

Original Research Article

Chemical composition and antimicrobial of essential oil from *Artemisia lehmanniana*

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Abstract

The plants of *A. lehmanniana* were gathered in May 2009 from the heights of 2300 m of Taftan Mountain located in Baluchestan. Its essential oil was extracted with an outcome of 1.34%w/w, using hydro distillation method. Then, using GC/MS technique, it was analyzed and 54 components were recognized that constituted 96.61% of the whole essential oil. The components that had the highest percentage were as Follow: 1, 8- Cineole (22.06%), (z)-2-Nonenal (12.11%), Nonaconsane (8.97%), -Terpineol (5.90%), 4-Terpineol (5.07%), Camphor (4.19%) and unknown compound (4.41%). Antimicrobial effects of this essential oil were exactly estimated and examined in Laboratory. Its sensitiveness (Minimal inhibition Concentration) to mentioned-micro-organisms in the following: *Staphylococcus aureus* (4µg/ml), *Salmonella typhi* (32µg/ml), *Escherichia coli* (32µg/ml), *Candida albicans* (8µg/ml), *Aspergillus Niger* (4 µg/ml), showed that the highest was recognized by comparing with standard samples with the way of dilution and minimum controlling concentration was calculated. As the results revealed, the micro-organism of *Pseudomonas aeruginosa* could be controlled in higher concentrations.

Keywords: *Artemisia Lehmanniana*, Essential oil, chemical composition, 1, 8- Cineole, antimicrobial, *Staphylococcus aureus*

Introduction

Infectious diseases are the most important prevalent (epidemic) diseases in the world that impose of financial burden to human communities. Artificial antibiotics played a significant role in treatment of infectious diseases the manifestation of microbial resistance against antibiotics resulted in inclination to use medicinal plants more than ever [1]. In Food industries peoples negative tendency to consume the Foods chemical preservatives used, has caused them to use plant resources as antimicrobial in addition to using them as taster [2]. The use of extracts and essential oils of plants as alternatives for artificial preservative materials has found its, place in Food industries properly. There for, in order to achieve natural antimicrobial substances researchers have paid their attention on refinement of essential oils and plant extracts [3]. In this field of research the results show that many plants of the families of umbelliferae, chicories and so on possess antimicrobial effects [4]. A new strategy in to confront drug resistance including finding bio-materials with antimicrobial properties as well as increasing interest in using natural drugs and medicinal plants [5].

Materials and Methods

Sampling

The Aerial parts species were collected from the heights of Taftan area and Tamamdan valley May 2009. All security consideration was followed during their transfer to the university of Sistan and Baluchestan. The sample of barium was sent to research Institute of forests and Rangelands in order to identify its scientific term [6].

Preparation of sample

The Collected were dried by sun light 10% moisture and in a humidity- free environment .Then the dried samples were ground into powder with a grinder.

Extraction of Essential oil

50 g of powder of prepared sample was put in a 2L-ballon with 300 mL water, and then the mixture was distilled twice. A Clevenger-type, apparatus extracted Essential oil in periods of 3.5 h. Using Hydro-distillation method, the obtained essential oil was collected in n-Hexane-solvent. Finally, the purified essential was maintained in a sample container in the temperature of 4 °C to become analyzed by GC/MS technique.

Gas chromatography / mass spectrometry (GC/MS) Analysis



The analysis of the essential oils was performed using a Hewlett-Packard 6890 Net work GC System, equipped with a HP-5MS capillary column, (60m* 0.25mm id, 0.25µm) and a HP 5973 mass selective detector in the electron impact mode. Helium was the carrier gas at 1 ml/min. injector and MS transfer line temperature were at 250 and 260 °C respectively. Column temperature was set at 40°C for 1 min, then programmed from 40°C to 250°C at a rate of 3°C/min, 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 retention times of C₈-C₂₆ that were injected after the oil at the same chromatographic conditions according to Van Den Dool method [7]. The linear retention indices for all the compounds were determined by injection of the sample with a solution containing a homologous series of C₈-C₂₆ n-alkenes. The individual constituents were identified by their identical retention indices, referring to known compounds from the literature [8] and also by comparing their mass spectra with either the known compounds or with the Wiley7 mass spectral database.

Method of Ager Dilution original

First, the mentioned micro-organisms were cultured on culture environment of Moeller Hinton (for bacteria) and sobered dextrose ager for fungi in order to obtain a fresh culture to fresh or to prepare) after 24 hours at 37 °C and 48 hours at 25 °C different concentrations of given essential oils were prepared in ager-having culture environment as a form of two Fold 1/2,1/2, so that the

dilutions were prepared from concentration of 256 mg/L (256,128,64,32,16,8,4,2,1,1/2). according to standard of NCCLS (or CLSI) bacterium should be added 10⁴ CFU/mL to achieve this. At first a suspension of half Mc. Farland was provided from bacteria, then it was diluted 10 times and then from each bacterium, 5 µL was taken. After, they were put on the water plots of Ager culture environment and different concentration of essential oils. To examine growth and not-growth of micro-organisms all the plots were kept at certain temperature after 24 hours for bacteria and 48 hours for fungi the results were analyzed [9,10].

