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Chemical Composition of the Essential Oils for *Anthemis melampodina* from North Saudi Arabia

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ABSTRACT

The chemical composition of essential oil of *Anthemsmela mpodina*is determined by GC/MS. The oil was obtained by hydro-distillation and SPME extraction methodsIn the SPME method, a total of 41 constituents were identified monoterpene hydrocarbons (88.89%) were the main class of compounds detected in the SPME method with β-pinene (35.29%), trans-ocimene(23.96%) and terpinolene(15.78%) being detected as the main constituents. On the otherhand, hydro-distilled oil was rich in oxygenated sesquiterpene(31.22%).

Key words: *Anthems melampodina*, Hydro-distillation, SPME, GC/MSmonoterpene hydrocarbons, oxygenated sesquiterpenes.

INTRODUCTION

Anthemis(belonging to the Astraceae family)is a genus of 210species offlowering plants. *Anthemis* plants are known to growwidely in Europe, south western Asia, northern and north eastern Africa and southern Arabia.The arabic names for this plant include Qahwiyan,Rebyan and Arbiyar[1]. In Saudi Arabia, the genus *Anthemis* is represented by 17 species distributed in the central, eastern and northern regions[2]. examples include *A. pseudocotula, A. bornmuelleri,A. cotula. A. odontostephena* and *A. melampodina*[3]. In folk medicine *Anthemis s*pecies are frequently used for the treatment of various ailments such as digestive problems, insomnia and toothache. Moreover, several studies also showed that different *Anthemis* species exhibited antiflammatory, antioxidant, antibacterial, antiproliferative and antispasmodic properties[4-8]. Most *Anthemis*species are economically important, due to their use in pharmaceutics, cosmetics and food flavoring[9-13]. Furthermore, chemical investigations on *Anthemis*species revealed that the genus is dominated by sesquiterpene lactones, flavonoids and polyacetylenes[14-20].*Anethimsmelampodina* is anannual, greyish-tomentose, herb with ascending branches from near the base. The flowers are white-yellow in color.This plant is known to grow widely in northern region ofSaudi Arabia including Hail[21].

In a continuation of an extensive work aimed at the investigation of the volatile constituents of aromatic plants from Saudi Arabia, the current investigation was designed to investigate the chemical composition of the essential oil *A. melampodina* obtained by two different extraction methods, the Solid Phase Micro-Extraction and hydro-distillation methods and compare the current findings with those obtained from other locations of the world.

EXPERIMENTAL

Plant Material

Aerial parts of *A. melampodina*were collected during the full flowering stageMarch 2012) from Hart Alrha - south Tabuk. The identity of the plant species was confirmed by Dr Jacob Thomas from the Herbarium Division, College of Science, King Saud University, Riyadh, KSA. A voucher specimenAnthps-PNU-013was kept in the Chemistry Department, College of Science, Princess NouraBint Abdel Rhman University, Riyadh, Saudi. The plant material was dried at room temperature until constant weight was obtained.

Hydro-distillation of plant material

Air dried flowering parts (150 g) were coarsely powdered and then hydro-distilled using a Clevenger apparatus for 3 h. The extraction was repeated twice and the obtained oils were pooled separately, dried over anhydrous sodium sulfate (Na_2SO_4) and stored at 4ºC in amber glass vials until analysis.

Solid Phase Micro Extraction of the volatile oils (SPME)

The Solid Phase Micro Extraction experiments were performed using SPME fiber assembly (Polydimethylsilocane/Divenylbenzene, PDMS/DVB; d_f 65 mm partially crossed-linked phase, fiber length 1 cm) and assemblies for manual sampling (Supelco, Bellefonte, PA, USA). Before measurements, the fiber was conditioned according to the producer's recommendations. About 0.1 mg of freshly powdered flowerswas introduced into 4.0 mL amber glass vials, tightly capped with PTFE-coated septa, and SPME extraction was performed for 2.0 min at room temperature. Desorption of the analytes was carried out at 240ºC for 60 s. Each sample was repeated twice.

