Antimicrobial activities of the essential oils of five *Salvia* species from Tehran province, Iran

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ABSTRACT

Essential oils are volatile, natural, complex compounds formed by aromatic plants as secondary metabolites. In nature, essential oils play an important role in protecting plants in form of antibacterials, antivirals, antifungals, insecticides properties and also against herbivores. They also may attract some insects to use them in dispersion of pollens and seeds, or repel undesirable others. Present study describes the results of our study on the chemical composition, and antimicrobial activity of the essential oils from aerial parts of aromatic plants which collected from province of Tehran. The aerial parts of plants were collected at full flowering stage. The essential oils were isolated by hydrodistillation and analyzed by combination of capillary GC and GC-MS. Also their antimicrobial activities were considered according to the disc diffusion method and MIC values. Finally, five plant species (*Salvia sclarea, salvia multicaulis, salvia verticillata, salvia limbata, Salvia choloroleuca)* which showed good significant antibacterial were presented. These five plants belong to labiates' family. Among these proposed plants *salvia multicaulis* and *Salvia sclarea* showed good antibacterial activity.

Keywords: Essential oils; *Salvia*; Antimicrobial effects; Tehran province

INTRUDUCTION

Finding the healing forces of nature always has been one of the oldest human ideas. Medicinal plants used as beverages, or as steamed or as balm for wounds have been common around the world. It is estimated that there are about 250 to 500 thousand plants on earth. A small percentage of this number (1 to 10 percent) is used as food for humans and animals and almost around the same number is used as medicinal plant [1].

Between countries, only China can be recognized as an old reservoir of using medical plant regulations. Chinese believed that "nature has a treatment for every disease" , and among medical miracles, including those not known yet, roots magic jeans Singh (*Panax ginseng*) is used as the curer of all the pains of infertility, and aging, to cancer [1, 2]. One of the problems that new medicine brought with its apparent advantages compared with traditional medicine, taking chemical drugs is increasing day by day problems that create excessively acute causes. Of course, noting that chemical drugs, mainly herbal formulas with the imitation of synthetic pharmaceutical laboratories are synthesized. Interestingly, many natural compounds obtained from plants, especially medicinal and aromatic plants show remarkable anti-bacterial activity [3].

It should be mentioned that today, antimicrobial active ingredients in essential oils and various extracts obtained from plant sources in various plants are frequently mentioned in sources. Therefore, in this study, antimicrobial properties of essential oils of several plant species have been mentioned.

MATERIALS AND METHODS

Collecting plants:

Proper time for gathering medicinal plants is when plant organs have a maximum active ingredient and this time for leaves and branches often coincided with the time of blossoming and

before the fruit ripened. For more importance to leaves and flowering plants in mint leaves dark, the time for collecting these plants is mainly in the spring. Collected plants (*Salvia sclarea, salvia multicaulis, salvia verticillata, salvia limbata, Salvia choloroleuca),* after a careful and scientific identification, are prepared as samples of herbariums and are kept in the Institute of Applied Science herbariums [4].

Extracting and identifying essences:

Method for extraction of essential oil distillation (Hydrodistillation) we spill some amount (approximately 100 grams) of dried and ground plant in 2 liters balloons and pour distilled water on it so that completely cover the plant. Essential oils was obtained with Clevenger apparatus. After assembling device we usually let the plant boil in water for 2 to 3 hours. Due to the oily nature of essences, obtained essential oil floats as a layer of oil on the water surface which can be easily separated. For dehydration of oil, salt less sodium sulfate was used. Obtained essential oil was poured in colorful small tubes and was kept in temperature - 20 ° C in the freezer until testing and analysis.

Essential oil analysis:

Common physical method for separation of essential oil components, is Gas Crowe Matography (GC) which is based on the sample release of two phases between residents (solid) and mobile phase (gas). Diagnosing obtained components occurs from the inhibition time. Then for detection and isolation of two components in the oil, two devices, gas chromatography and mass spectrometry as coupled, were used that the GC and GC-MS devices details are as follows: For GC analysis Thermoquest gas chromatograph equipped with DB-1 column, type the length of 60 meters and diameter 0/25Mm and thickness layer 0/25 Mm were used. Oven temperature of 60 ° C to 250 ° C with 4 degree per minutes speed increased. Part injection temperature 250 ° C and making clear 280 ° C and nitrogen azotes' carrier gas with a speed 1 / 1 ml min was used [7].

