>.

The effectiveness of the extract of the plant of the rosemary in the inhibition of microorganisms to contain the

compounds of tanning and soap and these compounds are effective inhibitory for a large number of microorganisms, including bacteria and fungi.<sup>(19)</sup>

We conclude from this that the volatile oil of the rosemary contains compounds found in the extract of the same plant and that the volatile oil has a clear inhibitory ability against the pathogenic bacteria used in the study. Therefore, it is necessary to encourage the production of therapeutic products belonging to plants of the oral species, such as rosemary and marrow, as well as conducting studies and research on the wider rosemary for the purpose of availing of its therapeutic properties.

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