GC-MS and GC-FID analysis

About 1 µl aliquot of each oil sample, diluted to 5 µl in GC grade *n*-hexane, was subjected to GC/MS analysis. The GC/MS analysis was performed using Varian Chrompack CP-3800 GC/MS/MS-200 (Saturn, Netherlands) equipped with DP-5 (5% diphenyl, 95% dimethyl polysiloxane) GC capillary column (30 m × 0.25 mm i.d., 0.25 µm film thicknesses), with helium as a carrier gas (flow rate 0.9 mL/min). The actual temperature in MS source was 180ºC and the ionization voltage was 70 eV. The column temperature was kept at 60ºC for 1 min (isothermal), and programmed to 246ºC at a rate of 3ºC/min, and kept constant at 246ºC for 3 minutes (isothermal). A hydrocarbon mixture of *n*-alkanes (C_8-C_{20}) was analyzed separately by GC/MS under the same chromatographic conditions using the same DP-5 column.

For the quantitative analysis (% area), a Hewlett-Packard HP-8590 gas chromatograph equipped with a split-splitless injector (split ratio 1:50) and an FID detector was used. The column was an optima-5 (5% diphenyl, 95% dimethyl polysiloxan) fused silica capillary column (30 m × 0.25 mm, 0.25 film thickness). The temperature of the oven was increased at a rate of 10ºC/min from 60ºC to 250ºC and then held constant at 250ºC for 5 min. Thetemperatures of the injector and detector were maintained at 250ºC and 300ºC, respectively. The relative peak areas of the oil components were measured and then used to calculate the concentration of the detected compounds. Each sample was analyzed twice.

Identification of the components

The components of the essential oils obtained from the SPME and hydro-distilled flowering parts of the plant were identified using the built in libraries (Nist Co and Wiley Co, USA) and by comparing their calculated retention indices relative to (C_8-C_{20}) *n*-alkanes literature values measured with columns of identical polarity (Adams, 2001), or with authentic samples. The compounds, *á*- and *â*-pinenes, *p*-cymene, limonene, linalool (Fluka, Buchs, Switzerland) and sabinene hydrate (Sigma-Aldrich, Buchs, Switzerland) were used as reference substances in GC/MS analysis. GC-grade

hexane and analytical reagent grade anhydrous Na_2SO_4 were purchased from Scharlau (Barcelona, Spain) and UCB (Bruxelles, Belgium).

RESULTS

The GC/MS analysis of the volatile potentials and hydrodistilled oil obtained from flowering parts of *A. melampodina*led to the characterization of total of 76 different components(Table 1).In the SPME method, 47 components amounting to 98.25 % of the total oil content were identified. Monoterpene hydrocarbons (88.89%)were the main contributors to the SPME volatiles and were dominated by b-pinene (35.29%), *trans*-ocinene (23.29%), terpinolene (15.7%), g-terpinene (6.84%) and *cis*-ocimene (5.23%). Oxygenated monoterpenesaccounted for 4.95% of the total oil content with tetrahydrolav and uledetected as a major components (0.94%). Sesquiterpene hydrocarbons were detected at much lower concentrations (2.51%) and the main contributors included b-sesquiphellandrene (1.23%) andguaiene (0.58%). In addition, oxygenated sesquiterpenes and aromatics compounds where detected in very low concentrations as compared to other classes (0.76% &0.74%., respectively).

