Chemical composition of essential oil from *Semenovia suffruticosa* and their antimicrobial's effects in drinking water

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ABSTRACT

This study reports the chemical composition and antibacterial activity of the essential oil from aerial parts *of Semenovia suffruticosa* (which is endemic to Iran). The essential oil of aerial parts extracted by hydro distillation method and was investigated using GC& GC-MS techniques. The oil yield (w/w %) was 0.77 on dry weight. Sixty components were recognized consists of 84.99 % of total oil .The major components found in the oil: cis-ocimene (19.31%), linalool (9.00), cinnamy valerate(8.19%), α-terpinolene (5.49%) ,6-amyl-α-pyrone(4.55%) and unknown compounds (1.70 %).The percentage of oxygenated terpenoids and total terpenoids in the essential oil are 22.69 and 61.42 respectively. Antimicrobial effects of this oil were examined according to Agar dilution method. The results of Minimal inhibition Concentration presented, in the following: *Staphylococcus aureu*s (8 $\mu g.mL^{-1}$), *Salmonella typhi* (32 $\mu g.mL^{-1}$), *Escherichia coli* (32 $\mu g.mL^{-1}$), *Candida albicans*(8 $\mu g.mL^{-1}$), *Aspergillus niger*(32 $\mu g.mL^{-1}$), shows that the highest dilution of essential oil has been on *Candida albican*s and the lowest dilution of essential oil that is capable of control on micro-organism, has been *pseudomonas aeruginosa* . The antimicrobial effects of essential oil have been demonstrated in drinking water using the heterotrophic plat technique. The increase of this essential oil among the samples resulted in decreasing the number of micro-organisms colonies.

Keywords: *Umbellifera* , Essential oil composition , Agar dilution method Heterotrophic plat count (H.P.C) technique.

1. INTRODUCTION

Eleven species of genus *Semenovia* are found in Iran, five of them are endemic[1]. *Semenovia suffruticosa* is a perennial plant species of *Apiaceae* (*Umbeliferae*) family that grows only in altitudes of 2300 - 2500m Taftan Mountain (Sistan & Baluchestan province, Iran). The plant *Semenovia suffruticosa* has comb-like leaves or bifurcate divisions and long petioles with hard pods. The stems are almost cylindrical trunk and 45 – 70 cm long, with low bifurcate branches, and sometimes with shallow grooves on the surface. Terminal Umbels are within 7 – 10 cm radiuses with almost equal parts and glabrous, and 40 - 50 cm long in fruit-containing state *Umbellules* have 12 - 15 flowers and peduncle is shorter thanthe ripe fruit *Apiaceae* family[2] plants contain compounds such as coumarin, furanocoumarin, cromenocoumarin, terpene, sesquiterpene, triterpenoid saponins and acetylene compounds[3].

The essential oils of *Semenovia suffruticosa* (Freyn Bornm) Manden. And *S.Tragioides* Boiss. Manden were extracted from the aerial parts by hydro distillation method .The subject of our previous has been studies, the major components were linalool (13.9%), lavandulyl acetate (11.5 %), (E)-β-ocimene (9.7%) and cinamy isovalerate (9.4%) in the latter[4].The antimicrobial tests were carried out at the department of biological sciences, north Tehran branch, and LA. University of Tehran using the following microorganisms: *Staphylococcus aureus*

