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Phytochemical and Biological Studies of Some Myrtus *(Myrtus communis L.)* Populations of South West Region of Zagros (Iran)

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Abstract

Myrtus communis L. is one member of the Myrtaceae family. It is one of the important medicinal plants in the Zagros region of Iran. The Essential oils include a large percentage of compounds called terpenes. In this study, the chemical composition of essential oils of Myrtus (*Myrtus communis L.*) was identified by the GC-MS method. Results showed that essential oils of populations from different regions have a series of similar compounds, but different in quality and quantity. In fact, they have several organic compounds such as hydrocarbons, alcohols, ketones, aldehydes, ethers, esters, oxides and other volatile oil and so on. Percentage of essential oils in different populations were variable. Essential oil of "Basht" population has 35 compounds such as 1,8-cineole, linalool, α-pinene and lynalyl acetate. Essential oils of "Dehno-Rostam" population has 30 compounds include 1, 8 cineole, linalool, α terpineol, α-pinene. Essential oils of "Sarab-Bahram" population has 19 compounds include α-pinene, 1,8-cineole, linalool, and α terpineol. Essential oils of "Keveshk" population has 29 compounds such as 1,8-cineole, α-pinene, linalool, and α-terpineol and Essential oils of "Tang-Tamoradi" population has 25 compounds include Eugenol, δ-3-carene, 1,8-cineole, and α-terpineol. Aqueous extracts of those populations were evaluated, and the results showed that they have antibacterial properties against *Escherichia coli, Bacillus subtilis* and *Pseudomonas aeruginosa*, but have not any antifungal activity (*Aspergillus oryzae*).

Keywords: *Myrtus communis L.;* Zagros; Essential oil; Antimicrobial; Antifungal

Introduction

Myrtus communis L. (Myrtaceae) is an evergreen shrub, typical of the Mediterranean regions, which grows spontaneously in many countries, the scientific name for this *Myrtus communis L.* is taken from its Persian name. It is traditionally used as an antiseptic, antimicrobial, disinfectant drug and hypoglycaemic agent [1]. Different parts of this plant find various widely uses in the food industry, such as for flavouring meat and sauces, and in the cosmetic industry and so on [2]. Common myrtle belongs to the Myrtaceae family, which include approximately 145 genera and over 5500 species [3]. The genus Myrtus includes the flowering plant with near 16 species reported in areas of the Middle East and Asia [4]. *M. communis L.* known in fact myrtle is one of the main aromatic and medicinal species of this family. It is an evergreen sclerophyll shrub or small tree, 1.8-2.5 m height, with a small bush and deeply fissured grove [5]. True Myrtle is characterized by its branches, which form a close full head, gross covered with ovate or lanceolate evergreen leaves. Their leaves are 3-5 cm long and include tannins, flavonoids and volatile oils [6]. This part is a so aromatic plant since of the high essential oil content in its leaf, flower and fruit glands. It has solitary auxiliaries white or rosy flowers followed by black a several-seeded berry, which is globular in shape with dark red to violet in color [7]. There are two major fruits according to whether the color dark or white. The dark color is more current, but there are as well cultivated white-colored types, which they have yielded much bigger fruit than their wild counterparts [8]. Myrtle grows abundantly from the northwestern to the eastern Mediterranean, including bordering countries and western Asia, also Aegean regions [9]. Myrtles native to Southern Europe, North Africa and western Asia. It is also distributed in Southern America, Northwestern Himalaya and Australia. Myrtle is cultivated in gardens, especially in the Northwest Indian region, because of its odor flowers [10]. Myrtle has been used since ancient times as a spice, also for medicinal, antiseptic, food preparation and spice purposes. Myrtle as a condiment finds no wide application because of its bitterness, despite the pleasant odor. The taste is very intense, quite nasty and strongly bitter, so its culinary application is limited to the region of origin, such as Italy [11,12]. Its berries and leaves are usually used for the industrial formulation of sweet liquors with advertising digestive properties [13,14]. Its leaves are very fragrant and have been extensively employed in the perfume and cosmetic industries, particularly in Portugal [15] and Turkey [6]. It is traditionally used as an antiseptic, disinfectant and hypoglycemic agent [1]. Generally, in folk medicine, a decoction of leaves and fruits is used orally for the treatment of stomach aches, hypoglycaemia, disbiosis, cough, constipation, poor appetite, as well as also externally for wound healing [16]. Essential oils are aromatic and volatile compounds found only in 11% of the plant kingdom [17]. Essential oils and their components can be very hopeful biological agents, to their relative safety, wide acceptation by consumers and exploitation of potential multi-purpose use [18]. They are stored in plants in special fragile secretory structures, such as glands, secretory hairs, secretory ducts, secretory pit or resin ducts [19-22]. The total essential oil content of plants is generally so low and scarcely more than 1% of biomass [23]. For example, the essential oil yields in leaf stalk and flower of *M. communis* var. Italical were respectively 0.61%, 0.08% and 0.30% (w/w) [24]. Essential oils are hydrophobic and thus only slightly solvable in water. They are soluble in alcohol, non-polar or weakly polar solvents, beeswax, and oils. Most essential oils are colorless or light yellow, liquid and have a lower density than water

