

## Antibacterial Activities of Volatile oils from mentha Piperia Against Growth of Pathogenic Bacteria

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### Abstract:

The study included the extraction of volatile oil from *Mentha piperita* which was 1.3 % in the leaves and flowers . Volatile oil of the *Mentha piperita* leaves had special aromatic odour, pale yellow color, slightly pungent taste . The specific gravity and refractive index were (0.9794) and ( 1.464) respectively.

The inhibition activity of the *Mentha piperita* Volatile oil extracts were studied on some pathogenic microorganisms like *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Proteus sp*, and *Klebsiella pneumoniae* . The result showed that the volatile oil had an inhibition effect on the growth of all microorganisms, and it gave the higher inhibition effect on the growth of *S. aureus* in which the inhibition zone reached to 25 mm. also the inhibition zoon on the growth of *K. pneumoniae* was 20 mm.and it was 17 mm. on the growth of *Salmonella.typhi* , while the effect was slight on the growth of *Proteus sp* and *E. coli* 12,10 mm. respectively.

The minimum inhibition concentration (MIC) and the minimum bactericidal concentration (MBC) of the volatile oil extract of the *Mentha .piperita* leaves, were determined. The value of (MIC, MBC) of *Staph. aureus* was 0.625 , 1.25 % and the value for (MIC, MBC) of *K. pneumoniae* , *Salmonella typhi* were 1.25 , 2.5 % for each of them, while the value of (MIC, MBC) of *Proteus sp* , *E. coli* were 2.5 , 5 % respectively .

**Key words :** *Mentha piperita* , extraction of volatile oil ,antibacterial

### Introduction:

Peppermints Latin name *Mentha piperita* ,comes from the Greek . Peppermints is currently one of the most economically important aromatic and medical crops produced in the U.S. The world production of peppermint oil is about 8000 tons per year [1]. Peppermints leaf and oil are used for folk medicine ,as flavoring agents , and in cosmetic and pharmaceutical products throughout the world [2] . Peppermints oil is the most extensively used of all the volatile oils [3].

Peppermints is taken internally as a tea , tincture ,oil, or extract , and applied externally as a rub or liniment .

Herbalists consider Peppermint an antiseptic, antimicrobial ,antispasmodic , antipruritic ,antiemetic carminative , diaphoretic ,mild bitter and anticatarrhal . Its oil vapor is used as an inhalant for respiratory congestion and treat coughs , bronchitis and inflammation of the oral mucosa and throat .It has been used traditionally to treat a variety of digestive complaints such as colic in infants , flatulence , diarrhea , indigestion , nausea and vomiting ,morning sickness and anorexia [4,5,6].

Peppermint is currently used to treat irritable bowel syndrome , Crohns disease ,ulcerative colitis ,gallbladder

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and biliary tract disorders and liver complaints [7,8]. Peppermint oil is used to relieve menstrual cramps [9]. Peppermint oil and menthol have moderate antibacterial effects against both Gram positive and Gram negative bacteria [10,11]. Peppermint extracts are bacteriostatic against *Streptococcus thermophilus* and *Lactobacillus bulgaricus* [12]. Menthol is bactericidal against *Staphylococcus pyogenens*, *Staphylococcus aureus*, *Serratia marcescens*, *Escherichia coli*, and *Mycobacterium avium* [12]. Peppermint oil are fungicidal against *Candida albicans*, *Aspergillus albus* and dermatophytic fungi [12,13,14]. Peppermint has significant antiviral activity [15]. Menthol is virucidal against *Influenza*, *Herpes* and other viruses *in vitro*. Aqueous extracts of Peppermint leaves were antiviral against *Influenza A*, *Newcastle disease virus*, *Herpes simplex virus*, and *Vaccinia virus* in egg and cell-culture systems [16].

## Material and Methods:

### 1- PLANT

Peppermint leaves and flowers were separated from branches and cutting to small pieces. Volatile oils were extracted by using the volatile oils distilling apparatus (Glavenger), which works by closed commercial distillation technique for separating volatile oil from the plant Peppermint. [17]

### 2- METHOD OF VOLATILE OIL EXTRACTION

Ten grams of the leaves and flowers were put in especial container of the distillation apparatus and water was added in a ratio of 1:10 (w:v). Distillation process was carried out for four hours, to obtain the volatile oil.

