



Chemical Constituents of the *Mentha Spicata* Volatile Oil and the Activity against Gram Positive and Gram Negative Bacteria in Sudan

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ABSTRACT

100g of dried leaves of *Mentha spicata* produced 1.1% of volatile oil by hydro distillation method. GC-MS, method used to identified the chemical compound of the volatile oil, it contained eighteen compounds, ten of them constituted as the major compounds, carvone (58.3%), *cis*-carveol 14.99%), limonene (8.5%), carvyl acetate (3.10%), α -pinene (1.22%), β -Pinene (2.11%), Menthone (1.08%), Menthol (1.01%), β -bourbonene (1.98) and *trans*-Caryophyllene (1.75). The spearmint volatile oil more actively against gram positive bacteria (*Staphylococcus aureus* and *Bacillus cereus*) than gram negative bacteria, (*Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*). The activity of antibiotic Chloramphenicol, Ciprofloxacin, Tetracycline Ceftriaxone, and Gentamycin against bacteria found differences inhibition zoon. Among all antibiotic, ciprofloxacin have more activity against bacteria while Ceftriaxone have the lower activity.

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Introduction

The family of Spearmint (*Mentha spicata*) is Lamiaceae, it is perennial herbs, the original regions of the plant Europe and the Middle East, Australia and South Africa, now a day the plant cultivated in many regions of the world.^(1,2) Spearmint have good aroma so that used in food industries as flavoring agent, plant widely used in pharmaceutical, folk medicine for treatment of liver disease fevers, abdominal pain such as vomiting, also used in manufacture of confectionary, chewing gum, toothpaste as favoring agent. Plant have more active against bacteria^(1,3,4) The spearmint volatile oils have antimicrobial effects and antioxidant^(5,6,7). Volatile oils of *Mentha spicata* have highly active against gram negative and gram positive bacteria, fungi, pest and insect^(8,9). The general chemical compound of spearmint menthol, menthone, carboxyl esters, particularly menthyl sacetate.⁽¹⁰⁾ Dried leaf also contain limonene, pulegone, caryophyllene and pinene.^(11,12) peppermint contain flavonoids such as eriocitrin, hesperidin, and kaempferol 7-O-rutinoside and terpenoids⁽¹³⁾. The volatile oil of mint (contain menthol) used as antidermatophytic leishmanicidal products⁽¹⁴⁾

Objective

This study to identify the volatile oil of *Mentha spicata* leaf extract constituents and the activity against bacterial, compare with the activity of antibiotics.

Materials and method

Plant material

Mentha spicata cultivated in the Demonstration Farms of Medicinal And Aromatic Plant Research Institute at Shambati (Sudan), the plant kept in carton bags for extraction after drying.

Microorganism

All the microorganisms used were culture in the Stak Laboratory (Khartoum) Sudan. Bacterial identification by conventional biochemical methods according to the standard microbiological techniques These microbes were *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebsiella pneumonia* and *Bacillus cereus*.

Extraction of Volatile Oil

Hydro distillation method

The dried whole plants (100g) subjected to hydro distillation for 6 hours using a Clevenger type apparatus; anhydrous sodium sulphate was used for drying the oil.⁽¹⁵⁾

Chemical components Identification

Sample Preparation

2g of the spearmint volatile oil was mixed thoroughly with 7ml of alcoholic sodium hydroxide (Noah). The mixture was then shake for 5 minutes. The content of the test tube was left to stand overnight. 1 ml of Super saturated sodium chloride (NaCl) was added and shaken, added 2ml of normal hexane and then the contents were shake thoroughly for three minutes. Then the n-hexane layer (the upper layer of the test tube) was formed, 5 μ l from the n-hexane extract was diluted with 5 ml of diethyl ether, filtered the mixture and dried with 1g of anhydrous sodium sulphate, and 1 μ l of the diluted sample was injected in the GC/MS instrument.

Method of analysis Gas Chromatography-Mass Spectrometry (GC- MS) Conditions

The identification of spearmint constituents were carried out by using Gas chromatography with mass spectrometry, model (GC/MS-QP2010-Ultra) Shimadzu Company, Japans with serial number 020525101565SA and capillary column (Rtx-5ms-30m x 0.25mm x 0.25 μ m). Injection of the sample by using split mode, the carrier gas

was helium passed with flow rate 1.61 ml/min, the rate of temperature was began from 6°C with flow rate 10c/min to 30°C as end temperature degree with 3 minutes hold time, the injection port temperature was 30°C, the temperature of the ion was 20°C and the interface temperature was 25°C. The spearmint volatile oil sample was analyzed by using scan mode in the range of m/z 40-500 charges to ratio and the total run time was 27 minutes. The identification of the compound by comparing their retention index and mass fragmentation patents with those are found in the library, the National Institute of Standards and Technology (NIST).