[25]. Essential oils are complex mixtures containing many diverse compounds. Chemically they are derived from terpenes and their oxygenated compounds [26]. The chemical composition of the myrtle essential oil has been explained by many authors [2,27-29]. Compounds that have been found in myrtle oils include E-2-hexenal, Z-3-hexenol, hexanol, tricyclene, α-thujene, α-pinene, sabinene, β-pinene, myrcene, δ-3-carene, α-terpinene, p-cymene, limonene, 1,8-cineole, E-β-ocimene, E-oxide, terpinolene, linalool, terpinene-4-ol, borneol, p-cymene-8-ol, α-terpineol, myrtenol, nerol, cis-carveol, geraniol, linalylacetate, bornyl acetate, eugenol, myrtenyl acetate, α-terpinyl acetate, geranyl acetate, neryl acetate, methyl eugenol, β-caryophyllene, α-humulene, allo-aromadendrene, germacrene-D, caryophyllene oxide and camphene.

Materials and Methods

Plant material and essential oil

In June with the use of plant distribution maps for *Myrtus communis L.* five regions for sampling was selected. The different geographical characteristics of these regions were measured. Latitude, longitude and altitude to the global coordinate system (GPS) were measured (Table 1). Then samples (leaf and twigs) were dried away from the sun for ten days. Dried leaves transferred to the Phyto chemistry Laboratory and extraction essential oils by Clevenger for 3 hours. Finally, essential oils after dehydration kept in the dark glass bottle in the refrigerator (in 4°C). Percentage of essential oils in this studied population were shown in Table 2.

Row	Name of Location	Altitude (m)	Latitude	Longitude
1	Basht	930	302029	511142
2	Dehno Rostam	810	301854	512158
3	Sar Ab Bahram	1037	300229	513330
4	Keveshk	1712	302650	512916
5	Tang Tamoradi	1623	303048	512509

Table 1: Geographical characteristics of studied regions.

Name of Location	Percentage of essential oils
Basht	2.2
Dehno-Rostam	2.1
Sarab-Bahram	1.6
Keveshk	2.0
Tang-Tamoradi	0.8

Table 2: Percentage of essential oils in studied populations.

Preparation of the extracts

The aqueous extract was obtained as follows: 80 ml of water added in 10 g of plant material in a round-bottomed flask, then put it on the electric mixer for 24 h. Then it was a smooth operation and filtered by Whatman paper No. 42.

Preparation of the culture medium

Based on the directions on the container culture media, culture media powder is dissolved in distilled water and heater stirred until the boil, Then the flask door closed with cotton for 20 min at 121°C inside the autoclave to be sterilized. After leaving autoclave poured about 15 ml of culture medium in each plate after cooling to keep in the refrigerator. Broth as the liquid culture medium is not necessary to pour plates can be directly stored in the fridge after sterilization and cooling.

Analysis of the essential oils

To determine the composition of the essential oils of the leaves was used gas chromatography and gas chromatography (Agilent, Model N 6890) connected mass spectrometry (GC-MS). HPS column (30 meter), its diameter was 250 micrometers and thickness of stationary phase 0.25 m. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. Diluted samples (1/100 in n-Hexan, v/v) of 1 µl were injected manually in split mode (split ratio 1/30). Oven temperature was programmed from 50 to 220°C at a rate of 5°C/min and from 220°C to 280°C at a rate 20°C/min and held at for 2 min. Oil constituents were identified by MS data obtained from Wiley (http://eu.wiley. com/wileycad/wileyTitle/productCd.htm) and NIST (http://www.nist.gov/srd/nist1a.htm) libraries.