For GC-MS analysis, Thermoquest-Finnigan Trace GC-MS system equipped with a DB-1 column 60 m in length and diameter of 0/25 Mm thickness layer and 0/25 Micrometers were used. Oven temperature from 60 ° C to 250 ° C with 4 degrees per minutes speed increased. Helium gas with speed 1 / 1 ml per minute and ionizing energy 70 electron volts in the coupled mass spectrometer with gas chromatograph was used [8].

Identifying compounds in the essential oil:

For identifying formed components of essences, these three methods were used: compared inhibition index (Retention index) of each essential oil components with inhibition indices reported in the resources (Adams 1995; Shibamoto 1987; Davis 1988), Comparison of mass spectra of each essential oil components with mass spectra available in libraries (Wiley and Terpenoid) GC-MS system and in some cases simultaneously with the injection of standard samples of known compounds, and lastly the work for formed essential compounds oil were done.

Antimicrobial tests:

In this study, four species of gram-positive and gram- negative bacteria, which all have been taken from bacterial banks that comes bellow:

(American Type Culture Collection) ATCC, (Persian type culture collection) PTCC(9 , 1) were used. Profiles of used standard bacterias are shown in the table 1.

Table 1- profiles of used standard bacteria.

Bacteria	**Gram activity**	**Standard number**
Staphylococcus aureus	+	ATCC 25923
Klebsiella pneumoniae	-	ATCC 10031
Escherichia coli	-	ATCC 25922
Streptococous mutans	+	PTCC 1219

Microbial culture:

By a sterile loop and under Laminar Air Flow some of each frozen bacteria were transferred to agar plates containing Mueller Hinton. Plates were placed to incubator for 24 hours at temperature 37 ° C so that completely grown bacteria and bacterium can be obtained from each bacteria. In next stage, four to six bacterial colonies from each bacteria by a sterile loop were transferred to bacterial tubes containing 5 ml culture of liquid medium muller- Hinton broth from bacteria's culture and were placed in the temperature 37 ° C until bacteria after 4 up to 6 hours reach to the exponential growth

phase. The turbidity of any tubes was compared with standard number of 0/5 Mc Farland that its turbidity is equivalent to $1/5 \times 10^8$ bacteria in an ml.

Determination of the anti- microbial properties of essential oil:

To determine the primary antibacterial properties of essences and their main components and their no-growth halos measurement methods, (Disk diffusion method) were used. Desired concentrations of pure essential oils and main composition poured on sterile paper disks (6 mm diameter), and then disks were placed on agar medium infected bacteria. The diameter of no growth halos was determined after 24 hours incubation at 37 ° C. The halo diameter was measured by Hi Antibiotic Zone Scale ruler and these results mean were recalculated for three times. Some of the standard antibiotics, including gentamicine and tetracycline were considered as a positive control [10] .

Determination minimum inhibitory concentration (MIC) of essential oil:

By this method, we could catch the essence effect and it's main properties on bacteria's MIC [3]. For this purpose, the 96 sinks microplates were used which we can simultaneously do MIC test on eight oil samples with that. In the first row, we pour 200 microliters and in other sinks, we pour 250 microliters microplate of Muller hinton broth culture. Then the desired concentration of oil in the first sinks of each row fixed and 100 micro liters from the first sink and poured in the second sink and then after a few times to fill and empty in other to mix essence with culture , by sampler 100 micro liters removed and poured into the third sink. This will continue to sink No. 11. We pour Stokes solution prepared from the bacteria with concentration of 1×10^6 CFU (Colony Forming Unit) 100 micro liters to any micro plate, except No. 11 sink throw each row. No. 11 of each Sink row is as the control medium containing only culture and essential oils. No. 12 of each Sinks row is as the essence control bacteria to determine bacteria turbidity which contains culture and bacteria. In the last stage, we put micro plates in incubator for 24 hours at 37 ° C. To determine the minimum inhibitory concentration for growth, the first sink that has no turbidity and in other words, the one with no bacterial growth, can be observed as the number of MIC [11].