### 3-DETERMINATION OF ANTIMICROBIAL ACTIVITY

#### • TEST ORGANISMS

The organism which used in this study were provided by the central laboratory / Ministry of Health / Iraq which included *E.coli*, *S.aureus*, *Klebsiella pneumoniae*, *Salmonella typhi*, *proteus sp*

#### • EFFECT OF VOLATILE OIL ON GROWTH OF THE MICROORGANISMS IN SOLID MEDIA.

The volatile oil was diluted in dimethyl sulphoxide (DMSO) using different dilution 100, 50, 25, 12.5 and 0.675. All dilution of oil was stored at 4°C until used. Bacterial suspension was prepared of 24 h old cultures ( $10^8$  cfu/ml) and plates inoculated with 0.1 ml. Two wells (5mm in diameter) were made in each plate using the cork borer. (DMSO) was used as control treatment, each treatment had 2 plates. The plate were incubated at 37°C for 18-24 h, then examined and the results recorded [18].

The inhibitory effect of the treatment against each test bacterium was determined by measuring the diameter of zones of inhibition (in millimeters)

#### • DETERMINATION OF (MIC), AND (MBC)

The MIC defined as the Minimum Inhibitory Concentration that inhibit most of the bacterial growth, while MBC defined as the Minimum Bacteriocidal Concentration, the MIC and BMC were determined by the broth macrodilution method, described by the National committee for clinical laboratory standards in 1993 as follow

Five different concentration of oil from the *Menthe piperita* were prepared which are (10,5,2.5, 1.25 and 0.625 %) by adding 1 ml of volatile oil in 10 ml TBS media ( test tube number 1 ) its concentration is 10% , than 5 ml transferred to test tube number 2 to get concentration 5% and proceeding transferring 5 ml to the remaining 4 test tube to obtain ( 2.5 ,1.25 and 0.625% ) ., thereafter these tubes sterilized using autoclave . 0.1 ml of bacterial inoculums w added to all test tubes , mixed well and incubation at 37 C for 24 hr.

The result were recorded according to the turbidity appearance and compare it with the control tubes .The control tubes number 1 (contain broth + bacterial inoculums) and control number 2 ( contain broth + volatile oil ).

MIC defined as it's the minimum concentration present turbidity appeared clearly to the eye in the cultured broth in which 0.1 ml from test tubes that not appearing any turbidity and spread on the nutrient agar plates by sterilized cotton swap stick then incubated plates at 37 C for 24 hr , the result recorded depending on existing of colonies (+) at least numbers .

The MBC defined as the minimum bacteriacidal concentration that kill 99.9% of bacteria by taking 0.1 ml from prepared tubes which its beyond the MIC tubes and its spread on the nutrient agar plates by sterilized cotton swap stick then plates incubated at 37 C for 24 hr . The result recorded by existing or not existing of bacteria growth .

### Results and Discussion:

Oil extracted from the plant parts (leaves) using a Clavinger, were found that the leaves contain oil about 1.3% (v:w) and this rate approaching with result of Anonymous [19], who

found that percentage of oil from the *Menthe piperita* , were rang between 1.2% - 1.5% .

Volatile Oil is Distinguish smell perfumed distinctive because it contains some of the compound with small molecular weights, such as alcohols, esters, phenols and Alaoxgenest compounds, which are rapid volatilization at room temperature ,is the smell of aromatic oils attributes diagnostic function of the fact that all oil has the distinct smell of him and therefore is a diagnostic plant containing the aromatic oils [20] .

Volatile Oil is Discriminate yellow color as volatile oils differ in the degree of natural colors are unrelated to either the color or pale yellow, as in oil output from the fruits of coriander and Anise.

Volatile Oil is Discriminate Baited bitterly, and characterized by volatile oil Baited ranging from sweet, warm harsh and Caustic [20].

We found that the density of the oil quality of the volatile oil of *Menthe piperita* was (0.9794) and depend on the quality density and type of vegetation depending on the source components of the turbine materials, if the quality density less than one correct density of less than water, as in most volatile oils, it leads to the buoyancy of oil on the surface water in the presence of high quantities of turbine and other alalevate on aromatic oil, if the quality density greater than one correct mean density greater than water, it leads to the deposition of volatile oil underground water and that the presence of high quantities of compound turbine. The specific gravity range to aromatic oils of all aromatic plants between (0.824-1.172) . [20], and this difference is due to genetic Group. The species differ from to anther through the physical attributes of the volatile oils because of

the difference in the quality and quantity of compounds components of the oil according to the different varieties of genetically as well as the impact of environmental conditions and farming techniques [ 21]

volatile oil of *Menthe piperita* value of the refractive index (1.464), and these are important qualities of character to determine the quality and purity of oils and fats [20]. The high value of refractive index of volatile oil is described qualities oil and increase the value of refractive index increased density oil also affected the value depending on the focus of its components, especially solid compounds[22].

Volatile oil examined to determine the effectiveness of this oil to the isolates bacteria under study results showed that the oil has antibacterial for the growth of all microorganisms tested table(1). Found the gram positive bacteria is the most affected by volatile oil since zone of inhibition of *S.aureus*. 25 mm, as in the figure(1), this may be attributed to the layer of peptedoglycan for the bacteria in gram positive more available contact to volatile oil, the bacteria not have protection against any material outside this layer [23].