Biological activity

In-vitro biological activity of the extracts against two Gram negative bacteria such as *Escherichia coli* (ATCC 25922) and *Pseudomonas aeroginosa* (ATCC 9027) and Gram-positive bacteria including *S. Bacillus subtilis* (ATCC: 6633) and also a fungal strain such as *A. oryzae*, were carried out using the disk diffusion method [30,31]. For this mean, the Petri dishes were prepared as follows: i) a layer of culture medium (Muller Hinton Agar (Merck, Germany) for bacterial strains and Sabouraud dextrose agar (SDA) for fungal strains was poured on the surface of plates, ii) 100 µl of spore suspension of bacteria and/or fungi including nearly 0.5 × 106 colony-forming units (CFU/ml) was inoculated on the surface of culture medium, iii) The prepared discs (that had been soaked in the various concentrations of Extracts; 12.5, 25 and 50 mg/ml in physiological saline) were placed at different positions on a surface of plates. Then, the plates were incubated at 37 °C for 24 h for bacterial strains and 27 °C for three days for *Aspergillus oryzae* [32,33]. Antimicrobial activities of extracts were evaluated based on the diameter of the zone of inhibition (mm) and listed in Table 8. Amoxicillin, Erythromycin, Fluoxamine and Cephalexin were used as reference bactericidal drugs (positive controls) Table 9.

Results and Discussion

The geographical characteristics of the study regions (latitude, longitude and altitude) were shown in Table 1. Essential oil extraction by Clevenger and dehydration by Na_2SO_4 then compounds in essential oils were identified by chromatography connected to mass spectrometry (GC-MS). Chemical compounds were identified in different populations was shown in Tables 3-7. In the essential oil of Basht population was identified 35 compounds and 1,8-cineole, linalool, α-pinene and lynalyl acetate have the highest percent (Table 3).

Row	Compounds name	Retention time (min)	Percent

1	Isobutyl isobutyrate	3.855	1.48
2	α–Pinene	4.288	7.98
3	Camphene	5.171	4.12
4	δ-3-carene	5.347	2.73
5	P-cymene	5.586	3.56
6	Unknown	5.864	1.30
7	1,8–cineol	6.355	12.89
8	Ocimene	7.241	0.78
9	γ-terpinene	7.330	0.53
10	Linalool oxide	7.510	0.25
11	α–terpinolene	7.825	0.33
12	Linalool	8.214	12.10
13	Allo ocimene	8.933	0.30
14	Trans-pinocarveol	9.305	0.39
15	Cis-verbinol	9.378	0.45
16	Unknown	9.585	0.44
17	Borneol	9.962	0.11
18	4-terpineol	10.213	0.70
19	A-terpineol	10.618	4.43
20	Unknown	10.699	1.52
21	Myrtenal	10.866	6.24
22	Trans-geraniol	11.568	0.68
23	Citronellol	11.745	0.75
24	Linalyl acetate	12.381	7.73
25	Bornyl acetate	13.088	0.24
26	Myrtenyl acetate	13.834	0.05
27	α-terpenyl acetate	14.909	6.15
28	Neryl acetate	15.073	0.95
29	Granyl acetate	15.218	0.17
30	Terpinyl propionate	15.743	4.50
31	Methyl eugenol	16.380	2.55
32	Trans-Caryophellene	16.864	2.08
33	α-Humulene	17.669	1.90
34	Caryo phellene oxide	20.952	2.15
35	Humulene oxide (II)	21.570	1.12

Table 3: Compounds in essential oils of Basht population.