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PTCC1113,*Staphylococcus epidermidis*PTCC1349, *Staphylococcus saprophytic* us PTCC 1379 (Gram-positive bacteria), *Salmonella typ*hi PTCC 1185, Shigella Flexnery PTCC 1234 and *Escherichia coli* PTCC 1330(Gram-negative bacteria) identified by Iranian research organization for sciences of technology (IROST)[5] . Bacteria, molds and yeasts that require organic carbon to grow are called heterotrophic. Most bacteria, including many of the bacteria associated with drinking water systems, are heterotrophic. Concentrations of heterotrophic bacteria are determined using a variety of commonly recognized international methods[6].NBH methods use colony formation in culture media to approximate concentrations of heterotrophic organisms in drinking water samples, where bacteria are more common than molds and yeasts. NBH methods do not provide an indication of the specific heterotrophic bacteria present or their sources[7].Heterotrophic bacteria are present in all types of water. In groundwater, concentrations of heterotrophic bacteria are generally low and stable over time. In surface waters and underground water under the direct influence of surface water, their concentrations vary and can be minimized through effective treatment. Treatment of drinking water does not eliminate or inactivate all heterotrophic organisms[8].As a result, these organisms pass through the treatment system to the distribution or plumbing systems. Heterotrophic bacteria can also enter the distribution system through open treated water tanks, during pipe repairs, or due to back-ups of pipeline projects or the addition of new piping systems[8,9] .The National Primary Drinking Water Regulations regulations established by the Environmental Protection Agency of the United States (US EPA) states that the concentration of heterotrophic bacteria is not necessarily an indicator of health effects, but a low concentration of bacteria Heterotrophs in drinking water is associated with good maintenance of the treatment system and distribution network. This regulation establishes that treatment techniques for surface and ground water subject to the influence of surface waters should be used to limit the concentration of heterotrophic bacteria in drinking water to less than 500 $CFU.mL^{-1}$, Measured using the standard method on agar incubated at 35 °C for 48 h[10] .This standard is not based on health considerations, but reflects the fact that at concentrations greater than 500 $CFU.mL^{-1}$, heterotrophic bacteria can interfere with certain methods of recovery of total coliforms and *E. coli* [11,12] .The Drinking Water Inspectorate of England and Wales, based on the Directive of the EU Council on the quality of water intended for human consumption[13], has not established limit Quantitative analysis of the concentrations of heterotrophic bacteria in drinking water, but stated that no abnormal changes in these concentrations should be observed in tap water, treatment plants or service tanks[14]. Measuring the heterotrophic plate count (H.P.C) is an analytical method that can be a useful operational tool for monitoring general bacteriological water quality through the treatment the process and in the distribution system. Each drinking water system will have a baseline range of H.P.C bacteria levels depending on the site-specific characteristics[15].

Image of *Semenovia suffruticosa* plant in Taftan Mountain (April month)

Materials and Methods

Plant material

The aerial parts of *Semenovia suffruticosa* (which is endemic to Baluchestan- Iran) were collected during the flowering stage from the heights of Taftan Mountain in Baluchestan (South eastern of Iran) in June 2010. Plant identification was carried out by Dr. Mozaffarian [16] Botanist in the Research Institute of Forests and Rangelands (Tehran-Iran).

Preparation of sample

The aerial parts were freeze-dried in the shade at the ambient temperature and stored in double-layer paper bags at the room temperature, protected from the direct light, until further analysis[17,18]. They were then sieved to particles with 0.5 mm sizes. All reagents used were of the analytical grade with the highest purity available.

Essential oil isolation

The essential oil was extracted by mixing e g.50 ±0.01g of plant powder with 400±0.1mL in 2 L- balloon of distilled water at 95 ^{0}C temperature for 2.5 h using a Clevenger-type apparatus based on the recirculation of water according to the method recommended in the European Pharmacopoeia[19] .The oil dried over anhydrous sodium sulfate .After filtration, the solvent was removed by distillation under reduced pressure in a rotary evaporator at 30 ^{0}C and the pure oil kept at 4 ^{0}C in the dark until the moment of analysis. After determining the optimum conditions such as water volume, kind of balloon, time of the process of essential oil tracting proper amount of the plant sample[20].

GC-MS Analysis

The analysis of the essential oil was performed using a Hewlett-Packard 6890 Network GC System, equipped with a 60m* 0.25mm id, 0.25µm an HP-5Ms capillary column, and a HP 5973 mass selective detector. Helium was the carrier gas at 1 ml.min^{-1}, the temperature was at 250 ^{0}C and 260 ^{0}C respectively. The column temperature was set at 40 ^{0}C for 1 min, then programmed from 40 ^{0}C to 250 ^{0}C at a rate of 3 ^{0}C.min^{-1}, and finally, held isothermally for 20 min for GC-MS detection an Electron Ionization

System was used with ionization energy of 70 eV.Retention indices were calculated by using the injector and MS transfer line retention times of C_8-C_{26} n- alkanes that were injected with the oil at the same chromatographic conditions according to Van Den Dool method[21].The individual constituents were identified by their identical retention indices, referring to known compounds from the literature and also by comparing their mass spectra with either at the compounds or with the Wiley7 mass spectral database[22].

Antimicrobial activity

The antibacterial, anti-yeast, and antifungal activities were observed by mean of Ager Dilution method within a concentration range of 0.5 -64 ($\mu g.mL^{-1}$). The Minimum Inhibitory Concentration (MIC) of the aerial parts oil was determined for four different bacteria.