As for the nature of gram negative bacteria had varying influence of oil in the inhibition bacteria has been a better effect on the growth of bacteria *Salmonella typhi* and *K. pneumoniae* rate zone inhibition 17,20 mm respectively , while the least effect of oil on the growth of bacteria *E. coli* and *Proteus sp* as rate zone inhibition 10, 12 mm respectively, as in the figure (1), and this is consistent with what was said by 22. That the captain of the oil fired plant type *Ocimum gratissimum* him effective disincentives to the cellular membrane of intestinal family and also

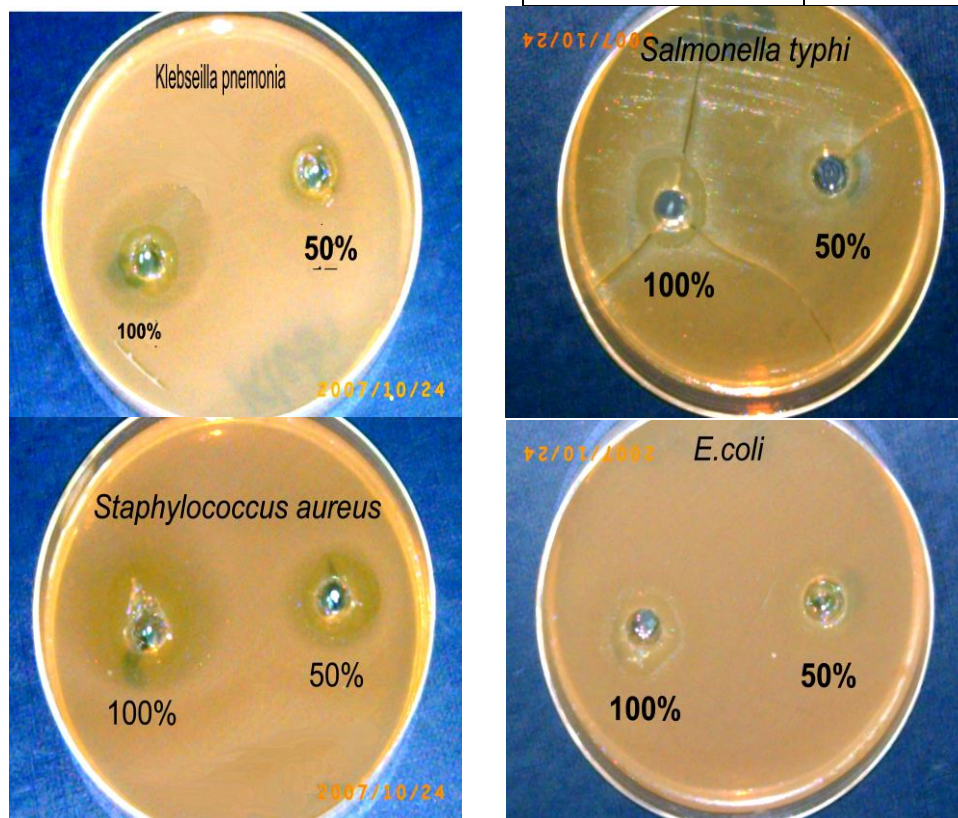
mentioned that the impact of oil on the growth of bacteria .

Results are shown in the table (2) The amount (MIC) of *S.aureus*, *K. Pneumoniae*, *S.typhi* , *P.sp* , *E.coli*. (0.625, 1.25 ,1.25, ,5,2.5 )% respectively, and thus clear that *S.aureus* is the most sensitive species compared to the other bacteria . While the minimum bacteriocidal concentration (MBC) (1.25, 2.5, 2.5, 5, 5)% respectively, of the species mentioned above, and Figure (2) inhibitor concentration and lower concentration level of the deadly. Sartoratto *et al.* [24] found values (MIC) to extract oil for the leaves the Rayhan *S.aureus* , *E. coli* and yeast *C. albicans* 0.7, 2, 2 mg / ml, respectively, while Mimica *et al* [25] founds volatile oil exhibited very strong antibacterial activity , in particularly against volatile oil , especially towards multiresistant strain of *shigella sonei* and *Micrococcus flavus* ATTC10,240 .The volatile oils of *M.piperita* and *M.longifolia* were found to be more active than the volatile oil of *M.aquatica* .Especially low MIC 4 microl /ml and 8 microl/ml were found with *M .piperita* oil against *Trichophyton tonsurans* and *Candida albicans* . whether Nakamura *et al.*, [23] found that the value (MIC) to extract oil for the leaves Rayhan kind of *Ocimum gratissimum* to *S.aureus*, *S.typhi*, *E.coli*, *K. pneumoniae* 0.75, 3, 6, 6 mg / ml, respectively, and the values (MBC) of the isolates itself (1.5, 6, 12, 12) mg / ml, respectively, and these results found that all these values are approaching what been obtained in this study and this explains that the aromatic oil impact on the broad spectrum of bacterial isolates that has any influence or discourage effective in killing bacteria even within lower concentrations. This is a clear signal that the volatile oils extracted from the

leaves *Menthe piperita* have a good characteristics of limiting the spread of pathogenic microorganisms to humans.