The dehno-Rostam population has 30 compounds and 1,8-cineole, linalool, α terpineol, α-pinene have the highest percent (Table 4). In Sarab-Bahram population was identified 19 compounds and α-pinene, 1,8-cineole, linalool, and α terpineol have the highest percent (Table 5). Keveshk population has 29 compounds and 1,8-cineole, α-pinene, linalool, and α-terpineol have the highest percent (Table 6). Tang-Tamoradi population has 25 compounds and Eugenol, δ-3-carene, 1,8-cineole, and α-terpineol have the highest percent (Table 7). The antibacterial activities of the extracts were listed in Table 8 as the diameter of inhibition zone from the growth (in mm). The results show that extracts have the good antibacterial activity against all studied bacteria strains. Antibacterial activities of Basht, Dehno-Rostam, Sarab-Bahram and Keveshk populations was similar and have most effect on *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*, respectively. Antibacterial activity Tangeh-tamoradi population has different and has the greatest effect on the *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*, respectively. Antibacterial activity of Amoxicillin, Erythromycin, Fluoxamine and Cephalexin were (positive controls) shown in Table 9. The Aqueous extracts of those populations have not any antifungal activity against *Aspergillus oryzae*.

Row	Compounds name	Retention time (min)	Percent
1	Isobutyl isobutyrate	3.770	2.4
2	Unknown	4.031	1.00
3	α-Pinene	4.233	10.32
4	β-Pinene	4.861	3.72
5	δ-3-careen	4.984	4.20
6	Unknown	5.206	2.70
7	T-β-Ocimene	5.315	2.82
8	P-cymene	5.561	6.80
9	1,8-cineol	6.315	16.02
10	cis-β-Ocimene	6.962	2.40
11	γ-terpinene	7.114	2.32
12	α-terpinolene	7.710	1.25
13	Linalool	8.161	14.54
14	Allo ocimene	8.826	0.62
15	Trans-pinocarveol	9.174	0.60
16	Undecane,5-methyl	9.305	0.72
17	Iso Borneol	9.768	0.56
18	4-terpineol	10.068	0.73
19	α-terpineol	10.744	11.10
20	trans-(+)-carveol	11.160	0.40
21	Trans-geraniol	11.470	1.11
22	Linalyl acetate	12.160	7.60
23	α-citral	12.496	0.16
24	Myrtenalacetate	13.824	1.11
25	Methyl granate	13.948	0.48

26	Neryl acetate	14.970	1.70
27	Methyl eugenol	16.294	0.08
28	Trans-Caryophellene	16.634	0.80
29	α-Humulene	17.481	0.91
30	Caryo phellene oxide	20.782	0.83

Table 4: Compounds in essential oils of Dehno-Rostam population.

Row	Compounds name	Retention time (min)	Percent
1	α-Pinene	4.515	33.9
2	P-cymene	5.581	9.22
3	1,8-cineol	6.415	22.1
4	α-terpinolene	7.623	1.3
5	Linalool	7.966	11.92
6	Undecane,5-methyl	8.559	1.23
7	Iso Borneol	9.630	1.21
8	4-terpineol	9.927	1.2
9	α-terpineol	10.324	3.92
10	Nerol	11.184	1.05
11	Linalyl acetate	11.930	2.82
12	Trans anethole	12.779	1.03
13	Methyl granat	13.707	0.92
14	Myrtenel acetate	13.835	1.05
15	Methyl eugenol	15.879	1.9
16	Trans-Caryophellene	16.497	1.2
17	α-Humulene	17.375	1.12
18	Caryo phellene oxide	20.726	1.46
19	Humulene oxide (II)	21.30	1.45

Table 5: Compounds in essential oils Sarab-Bahram population.

Row	Compounds name	Retention time (min)	Percent
1	Iso butyl iso butyrate	3.912	2.34
2	α-Pinene	4.365	13.2
3	β-Pinene	4.869	6.32
4	pseudo limonene	5.079	6.28
5	δ-3-careen	5.279	2.6
6	Unknown	6.019	2.61
7	1,8–cineol	6.393	15.21
8	β-Ocimene	6.748	3.12
9	γ-terpinene	6.986	2.72
10	α-terpinolene	7.647	1.2
11	Linalool	8.088	1.8
12	Allo ocimene	8.645	1.42
13	Unknown	8.919	1.02
14	Trans-pinocarveol	9.025	1.14
15	Undecane,5-methyl	9.203	1.31
16	4-terpineol	9.950	0.7
17	α-terpineol	10.404	12.23
18	Linalyl acetate	12.079	9.94
19	Trans anethole	12.811	1.0
20	Myrtenal acetate	13.850	1.23
21	Neryl acetate	14.818	1.31
22	Unknown	14.934	0.88
23	Granyl acetate	15.407	2.9
24	Unknown	15.830	2.8
25	Methyl eugenol	15.953	0.91
26	Trans-Caryophellene	16.550	0.42
27	α-Humulene	17.413	1.4
28	Caryo phellene oxide	20.701	1.04
29	Humulene oxide (II)	21.343	0.95

Table 6: Compounds in essential oils of Keveshk population.