Ager Dilution method

Firstly, the mentioned micro-organisms were cultured on culture environment of Muller- Hinton (for bacteria) and sabred Dextrose Ager for fungi in order to obtain a fresh culture to fresh or to prepare) after 24 h at 37 ^{0}C for bacteria and 48h at and sabred Dextrose Ager for fungi in order to obtain a fresh culture to fresh or to prepare after 24 h at 37 ^{0}C for bacteria and 48 h 5 ^{0}C for fungi. The different concentrations given of essential oil were prepared in Ager-having culture environment as a form of two fold 1/2,1/2 ,so that the dilutions were prepared from the concentration of 256 $\mu g.mL^{-1}$ (256,128,64,32,16,8,4,2,1,1/2) according to standard of NCCLS(or CLSI) bacterium should be added 10^4 $CFU.mL^{-1}$. To achieve this at the first a suspension of half Mc. Farland was provided from bacteria, and it was diluted 10 times and then from each bacterium, 5 µg was taken. Thereafter, they were put on the water plots of the Ager culture environment and the different concentration of essential oils. To examine growth and not-growth of micro-organisms, all the plots were kept at certain the temperature. After 24 h for bacteria and 48 h for fungi, the results were analyzed. All experiments were repeated for at least 3 times[23].

Heterotrophic plat count

The Heterotrophic plat count (H.P.C), represents an indication of aerobic and anaerobic bacteria that derive their energy and carbon from organic compounds. The number of these bacteria depends on the composite of culture environment, ancubation period (1-7 d) and the temperature of incubation (20-35) ^{0}C. This group consists of *pseudomonas aeruginosa, Stahylococcus aureus, Escherichia coli, Aspergillus niger* microorganisms. H.P.C bacteria in drinking water is variable and mostly under the influence of the temperature, remained chlorine existence and the concentration of absorbable organic materials. The amount of H.P.C should not exceed 5oo organisms/ml. The H.P.C is a useful parameter to evaluate water quality in distribution systems and water filtering house[24].Utilization of antimicrobial effects (H.P.C technique) of this essential oil was tested for samples of city-sewage (water and waste water). The effective essential oil concentration for its anti-microbial property has been tested in the H.P.C test with 0.1, 0.3, 0.5, and 0.7, 1.1(mL) concentration for *Semenovia suffruticosa* oil (figure 1). All experiments were repeated for at least 3 times.

Statistical analysis

The measurements was done in triplicates to test the reproducibility of them. All results are presented as mean ±S.E. Statistical analyses were performed by student's t-test. The values of $P<0.05$ were considered statistically significant.

Results and discussion

The essential oil of *Semenovia suffruticosa* was extracted by hydro distillation method. The oil yield(w/w%) was 0.77 based on dried weight of sample. The chemical composition of the oil was investigated using the GC-MS technique. Sixty compounds amounting

to about 84.99 % of the oil were identified, are: the major components found in the oil:cis-ocimene (19.31%) ,linalool (9.00 %),cinnamy valerate (8.19%), α-terpinolene (5.49%),6-amyl-α-pyrone (4.55%),isobutyl-isovalerate(3.71%),γ-terpinene(3.68%),α-bisabolol (2.93%) and unknown compounds (1.70%)(Table 1). The percentage of total monoterpenes and total sesquiterpene in the essential oil are 53.50and 7.92 respectively.

Table(1)

Chemical composition of the essential oil of *Semenovia suffruticosa*

Compound	RI	%
α-pinene	906	0.17
sabinene	946	0.39
1,8-Cineole	948	0.72
3-octanone	956	0.09
β-myrcene	960	0.46
isobutyl isobutyrate	977	3.71
δ-3-carene	983	0.17
α-terpinene	989	0.05
m-methylanisole	992	0.09
o-cymene	998	1.12
limonene	1002	0.24
cis-ocimene	1014	19.31
n-butyl isovalerate	1017	0.28
β-ocimene Y	1020	0.98
γ-terpinene	1035	3.68
p-cresol	1055	1.76
α-terpinolene	1065	5.49
linalool	1078	9.00
amyl isovalerate	1081	2.77
allocimene	1098	0.21
amyl valerate	1116	0.45
pulegone	1117	0.23
ethyldimethylthiophene	1146	4.64
1,8-metnthadien-4-ol	1153	0.17
4-terpineol	1155	0.09
p-cymen-8-ol	1160	0.27
α-terpineol	1166	0.11
trans-2,6-dimethyl-3,5,7-octatriene-2-ol	1169	0.14
p-allylanisole	1171	0.17
cis-2,6-dimethyl-3,5,7-octatriene-2-ol	1177	0.15