Results and Discussion

The chemical composition of the oil was investigated using GC/MS technique. 54 compounds (comprising about 96.61% of the oil were identified, for essential oil of *A. Lehmanniana* collected in May 2009,. According to Table1, the components having the percentage were including: 1, 8- Cineole (22.06%), (z)-2-Nonenal(12.11%),Nonaconsane (8.97%), -Terpineol (5.90%), 4-Terpineol (5.07%), Camphor 4.19%),Geranyl acetate (3.45%),Borneol(3.11%),Chrysanthenone(2.73%) and unknown compound(4. 41%). According to Table 2, 57.65 % of them consisted of oxygenated Terpenoids. Antimicrobial effects of this essential oil were examined according to the Agar. Dilution on *staphylococcus aureus* (4µg/ml), *Salmonella typhi* (32µg/ml), *Escherichia coli*, (32µg/ml) *Candida- albicans*(8 µg/ml) *Aspergillus nige*(4 µg/ml).

Table1. Chemical composition of essential oil from *Artemisia Lehmanniana*

Name of Compound	RI	%	Name of Compound	RI	%
Cyclohexane	610	0.58	p-menth-2-en-1-ol	1095	0.45
Methyl isopropyl ketone	619	0.21	Chrysanthenone	1098	2.73
Heptane	646	0.13	Camphor	1121	4.19
Methylcyclohexane	681	0.06	Borneol	1142	3.11
3-Methyl-2-butenal	746	0.09	4-Terpineol	1152	5.07
2,5-Dimethyl-3-ethylfuran	813	0.05	-Terpineol	1165	5.90
Tricyclene	894	0.04	Car-3-en-2-one	1206	0.82
-Thujene	898	0.15	Carvone	1215	0.29
-Pinene	906	0.38	Chavicol	1221	0.80
Camphene	922	0.66	Lavandulyl acetate	1251	1.29
Sabinene	945	0.29	Bornyl acetate	1254	0.63
β-Pinene	950	0.21	Eugenol	1319	1.01
β-Myrcene	959	0.11	Geranyl acetate	1336	3.45
Unknown	962	0.71	Methyl Cinnamate	1343	0.52
Isoamyl butyrate	980	0.28	Methyleugenol	1357	2.43
Delta-3-Carene	982	0.10	Cis-Jasmone	1359	1.97
2-Methylbutyl		0.13			0.50
iso butyrate	984		Trans-Caryophyllene	1382	



-Terpinene	988	0.76	Homoadamantane	1435	1.57
m-Cymene	997	0.40	(z)-2-nonenal	1510	12.11
1,8-Cineole	1007	22.06	Unknown	1524	1.60
1,6-Dioxaspiro [4.4]nonane, 2,2,9-trimethyl	1026	0.18	Phenyl ethyl tiglate	1550	0.72
Gamma-Terpinene	1030	1.38	Spathulenol	1554	0.38
Cis-Sabinene hydrate	1040	1.17	Caryophyllene oxide	1561	0.46
-Terpinolene	1059	0.38	Methyl jasmonate	1612	1.58
4,7,7-Trimethylbicyclo [3.3.0]octan-2-one	1064	2.10	-Bisabolol	1647	0.49
Linalool	1069	0.89	unknown	1678	2.10
β -Terpineol	1071	1.05	Nonacosane	1874	8.97
Filfolone	1076	0.33			

Table 2. Chemical composition of the Essential oil from *A. Lehmanniana* by chemical class

Name of chemical class	Percent of chemical class of essential oil from Aerial parts
Monoterpene Hydrocarbons	4.86
Oxygenated - Monoterpenes	56.32
Sesquiterpene hydrocarbons	0.50
Oxygenated Sesquiterpenes	1.33
Other Hydrocarbons	9.10
Other Oxygenated	22.26
Other. compounds	1.57
unknown	4.41
T.H	5.36
O.T	57.65
T.T	62.66
T	100.02

Table 3. Presentation of minimal inhibition concentration for microorganisms

micro-organism	<i>Staphylococcus aureus</i>	<i>Salmonella typhi</i>	<i>Escherichia coli</i>	<i>Candida albicans</i>	<i>Aspergillus niger</i>
MIC(μ g/ml)	4	32	32	8	4

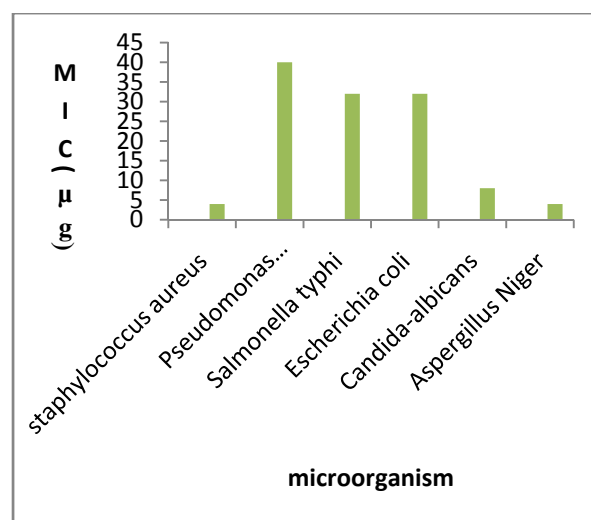


Figure 1. The variation of minimal inhibition concentration for microorganisms

Conclusion

The essential oil of the plant *A. Lehmanniana* was extracted with a yield of 1.34% w/w. The essential oil was analyzed using GC/MS technique. The oxygenated Terpenoids compounds had 57.65 percent of the whole essential oil according to Table 2 and Fig.1. Microorganisms of *Salmonella typhi* and *Escherichia coli* in the lowest dilution and microorganisms of *Staphylococcus aureus* and *Aspergillus niger* in the highest dilution were bound by essential oil. This proper antimicrobial feature of essential oil was in full adaptation with the Oxygenated Terpenoids.

Acknowledgements

The author is grateful to Dr. Sardashti and Dr. Fazeli from university of Tehran for cooperation in this work

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