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ESSENTIAL OILS OF *MALVA SYLVESTRIS* L. AND ITS ANTI-MICROBIAL ACTIVITY

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ABSTRACT

The essential oil obtained by hydro-distillation of the aerial parts of *Malva sylvestris* L. were analyzed by GC and GC/Ms. The oil was characterized by higher amounts of 1, 8-cineol (18.27%), α-terpinyl acetate (14.8%), α-cadinol (11.5%) which was among the twenty-three components comprising 95.5% of the total oil detected. The Ether extract of the same plant (aerial parts of *Malva sylvestris*) was also analyzed by GC and GC/MS. The oil from the Ether extract was characterized by higher amounts of 1, 8-cineol (23.4%), α-terpinyl acetate (20.0%), α-cadinol (11.7%) and γ-cadinene (9.0%) were among the 18 constituents representing 89.8% of the total oil detected. The aim of present study was to identification of naturally occurring non-polar compounds by two methods (Water-distilled hydrodistillation and Ether extraction) from the aerial parts of *Malva sylvestris* L. which has been collected in Khalkhal, west- Azarbayjan, Iran, 2014. The anti-bacterial activations of the aerial parts by hydrodistillation and ether extraction of *Malva sylvestris* using MIC method. The growth inhibitory zone (mm) was also measured.

KEYWORD: *Malva sylvestris* (Malvaceae), Essential oils composition, Anti-bacterial activity.

INTRODUCTION

Malva sylvestris is a species of the mallow genus *Malva* in the family of Malvaceae and is considered to be the type species for the genus. Known as common mallow to English speaking Europeans,[1] it acquired the common names of cheeses, high mallow and tall mallow (mauve des bois by the French)[2] as it migrated from its native home in Western Europe, North Africa and Asia through the English speaking world.[3]

M. sylvestris is a vigorously healthy plant with showy flowers of bright mauve-purple, with dark veins; a handsome plant, often standing one meter high and growing freely in fields, hedgerows and in fallow fields.[4]

Renewed interest in traditional pharmacopoeias has meant that researchers are concerned not only with determining the scientific rationale for the plant's usage, but also with the discovery of novel compounds of pharmaceutical value. Instead of relying on trial and error, as in random screening procedures, traditional knowledge helps scientists to target plants that may be medicinally useful.[5]

The plant flowers are used as a remedy for out wound, eczema, dermal infected wounds, bronchitis, digestive problem and inflammation.[6]

The anti-bacterial activity of the oils from *Malva sylvestris* were determined by measuring the growth inhibitory zone against their Gram-positive and four Gram-negative bacteria. The Gram-positive bacteria included *Bacillus subtilus* (ATCC 6633), *Staphylococcus aurous* (ATCC 25923), *Staphylococcus epidermidis* (KCTC 1917) and Gram-negative bacteria included *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella paratyphi B* (KCTC 12025).

EXPERIMENTAL

Plant material

The aerial parts of *Malva sylvestris* L. were collected in Khalkhal, west- Azarbayjan, Iran, at flowering stage. Then, the plants were dried in out of sunlight at room temperature. Voucher specimens were deposited of the herbarium of the Research Institute of Forest and Langelands (TARI), Tehran, Iran.

Oil Isolation

The air-dried aerial parts of *Malva sylvestris* (88g) were subjected to hydrodistillation using Clevenger-type

apparatus for 3h. After decanting and drying of the oil over anhydrous sodium sulfate, the corresponding oil was isolated in yield of 0.5% (w/w).

In addition the air-dried plant material (85g) was extracted with ether at room temperature for 12 hours. After filtration and evaporation, the result was subjected for the analysis.

Analysis

The oils were analyzed by GC and GC/MS. The GC analysis was performed on a Shimadzu GC-15A equipped with a split/splitless injector (250ºc) and a flame ionization detector (250ºc). N_2 was used a carrier gas (1ml/min). The capillary column used was DB-5 (50 m × 0.2 mm, film thickness 0.32 µm). The initial column temperature was 60ºc (held 3 min), then heated to 220ºc with a 5ºc/min rate and kept constant at 220ºc for 5 min.

Quantitation date was obtained from GC (FID) area percentages without the use of correction factors. GC/MS analysis was carried out using a Hewlett-Packard 5973 mass selective detector (MSD) coupled with a Hewlett-Packard 6890 gas chromatograph equipped with an HP-5 MS column (30 m × 0.25 mm, film thickness 0.25 µm). Temperature was programmed an above with He as the carrier gas. Mass spectra were achieved at 70 eV.

RESULTS AND DISCUSSION

Identification of the constituents was based on comparison of their mass spectra and retention indices with these obtained from authentic samples [7] and Wiley library spectra. The quantification of the components was performed on the basis of their GC peak area, which were identified via FID using a Shimadzu C-R4A chromate pac.