Compound	RI	%
cis-3-hexyl valerate	1201	0.63
hexyl isovalerate	1207	1.77
trans-3-hexyl valerate	1208	0.11
cis-4-decen-1-ol	1222	0.06
trans-4-decen-1-ol	1232	0.05
4-hydroxy-3-methylacetophenone	1250	0.34
eugenol	1318	0.09
α-copaene	1340	0.41
pentanoic acid,phenylmethyl ester	1350	0.85
methyleugenol	1358	2.19
Trans-caryophyllene	1374	0.31
unknown	1383	0.85
unknown	1412	0.22
trans-beta-farnesene	1419	0.28
unknown	1423	0.28
6-amyl -α -pyrone	1438	4.55
Granyl-isovalerate	1441	1.28
germacrene D	1458	0.25
zingberene	1462	0.12
lavanduilyl acetate	1470	0.20
bicyclogermacrene	1473	0.86
δ-cadinene	1494	0.79
cis- α-bisabolene	1506	0.47
germacrene B	1535	0.18
geranyl butyrate	1541	0.14
spathulenol	1555	0.14
geranyl isovalerate	1566	0.14
m-methylstyrene	1573	0.27
cinnamyl isovalerate	1589	0.27
valencene	1599	0.17
unknown	1621	0.35
cinnamyl valerate	1653	8.19
α-bisabolol	1658	2.93
angecin	1750	0.07
ficusin	1803	0.03
1-hxadecene	1827	0.05

Table (2)

Chemical composition of the essential oil of *Semenovia suffruticosa* by chemical class

Chemical class	Number of compounds	Percent of chemical class
Total monoterpenes	27	53.50
Hydrocarbon monoterpenes	12	32.37
Oxygenated monoterpenes	15	21.13
Total sesquiterpenes	13	7.92
Hydrocarbon sesquiterpenes	10	6.36
Oxygenated sesquiterpenes	3	1.56
Other hydrocarbon compounds	2	0.32
Other oxygenated compounds	17	18.61
Other compounds	1	4.62
Unknown compounds	4	1.70
Hydrocarbon terpenoids	22	38.73
Oxygenated terpenoids	18	22.69
Total terpenoids	40	61.42
Total without unknown compounds	60	84.99

Table (3)

The presented of Minimal inhibition Concentration of the essential oil from aerial parts of *Semenovia suffruticosa* for microorganisms

Microorganis	**Bacterial**				**yeast**	**Fungus**
Essential oil	*Salmonella typhi*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*	*Aspergillus niger*
Aerial parts	32 $\mu g.mL^{-1}$	32 $\mu g.mL^{-1}$	32 $\mu g.mL^{-1}$	8 $\mu g.mL^{-1}$	8 $\mu g.mL^{-1}$	32 $\mu g.mL^{-1}$

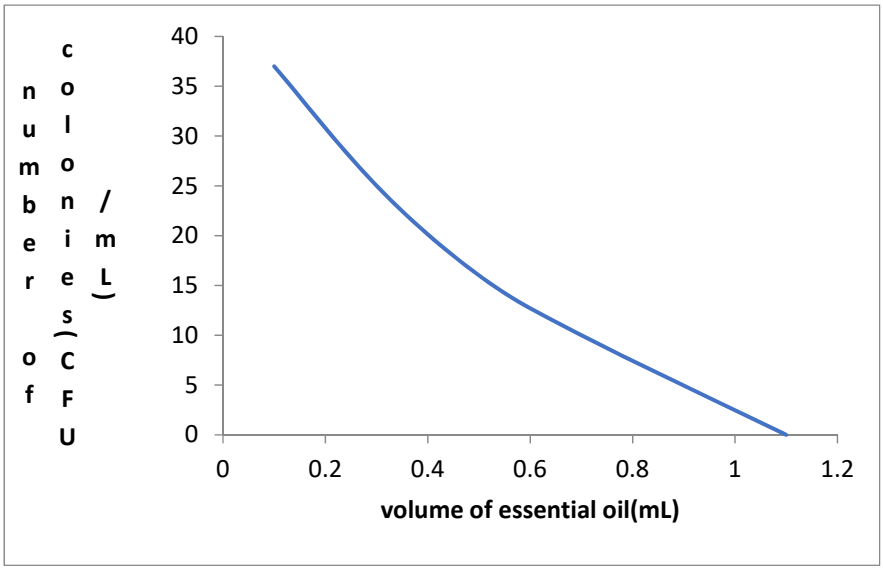

Figure (1): The variation of number of colonies as rate a function the variation of volume of the essential oil of *Semenovia suffruticosa*

The results of this examination are completely adapted with results of GC-MS technique of essential oil that confirms the existence of 22.69 % oxygenated terpeniods.In the essential oil of plant, this is oxygen-containing terpenoid compounds, in remarkable rate.So *Semenovia suffruticosa* essential oil has a good percent of total terpenoids (Table 2). Antimicrobial effects of this essential oil were exactly estimated and examined in laboratory.It,s sensitiveness(Minimal Inhition Concentration) to mentioned-micro-organisms in the following: *staphylococcus aureus*(8 $\mu g.mL^{-1}$), *Pseudomonas aeruginosa* (32 $\mu g.mL^{-1}$), *Salmonella typhi*(32 $\mu g.mL^{-1}$), *Escherichia coli* (32 $\mu g.mL^{-1}$), *Candida albicans*(8 $\mu g.mL^{-1}$), *Aspergillus niger*(32 $\mu g.mL^{-1}$), shows that the highest was recognized by comparing with standard the samples in the way of dilution, and minimum controlling the concentration was calculated *Staphylococcus aureus* and *Candida albicans* (Table 3).