**Table (1) Effect of extract volatile oil (concentration of 50% ) in the growth bacteria in solid media**

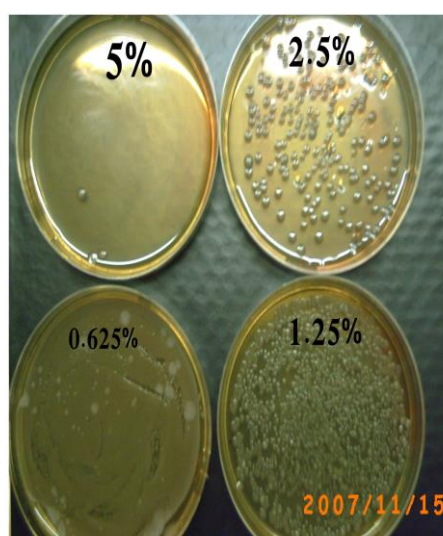
Bacteria	Inhibition zone(m)
<i>Staphylococcus aureus</i>	25
<i>Klebsiella pneumoniae</i>	20
<i>Salmonella typhi</i>	17
<i>Proteus.sp</i>	12
<i>Eserichia coli</i>	10



**Fig. (1) Effect of volatile oil (100 ,50)% on bacterial growth**

**Table (2) minimum inhibitor concentration and minimum bacteriocidal concentration to extract oil from *Menthe piperita* leaves**

Bacteria	MIC	MBC
<i>S.aureus</i>	0.625	1.25
<i>K.pneumoniae</i>	1.25	2.5
<i>S.typhi</i>	1.25	2.5
<i>Proteus.sp</i>	2.5	5
<i>E.coli</i>	2.5	5



**Fig. (2) minimum inhibitor concentration and minimum bacteriocidal concentration to *E.coli* .**

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## تأثير الزيوت الطيارة المستخلصة من نبات النعناع المحلي على نمو البكتيريا الممرضة

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### الخلاصة

تضمنت الدراسة إجراء استخلاص الزيت الطيار من نبات النعناع إذ بلغت نسبته 1.3% في الأوراق و الأزهار. تميز هذا الزيت برائحته العطرة وبلونه الأصفر الباهت وطعمه اللاذع ، وبلغت كثافته النوعية (0.9794) ومعامل انكساره (1.464).

درست الفعالية التثبيطية لمستخلصات الأوراق والزيت الطيار لنبات النعناع بطريقة الانتشار بالحفر على بعض الأحياء المجهرية الممرضة مثل *Staphylococcus aureus* ، *Salmonella typhi* ، *Klebsiella pneumoniae* ، *proteus sp* ، *Escherichia coli*

أظهرت النتائج أن للزيت الطيار تأثيراً مضاداً لنمو جميع الأحياء المجهرية المختبرة وقد أعطى أعلى تأثير تثبيطي على نمو بكتريا *S. aureus* إذ بلغ قطر منطقة تثبيط النمو (25) ملليمتر، وكذلك على نمو بكتريا *S.typhi* و *K. pneumoniae* إذ بلغ معدل قطر التثبيط ( 20,17) ملليمتر على التوالي ، وبلغ معدل قطر التثبيط (12) ملليمتر على نمو بكتريا *Proteus sp* ، بينما كان التأثير طفيفاً على نمو بكتريا *E. coli* (10) ملليمتر.

تم في هذه الدراسة كذلك تحديد قيمة التركيز المثبط الأدنى (MIC) (Minimum Inhibitory Concentration) والتركيز القاتل الأدنى (MBC) (Minimum Bactericidal Concentration) لمستخلص الزيت الطيار لأوراق نبات الريحان إذ بلغت قيمة MIC ، MBC لبكتريا *Staph. aureus* (0.625 ، 1.25) % وكانت قيمة MIC ، MBC لبكتريا *K. pneumoniae* و *S.typhi* (1.25 ، 2.5) % لكل منهما ، في حين بلغت قيمة MIC ، MBC لبكتريا *P. sp* , *E.coli* (2.5 ، 5) % لكل منهما .