RESULTS:

Hydrodistilled essential oils of five examined *Salvia* species was represent in table 2-6.The results of antibacterial activity of the essential oils according to the disc diffusion method and MIC values indicated that all the samples have moderate to high inhibitory activity against tested bacteria except for *K. pneumoniae* which was totally resistant (Table 7).

Table 2- List of plant essential oil compounds of *Salvia limbata*

Compound	RI	% of the oil
Pinene	938	24.4
Camphene	950	1.9
Sabinene	972	3.9
Pinene	981	21.9
Phellandrene	1002	0.3
Carene	1011	0.3
Terpinene	1013	0.3
p-Cymene	1018	0.4
1,8-Cineole	1029	7.7
Terpinenec	1053	0.8
Terpinolene	1085	1.7
Campholenal	1112	0.2
trans-Pinocarveol	1132	0.6
Verbenol	1136	0.5
Pinocarvone	1147	0.2
p-Menth-1-en-8-ol	1153	0.2
Borneolc	1158	1.9
p-Cymen-8-ol	1166	0.1
4-Terpineol	1171	1.0
Terpineol	1171	0.8
Myrtenol	1187	0.3
Bornyl acetate	1276	2.1
Terpinenyl acetate	1337	1.0
Elemene	1341	0.1
Copaene	1383	0.4
Elemene	1393	0.3
Germacrene D	1489	3.8
Spathulenol	1583	8.1

Table 3- List of plant essential oil compounds of *Salvia multicaulis*

Compound	RI	% of the oil
Tricyclene	926	1.4
α-Pinene	936	11.5
Camphene	949	7.5
Sabinene	970	3.0
β-Pinene	978	6.6
Myrcene	982	0.9
ρ-Cymene	1015	3.7
1,8-Cineole	1025	17.0
trans-β-Ocimene	1037	0.7
γ-Terpinene	1052	2.7
α-Terpinolene	1081	0.2
Linalool	1084	1.2
trans-Pinocarveol	1128	0.8
trans-Verbenol	1132	0.5
Borneol	1155	2.7
Teroin-4-ol	1166	2.0
Myrtenal	1175	1.1
Myrtenol	1182	0.6
Tridecane	1285	1.0
Eugenol	1322	0.7
α-Copaene	1385	0.7
Tetradecane	1395	1.9
β-Caryophyllene	1430	8.9
α-Humulene	1460	0.6
Germacrene D	1485	2.4
Bicyclogermacrene	1499	1.5
Spathulenol	1573	2.3
Caryophyllene oxide	1581	1.8
Epoxy allo-aromadendrene	1619	1.1
β-Eudesmol	1645	1.0
α-Bisabolene oxide	1661	0.5
Platambin	1842	0.5

Table 4- List of plant essential oil compounds of *Salvia verticillata*

Compound	RI	% of the oil
α-Pinene	936	1.0
Camphene	949	3.0
Sabinene	970	5.2
β-Pinene	978	10.4
Myrcene	982	0.9
ρ-Cymene	1015	2.7
1,8-Cineole	1025	3.2
trans-β-Ocimene	1037	1.5
γ-Terpinene	1052	1.7
α-Terpinolene	1081	0.2
trans-Pinocarveol	1128	0.6
trans-Verbenol	1132	0.5
Borneol	1155	2.7
Teroin-4-ol	1166	2.0
Myrtenal	1175	1.4
Tridecane	1285	1.0
Eugenol	1322	0.7
α-Copaene	1385	0.7
Tetradecane	1395	4.9
β-Caryophyllene	1430	31.5
α-Humulene	1460	0.6
Germacrene D	1485	16.2
Bicyclogermacrene	1499	1.5
Spathulenol	1573	1.3
Caryophyllene	1581	1.8
β-Eudesmol	1645	1.0
α-Bisabolene oxide	1661	0.3

Table 5- List of plant essential oil compounds of *Salvia sclarea*

Compound	RI	% of the oil
α-Pinene	936	0.8
Camphene	949	0.9
β-Pinene	978	0.6
Myrcene	982	0.1
Limonene	1018	1.7
1,8-Cineole	1025	0.3
β-Ocimene	1037	0.7
α-Terpinolene	1018	0.4
Linalool	1084	9
Borneol	1155	1.2
α-Terpineol	1166	7.4
Terpinen-4-ol	1171	0.9
Meral	1215	1.9
Geraniol	1253	6.8
Bornyl acetate	1285	0.1
Carvacrol	1281	trace
Tridecane	1285	0.7
Eugenol	1322	0.9
α-Copaene	1385	0.7
β-Caryophyllene	1430	9
α-Humulene	1460	1.4
Germacrene D	1485	9.8
Spathulenol	1573	0.3
Caryophyllene oxide	1581	3.8
Epoxy allo - aromadendrene	1619	2.1
β-Eudesmol	1645	0.5
Sclareol	223	11