Sensitivity test of volatile Oil against bacteria

A disc diffusion technique was used for the sensitivity test of the volatile oil of *Mentha spicata*. using Sterile 5 mm diameter paper disc soaked with the volatile oil was placed gently on the media, which had been freshly inoculated with each of the organisms. Incubation time 24 hours at 37 C°. Recorded the result by inhibited zoon by the oil, the same method was used for microbial sensitivity test of the antibiotic⁽¹⁶⁾.

Results and Discussion

100g of the dried leaves of *Mentha spicata*, produced 1.1%of concentrated volatile oil. Table.1 show that the chemical constituent of the volatile oil of *Mentha spicata* L. in the order of the retention times. 18 constituents were identified, α -pinene (1.22%), β -pinene (2.11%), α -Myrcene (0.45%), limonene (8.5%), γ -Terpinene (0.31%), trans-Sabinene hydrate(0.31%), Menthol(1.01%), Cis-carveol (14.99%), Carvylacetate (3.10%), carvone (58.3%), Menthone (1.08%), β -bourbonene (1.98%) trans-Caryophyllene, (1.75%), β -Cubebene (0.16%), γ -Muurolene (0.25%), Germacrene-D (0.64%), γ -Cadinene (0.12%) and β -cubebene (0.31%). Table 2, show that the activity of the volatile oil of *Mentha spicata* against different Six pathogenic organisms gram positive *Staphylococcus aureus* and *Bacillus cereu*, gram negative bacteria *Escherichia coli*, *Pseudomona aeruginosa*, *Salmonella typhimurium*, *Klebsiella pneumonia*, (the concentration of the volatile oil 100,50, 25 and 12.5 mg/ml) there was differences among bacteria, the high activity was detected against gram positive bacteria *Staphylococcus aureus* and *Bacillus Cereus* the lowest one against gram negative bacteria *Klebsiella pneumonia*, these results conformity with obtained in previous studies⁽¹²⁾ who found that higher antimicrobial activity against the gram positive microorganisms. and the bacteria was inhibited at the high concentration of the extract Table 3. detected the inhibition zone by the antibiotic shows that there was differences among them, ciprofloxacin more active against bacteria while Ceftriaxone gave lowest one, within the bacteria *Salmonella typhimurium* gave high inhibited zone by antibiotic and the *Klebsiella pneumonia* gave lower inhibition, there was no high different between antibiotic and volatile oil of *Mentha spicata* in this study.

Conclusion

This work conclude that the volatile oil of *Mentha spicata* leaf extract highly effective against pathogenic bacteria, and have no different between it and antibiotic in the activity against microorganism,

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Table 1. Chemical composition of *Mentha spicata* volatile oil

No	RT	Compound	Concentration%
1	8.30	α -pinene	1.22
2	9.66	β -Pinene	2.11
3	9.87	α -Myrcene	0.45
4	11.40	Limonene	8.50
5	12.21	γ -Terpinene	0.31
6	12.29	trans-Sabinene hydrate	0.31
7	15.48	Menthol	1.01
8	17.10	Cis-carveol	14.99
9	17.15	Carvylacetate	3.10
10	18.65	Carvone	58.3
11	19.20	Menthone	1.08
12	21.20	β -bourbonene	1.98
13	22.45	trans-Caryophyllene	1.75
14	22.71	β -Cubebene	0.16
15	23.56	γ -Muurolene	0.25
16	23.74	Germacrene-D	0.64
17	24.46	γ -Cadinene	0.12
18	23.45	β -cubebene	0.31

Table 2. Inhibition zone (in mm) of bacteria by different concentrations of the *Mentha spicata* volatile oil

Microorganism	Inhibition zone by different Concentration of the <i>Mentha spicata</i> volatile oil (μ g/disc)			
	100	50	25	12.5
Salmonella typhimurium	16	13	11	11
Staphylococcus aureus	21	17.6	13.9	12
Pseudomonas aeruginosa	19.8	14	12	10
Escherichia coli	13.9	10.5	10	9
Klebsiella pneumonia	13.8	10	9	9
Bacillus Cereus	20	17	14	13

Table 3. Inhibition zone of bacteria (in mm) by different antibiotics

Bacterial test	Inhibition zone by Antibiotics (μ g/disc)				
	CIP	G	Chlora	Tetr	CEF
Salmonella typhimurium	26	16	20	10	13
Staphylococcus aureus	21	10.3	21.2	14.1	11
Pseudomonas aeruginosa	26	10	13.9	13.8	16.1
Escherichia coli	16	12	14	10	11
Klebsiella pneumonia	15.1	12	9	11	7
Bacillus cereus	21	14	10	13	6

CIP=Ciprofloxacin (5 μ g), G=Gentamycin (10 μ g), CEF= Ceftriaxone (30 μ g), Chlora =Chloramphenicol (10 μ g), Tetr =Tetracycline (25 μ g).

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