Row	Compounds name	Retention time (min)	Percent
1	α-Pinene	4.240	3.92
2	β-Pinene	4.370	4.54
3	δ-3-careen	4.856	10.96
4	t-β-ocimene	5.459	2.37
5	Unknown	5.606	5.53
6	Unknown	6.228	2.15
7	1,8-cineol	6.441	10.32
8	α-terpinolene	7.506	1.22
9	Linalool	8.039	5.56
10	Allo ocimene	8.804	0.95
11	Camphor	9.353	3.54
12	Iso Borneol	9.960	4.51
13	α-terpineol	10.724	8.25
14	Unknown	11.152	4.43

15	Linalyl acetate	12.224	7.47
16	Bornyl acetate	12.883	0.76
17	Myrtenyl acetate	13.989	0.62
18	Eugenol	15.610	11.5
19	Granyl acetate	16.28	2.04
20	Methyl eugenol	16.430	2.93
21	Trans-Caryophellene	16.866	2.82
22	α-Humulene	17.626	0.48
23	Caryo phellene oxide	20.094	1.05
24	Unknown	20.899	2
25	Unknown	24.437	0.08

Table 7: Compounds in essential oils of Tang-Tamoradi population.

Name of Location	Gram negative						Gram positive		
	Escherichia Coli			*Pseudomons aeruginosa*			*Bacillus subtilis*		
	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)	50 (mg/mL)	25 (mg/mL)	12.5 (mg/mL)
Basht	13.72	10.10	7.66	18.76	17.53	14.53	11.48	9.77	8.46
Dehno-Rostam	15.14	12.48	9.80	17.88	14.64	11.88	12.85	12.41	9.30
Sarab-Bahram	12.99	9.65	6.88	14.06	11.53	8.38	12.45	11.51	9.00
Keveshk	13.21	9.57	6.00	15.20	11.20	9.66	13.04	9.03	6.83
Tang-Tamoradi	10.80	9.73	6.00	15.49	13.07	9.28	12.21	10.66	9.03

Table 8: Diameter of zone of inhibition (mm) of extracts in studied populations.

Antibiotics	*Escherichia Coli*	*Pseudomonas aeruginosa*	*Bacillus subtilis*
Amoxicillin (30 µg)	24.00	22.63	18.15
Erythromycin (15 µg)	20.33	21.27	27.44
Fluoxamine (15 µg)	33.33	23.11	38.37
Cephalexin (25 µg)	19.1	15.00	35.67

Table 9: Diameter of the zone of inhibition (mm) of Antibiotics.

Conclusion

This paper deals with the Environmental variation of the essential oil *M. communis L.* grown in South West region of Zagros. It should be noted that plants from this location are examined for the first time. The monoterpene fraction is the main chemical group of the essential oil in all populations. Essential oils of different populations have a series of compounds are similar and different in terms of quality and quantity. The essential oils are made of a complex mix of chemical compounds that those are different in terms of chemical compounds. In fact, there are a variety of organic compounds such as hydrocarbons, alcohols, ketones, aldehydes, ethers, esters, oxides and other volatile oil and so on. Percentage of essential oil in different regions is different. The results show that extracts have the good antibacterial activity against all studied bacteria strains. Antibacterial activities of Basht, Dehno-Rostam, Sarab-Bahram and Keveshk populations were similar and have the most effect on *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus subtilis*, respectively. The antibacterial activity of the Tangeh-Tamoradi population was different and has the greatest effect on the *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli*, respectively. The Aqueous extracts of those populations have not any antifungal activity against *Aspergillus oryzae*. This study shows that the combination of some Myrtus (*Myrtus communis*) populations in a different area can vary in terms of quality and quantity that these differences could be due to genetic or non-genetic factors. The result shows the aqueous extracts of *Myrtus communis L.* populations weren't a good alternative for antibiotics. The present results exhort additional and more in-depth studies on the phenolic, fat acid, glucoside and terpenoids composition of the plant extracts and assessment of the antibacterial activity of each compound separately also other biological properties.

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