Table6- List of plant essential oil compounds of *Salvia chloroleuca*

Compound	RI	% of the oil
Tricyclene	926	1.4
α-Pinene	936	9
Camphene	949	3.2
Sabinene	970	5
β-Pinene	978	10.6
Myrcene	982	0.9
ρ-Cymene	1015	3.7
1,8-Cineole	1025	9
trans-β-Ocimene	1037	0.7
γ-Terpinene	1052	2.7
α-Terpinolene	1081	0.2
Linalool	1084	1.2
trans-Pinocarveol	1128	0.8
trans-Verbenol	1132	0.5
Borneol	1155	2.7
Teroin-4-ol	1166	2
Myrtenal	1175	1.1
Myrtenol	1182	0.6
Thymol	1265	0.3
Carvacrol	1281	7.9
Tridecane	1285	1
Eugenol	1322	0.7
α-Copaene	1385	0.7
Tetradecane	1395	4.9
β-Caryophyllene	1430	9
α-Humulene	1460	0.6
Germacrene D	1485	6.4
Bicyclogermacrene	1499	1.5
Spathulenol	1573	3.3
Caryophyllene oxide	1581	3.8
Epoxy allo-aromadendrene	1619	1.1
β-Eudesmol	1645	1
α-Bisabolene oxide	1661	0.5
Platambin	1842	0.5

Table 7. The results of the proposed species on tested case microorganisms

Species of microorganisms	***S.limbata***		***S. multicaulis***		***S. verticillata***		***S. sclera***		***S. choloroleuca***	
	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
S. aureus	8	›15	15	7.5	13	15	15	7.5	15	7.5
K. pneumoniae	0	-	10	15	9	›15	10	›15	12	15
E. coli	10	›15	13	15	12	15	14	15	14	15
S. mutans	-	-	9	›15	0	-	0	-	0	-

DISCUSSION

The results showed that the oil exhibited high antimicrobial activity against Gram positive bacteria and low activity against the Gram negative organisms.

The mechanisms antimicrobial activities of essential oil and extract are not still fully understood. Inhibitory effects of some plant essential oils on the growth of pathogenic microorganisms is well documented and the phenolic components are chiefly responsible for such properties [5, 8]. Essential oils are hydrophobic compounds a vital characteristic which enables them even in a low pH, to break through the lipids of the bacterial cell membrane and mitochondria which results in disturbing the structure and rendering them more permeable and as a result leads to death of them. Considering the large number of different groups of chemical compounds present in the essential oils, it is most likely that their antibacterial activity is not attributable to one specific mechanism rather against several targets in the bacterial cell [8].

The essences are volatile and aromatic compounds which are ingredient of Trapani herbal compounds that are the most diverse. generally, the monotrepens and sesqueterepens are called volatile essences (essential oil). Usually these compounds are extracted from distillation water. These compounds play an important role in protecting plants against vegetarians and pathogenic fungi [12] and also in attracting pollinators and signaling between plants. Today, the properties of these compounds such as: anti-bacterial, anti-fungal, anti-viruses, anti-inflammatory are antioxidant are indicated. In addition to the medical roles, these compounds have a huge usage in perfumery and cosmetic industries and also protecting the canned food industry.Among 3000 Essence that are already known only 300 of them have found economic value [6 , 8].

Anti-bacterial effect of essences, in extensive studies which is done on different organisms has been proved. These mentioned microorganisms include: nutritional poisonous organisms, Micotoxigenic string fungi, pathogenic and dimorphic yeasts, and animal and plant viruses [12, 13].

Among the five proposed species, all species have shown good antibacterial results. Considering results of this research and work done by other researchers has shown that gram-positive bacteria were more sensitive to essential oil in comparison with Gram-negative bacteria.

CONCLUSION

The biological activity of the essential oil obtained from five *salvia* species showed significant antibacterial activities.

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