GC/MS analysis of the hydro-distilled oil obtained from the flowering aerial parts of *A. melampodina* resulted in the identification of a total of 45 components which amounted to 97.86 % of the total oil content (Table 1). Careful analysis of the GC/MS spectrum revealed marked qualitative and quantitative differences in the chemical composition between the SPME and the hydro-distilled essential oil (Figures 1&2). The hydro-distilled oil was dominated by oxygenated sesquiterpenes that accounted for 31.22 % of the total oil content. This fraction was dominated by intermedeol (11.69%), methyl-2-epilasmona (4.19%), Z-a-santalol acetate (2.11%) and eudesmo-7(11)-en-4-o (2.11%). Monoterpene hydrocarbon accounted for (29.97%). This fraction was represented bya-pinene (15.33%) followed by myrecen (5.70%) and limonene (3.18). Oxygenated monoterpenesamounted to 20.72%andborneol (7.31%) was detected as the main compound of this fraction. The hydro-distelledoil contained sesquiterpene hydrocarbons(7.28%) with b-selinene(2.06%) ,trans-b-farnesene(1.60%),trans-caryophyllene(1.59%), germacrene D(1.26%) being detected as the main contributors to this fraction. Diterpene hydrocarbon amounted to 6.39% of the total oil content and were represented bypimaradiene(3.63%),dolabradiene (2.02%).The hydro-distilled oil contained also aliphatic hydrocarbons and their derivative (1.26 %) and aromatic compounds (1.01%). The findings of the current investigation clearly indicated that the chemical composition of *A.melampodina* varies with the extraction method employed[22].

Previous studies on the essential oil of *A. melampodina*collected in Egypt(1989) revealed to the isolation ofSesquiterpene lactones ,Sterols, flavonoid[22]. Again in Egypt (2002) The essential oil of the *A. melampodina* was characterized by the presence of a high percentage of monoterpene hydrocarbons (49.94%) while asesquiterpene hydrocarbons and oxygenated sesquiterpenes

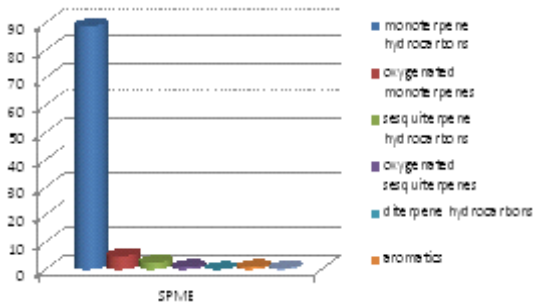

Fig. 1: Variation in the % composition of the essential oil of *A. melampodina*from Saudi origin obtained by SPME method.

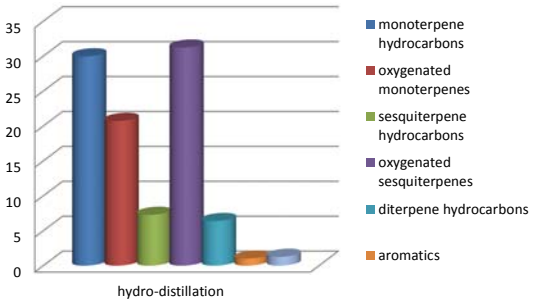

Fig. 2: Variation in the % composition of the essential oil of *A. melampodina*from Saudi origin obtained by hydro-distillation extraction method

reported only the yield (0.03%) 7.41% and 11.43% of the oil while .In addition the essential oil *of A. Melampodina* was showed moderate larvacidal activity (LC(50) 139.42 ppm against culexpipens[23]. Hence, the objective of the present study was undertaken to identify the chemical composition oil of *Anthemismelampodina.* plant collected from Tabouk.

Table 1:

No	Lit RI	exp RI	Compound	% SPME composition	% Distillation composition
1	915	926	amyl acetate	0.35	-
2	939	935	α-pinene	-	15.33
3	954	953	camphene	-	0.33
4	975	975	sabinene	-	0.86
5	979	981/987	β-pinene	35.29	2.15
6	991	990	myrecene	-	5.70
7	1003	1010	α-phellandrene	-	2.09
8	1009	1015	hexyl acetate	0.03	-
9	1025	1027	p-cymene	-	0.56
10	1029	1032/1027	limonene	1.78	3.18
11	1037	1032?	cis-ocimene	5.23	-
12	1031	1035	1,8-cineol	-	6.87
13	1050	1045	trans-ocimene	23.96	-
14	1060	1062	γ-terpinene	6.84	-
15	1070	1074	cis-sabinene hydrate	-	0.33
16	1082	1082	p-tolualdehyde	0.64	-
17	1089	1088	terpinolene	15.78	-
18		1094	α-campholenal	-	0.32
19	1121	1105?	endo-fenchol	0.15	-
20	1103	1105	isoamylisovalerate	-	0.48
21	1121	1118	sabina ketone	0.15	-
22	1134	1131	1-terpineol	0.05	-
23	1144	1145	cis-β-terpineol	0.08	-
24	1145	1151	trans-verbenol	0.10	0.76
25	1162	1162	tetrahydrolavandule	0.94	-
26	1170	1168	pinocampheol	0.30	-
27	1175	1173	isopinocamphone	0.22	-
28	1169	1177	borneol	0.10	7.31
29	1180	1182	isopinocampheol	-	1.46
30	1189	1190	α-terpineol	0.16	-
31	1196	1200	myrtenol	0.10	0.50
32	1229	1227	Z-ocimenone	0.07	-
33	1230	1232	nerol	-	2.35
34	1245	1237	2Z-hexenyl isovalerate	-	0.79
35	1239	1238	isoborneolformate	0.42	-
36	1249	1242	perilla ketone	0.22	-
37	1253	1251	pipertinoe	0.09	-
38	1264	1259	2E-deceneal	0.03	-

39	1272	1284/1286	perilla aldehyde	0.16	0.34
40	1298	1296	geranylformate	1.65	-
41	1354	1350	2-phenylethyl propanoate	0.11	-
42	1393	1399	Z-jasmone	-	0.81
43	1419	1423	trans-caryophyllene	-	1.59
44	1457	1455	trans-b-farnesene	-	1.60
45	1432	1447	β-copene	0.06	-
46	1457	1454	α- patchoulene	0.07	-
47	1460	1461	all-aromadendrene	0.14	-
48	1485	1485	germacrene D	-	1.26
49	1493	1493	guaiene	0.58	-
50	1490	1492	β-selinene	-	2.06
51	1500	1500	bicyclogermacrene	-	0.44
52	1514	1517	γ-cadinene	-	0.33
53	1523	1527	β-sesquiphellandrene	1.23	-
54	1531	1531	trans-g-bisabolene	0.04	-
55	1536	1535	sliphiperfol-5-en-3-ol B	0.07	-
56	1550	1554	elemol	-	1.05
57	1561	1558	germacrene D	0.31	-
58	1569	1566	longipinanol	0.42	-
59	1585	1573	globulol	0.22	-
60	1567	1578	3Z-hexenyl benzoate	-	0.45
61	1578	1582	spathulenol	-	2.51
62	1583	1587	caryophyllene oxide	-	0.86
63	1601	1591	guaiol	0.06	-
64		1596	carotol	0.06	-
65	1637	1634	gossonorol	-	1.11
66	1632	1638	γ-eudesmol	-	1.79
67	1640	1648	tau-cadinol	-	3.00
68	1667	1662	intermedeol	-	11.69
69	1679	1681	methyl-z-epijasmonate	-	4.19
70	1685	1689	5-neocedranol	-	0.58
71	1700	1692	eudesm-7(11)-en-4-ol	-	1.03
72	1779	1175	Z-α-santalol acetate	-	2.11
73	1807	1818	nootkatone	-	1.30
74	1906	1909	isopimara-9(11),15-diene	-	0.74
75	1950	1951	pimaradiene	-	3.63
76	1696	1958	dolabradiene	-	2.02
	monoterpene hydrocarbons			88.89	29.97
	oxygenated monoterpenes			4.94	20.72
	sesquiterpene hydrocarbons			2.51	7.28
	oxygenated sesquiterpenes			0.76	31.22
	diterpene hydrocarbons			0.00	6.39
	aromatics			0.74	1.01
	aliphatic hydrocarbons and their derivatives			0.40	1.267
	total			98.25	97.86

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