Twenty-three components were identified in the oil obtained by hydrodistilled water which representing about 88.5% of the oil. This oil contained about 49.9% of monoterpenes with 1, 8-cineol (18.4%), α-terpinyl acetate (14.8%) and sabinene (2.43%) as the main monoterpenes and about 38.5% sesquiterpenes with α-cadinol (11.5%), γ-cadinene (8.7%) and epi-α-murolol (8.4%) as the main components identified in the oils can be seen in Table 1 and 2.

Table 1: Chemical composition (%) of the essential oil from water distillation of *Malva sylvestris* L.

No.	Compound	R.T	KI	Percentage
1	α-Pinene	9.94	939	0.93
2	Sabinene	10.75	975	2.32
3	β-Pinene	10.88	979	0.95
4	O-Cymene	11.81	1026	0.69
5	1,8-Cineol	12.06	1031	18.14
6	γ-Terpinene	12.66	1059	0.43
7	Linalool	13.48	1096	1.94
8	α-Terpineol	15.47	1188	1.33
9	(E)-Anethole	17.23	1284	0.91
10	Thymol	17.70	1290	1.19
11	Carvacrol	17.86	1299	1.72
12	α-Terpinyl acetate	18.70	1349	14.87
13	Methyl eugenol	19.33	1403	3.5
14	Geranyl acetone	20.42	1455	0.99
15	Germacrene- D	21.39	1485	0.74
16	Selinene	21.66	1490	3.22
17	δ-Cadinene	21.91	1513	1.14
18	γ-Cadinene	22.05	1523	8.71
19	(cis)- Cadin-4-en-ol	22.31	1636	1.31
20	Spathulenol	22.96	1577	2.54
21	Caryophllene oxide	23.06	1582	0.98
22	epi-α-Muurolol	23.98	1642	8.45
23	α-Cadinol	24.19	1654	11.5
Total				88.5

Table: 2 Chemical composition (%) of the ether extraction of *Malva sylvestris* L.

No.	Compound	R.T	KI	Percentage
1	Sabinene	10.75	975	2.55
2	β-Pinene	10.88	979	0.83
3	O-Cymene	11.81	1026	0.65
4	1,8-Cineol	12.06	1031	23.41
5	γ-Terpinene	12.66	1059	0.25

6	Linalool	13.48	1096	1.99
7	α-Terpineol	15.47	1188	1.14
8	(E)-Anethole	17.23	1284	0.54
9	α-Terpinyl acetate	18.70	1349	19.97
10	Methyl eugenol	19.33	1403	3.57
11	Geranyl acetone	20.42	1455	0.97
12	Germacrene- D	21.39	1485	0.49
13	β-Selinene	21.66	1490	2.91
14	δ-Cadinene	21.91	1513	0.83
15	γ-Cadinene	22.05	1523	9.05
16	Caryophllene oxide	23.06	1582	0.42
17	epi-α-Muurolol	23.98	1642	8.48
18	α-Cadinol	24.19	1654	11.76
Total				89.81

Antibacterial assay

The anti-bacterial activity of the oils from *Malva sylvestris* were determined by measuring the growth inhibitory zone against their Gram-positive and four Gram-negative bacteria. The Gram-positive bacteria included *Bacillus subtilus* (ATCC 6633), *Staphylococcus aurous* (ATCC 25923), *Staphylococcus epidermidis* (KCTC 1917) and Gram-negative bacteria included *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella paratyphi B* (KCTC 12025).

The bacteria were obtained from the Research Center of Science and Industry, Tehran, Iran. Then micro-organisms (obtained from enrichment culture of the micro-organisms in 1ml of Mueller-Hinton broth, Merck, Germany medium.

After drilling 50ml of solutions with effective concentration in DMSO (Dimethyl Sulfoxide, Merck, Germany) were poured in each disk. After incubation of 37ºc during 24h. The inhabitation zone diameter was measured (Table 3).

Table 3: Antibacterial activity of the hydrodistilled water and ether extraction oils of the aerial parts of *Malva sylvestris* L.

Bacteria	Gram +/-	Hydrodistilled MIC	Water IZ	Ether MIC	Extraction IZ
Staphylococcus aurous (ATCC 25923)	+	250	21	300	18
Bacillus subtilus (ATCC 6633)	+	128	19	380	12
Staphylococcus epidermidis (KCTC 1917)	+	35.5	24	32.5	24
Enterococcus faecalis (ATCC 29212)	-	500	17	340	18
Escherichia coli (ATCC 25922)	-	500	18	500	17
Pseudomonas aeruginosa (ATCC 27853)	-	250	21	55	7
Salmonella paratyphi B. (KCTC 12025)	-	62.5	22	61	20

IZ: Inhibition zone (mm)
MIC: Minimum Inhibitory concentration (1g/ml)

Gram (+) zone sensitive ≥ 21mm
Gram (-) zone sensitive ≥ 20mm

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