The following microorganisms:*Staphylococcus aureus*(gram-positive bacteria), *Pseudomonas aeruginosa* ,*Salmonella typhi* and *Escherichia coli* (gram-negative bacteria) , *Candida albicans* (anti-yeast) , *Aspergillus niger* (antifungal), shows that the highest dilution of capable of the control on micro-organism has been *pseudomonas aeruginosa*. Comparison of the results showed that the antimicrobial feature of the oil is much greater oil due to its more combinations of oxygenated terpenoids, for instance linalool, 4- terpinol and alpha-terpineol**.**

Antimicrobial effects of this essential oil on water pollution were tested according to figure 1.In water polluted, H.P.C levels (number of colonies) are generally high. In water polluted under the direct the influence of different volumes of addition essential oil H.P.C bacteria concentration can be variable but are minimized through effective disinfection[15, 24].

Conclusion

In this study was to determine the essential oil antimicrobial effect was used of Agar dilution method.For the purposes of this standard six-microbial strains (a Gram-positive, four bacteria Gram in the negative,and two fungus strain) were used, use standard strains with the same identification code genetic microorganisms were identified to ensure that guarantee a reproducibility of results and possibility to compare the results of this

research is with the results of other researchers. So Gram-positive bacteria than Gram-negative bacteria and fungi, *Candida*. *albicans* is more sensitive to oil than the oil is more sensitive fungus *Aspergillus niger*. Lipid outer membrane of bacteria a Gram positive has pores that are called purines. This high-water conduit by multiple membrane proteins created and just let the free dissemination of hydrophilic molecules into the oil. So cannot easily hydrophobic properties pass of Gram-negative bacterial cell membrane. Anti-microbial experiments suggest that the essential oil of this plant can be used in filtration of water and waste water. Heterotrophic plat count bacterial growth in drinking-water shows this property[25].

List of Abbreviations

GC-MS: Gas chromatography/Mass spectroscopy technique **^{0}C:** Degrees a Celsius **mL:** Mili liter **min:** Minute **w/w%:** Weight / Weight percent **CFU.mL^{-1}**: Colony Forming Unit for liquids **Chemical class:** classification of compounds **MIC:** Minimal inhibition Concentration.

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التركيبات الكيميائية لزيت *semenoyasofroticosa* العطري وآثارها المضادة للميكروبات في الماء

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ملخص

وأفادت هذه الدراسة عن مركبات كيميائية ونشاط مضاد للبكتيريا للنفط الأساسي في براعم مصنع سيمينوياسوفروتيكا، الأصلي في إيران، وقد استُخرجت الزيوت الأساسية من البراعم بواسطة طريقة تقطير المياه وتم التحقيق فيها بواسطة الكروماتوغرافيا الغازية وتقنيات الكروماتوغرافيا الغازية المقترنة بالتقنيات الطيفية الكتلية. أظهرت النتائج أن المركبات الرئيسية التي بها أعلى نسب هي cis-ocimene 19.31 ٪، لينا لول 9٪، سينناميل فاليرات 8.19٪، إلفا تيربينولين 5.49٪، 6-إميل ألفا بيرون 4.55٪، وكانت النسبة لمركبات غير معروفة 0.85٪. كما تبين أن أعلى تركيز من النفط الأساسي على المبيضات ألبيكان وأدنى تركيز من النفط العطري قادرة على السيطرة على الكائنات الحية الدقيقة التالية: Staphylococcus aureus (8 µg/ml), Pseudomonas aeruginosa (32 µg/ml), Salmonella typhi (32 µg/ml), Escherichia coli (32 µg/ml), Candida albicans (8 µg/ml), Aspergillus niger(32 µg/ml) وزيادة الزيوت الأساسية بين العينات أدى إلى انخفاض في عدد مستعمرات الكائنات الحية الدقيقة.

الكلمات الدالة: Semenoyasofroticosa - مركبات النفط الأساسية – طريقة التخفيف أغار – عدد تقنية هيتروفويك .

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