

## STUDY OF THE COUMARIN AND VOLATILE OIL COMPOSITION FROM AERIAL PARTS OF *ACHILLEA MILLEFOLIUM* L.

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Aerial parts of *Achillea millefolium* L. (Fam. Asteraceae) were exhaustively extracted with ethanol (90%) and then the extract was fractionated into coumarin and unsaponifiable fractions. The coumarin fraction led to the isolation of scopoletin I, umbelliferone II and aesculetin III, whereas b-amyryn, a mixture of steroidal components were identified using GLC. Besides, the volatile oils from the leaves and stems of *A. millefolium* were separately analyzed using GC/MS chromatographic analysis.

**Key words:** Asteraceae, *Achillea millefolium* L., Coumarins, Sterols, Volatile oil.

### Introduction

*Achillea millefolium* L. is one of the commonest weed of the Asteraceae family, used in folk medicine for rheumatic pains, gastrointestinal disturbances, fever, diarrhoea, amenorrhoea and as an anthelmintic, antispasmodic, analgesic, haemostatic, (Snajder 1951; Vogel 1970; Lewis and Elvin-Lewis 1977; Noerman 1977), it is used as a drink i.e. tea and as lotions or ointments (Millsbaugh 1974).

The plant contains many constituents: volatile oils, coumarins, sesquiterpenes, sterols, alkaloids, flavonoids, steroidal saponins and fatty acids (Chandler *et al* 1982a & b; Schulz and Albroscheit 1988; Hausen *et al* 1991; Tozyo *et al* 1994; De Pasquale *et al* 1994; Glasl *et al* 1999). From *A. arabica*, scopoletin, umbelliferone and isopimpinellin have been isolated (Khalil *et al* 1981), from *A. erba-rotta* scopoletin (Nano *et al* 1981), from *A. bisserato* scopoletin, scopolin and scoparone have been identified (Davidyants 1982); from *A. krashennikovii*, umbelliferone, scopoletin and isoscooletin have been isolated (Shmatova *et al* 1985).

Reviewing the literature of coumarins (Snajder 1951; Vogel 1970; Lewis and Elvin-Lewis 1977; Noerman 1977), it can be noticed that the coumarin contents of *A. millefolium* have not been investigated before, even if coumarins were detected in some phytochemical screenings.

The volatile oils from leaves and flowers of *A. millefolium* were found to contain 1- $\alpha$ -pinene, d- $\alpha$ -pinene, 1-limonene, 1-borneol, bornyl acetate, 1-camphor, cineol, formic acid, salicylic acid, butyric acid and isovaleric acid (Miller 1916).

Comparative study between oil from flowers and leaves of *A. millefolium* during flowering stage and vegetative phase was carried out by means of GC/MS analysis (Fegueriedo *et al* 1992). Another GC/MS study of *A. millefolium* oil demonstrated the presence of ascrinol, cineol, p-cymene,  $\alpha$ -terpinene and camphor (Chatzapoulou *et al* 1992).

### Experimental

#### A. Apparatus and Equipments

- 1-M.P<sub>s</sub> were uncorrected and performed on Buchi 510.
- 2-U.V. -visible recording spectrometer 240.
- 3-I.R was recorded with Perkin Elmer 580 spectrophotometer.
- 4-MS was determined with Mass spectrophotometer Mat 112.
- 5-GLC chromatography used was Varian 3700 and the conditions of sterols are:

Column; 5%OV-101, 2 m x 0.25 ID  
Detector; Flame ionization detector  
Gase flow rates: N<sub>2</sub> 30 ml min<sup>-1</sup>, H<sub>2</sub> 30 ml min<sup>-1</sup> and air 300 ml min<sup>-1</sup>

Temperature programming: 240 (1 min., 10°C min<sup>-1</sup>), 280° (30 min.)

Injection part: 270°; Detector temperature: 280°  
Chart speed: 0.5 Cm min<sup>-1</sup>.  
Sensitivity: 10<sup>-9</sup> x 32

6-GC/MS chromatography used was varian 3400 under the conditions:

Apparatus: GC/MS finnigrn mat 7000

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Work station:	Digital DEC 3000
Column:	DB-5 (5%-phenyl)methylpoly siloxane (I.D) 0.25
Column length:	30 m
Carrier gas:	He-gas
Flow rate:	Column head pressure 13 Ps, 1 ml min <sup>-1</sup>
Sample size:	1 µl - splitless
Injector temp.:	230°
Ion source temp.:	180°

Programmed temperature at 50° for 5 min then increased to 260° within 42 min and lasting for 5 min.

MS conditions: electron ionization voltage 70 ev, scan range 39-250.

*B. Plant material.* *A. millefolium* L. aerial parts (*viz.* leaves and stems) were collected in November, 1997 (vegetative stage) from the experimental farm, Faculty of Pharmacy, Cairo University and authenticated by Dr. M.El-Gebaly, Chemistry of Natural and Microbial Products Dept., NRC, Cairo, Egypt. A voucher specimen is deposited at the herbarium of the NRC.

*C. Study of the coumarin content.* The dried powder of the aerial parts of *A. millefolium* (2 kg) was exhaustively extracted with ethyl alcohol (90%) and concentrated to 1 litre. The concentrated alcoholic extract was treated with an equal volume of 10% KOH solution at room temperature for 1h, shaking from time to time to open the  $\delta$ -lactone ring and afforded coumarinic salts soluble in water; the alkaline aqueous solution was defatted with ether. The alkaline aqueous layer was acidified with diluted HCl and refluxed on heating mantle for 2h; after cooling, it was extracted with ether. The ether extract was washed with water, dried over anhydrous sodium sulphate and evaporated till dryness to give the coumarin fraction (Abu-Mustafa *et al* 1964). The crude coumarin fraction 12.5 g was subjected to column chromatography using silica gel (375 g, 70-230 mesh ASTM) that was eluted with 0.5% MeOH in benzene to afford a semicrystalline residue which upon crystallization from methanol gave white needles (31 mg), m.p. 204-206°C, ( $R_f$  0.32) and bluish violet fluorescence under U.V. and brown colour upon spraying with I<sub>2</sub>/KI reagent; its spectral data are: UV,  $\lambda_{max}$  350, 300, 250 nm, which are characteristic of 6,7-dioxycoumarin (Murray *et al* 1982). IR, 1720 cm<sup>-1</sup> (C=O), 1625, 1580, 1510 and 970 cm<sup>-1</sup> (aromatic residue), 3415cm<sup>-1</sup> aromatic OH also M<sup>+</sup> at m/e 192. From the above data and by direct comparison of m.p. and mixed m.p., the compound showed to be scopoletin I.

Further elution of the column with 1% MeOH in benzene gave comp. II ( $R_f$  0.25) which was crystallized from MeOH as

needles (25 mg), m.p. 224-26°C; it gave brilliant blue fluorescence under UV and no colour when sprayed with I<sub>2</sub>, UV,  $\lambda_{max}$  205, 215, 242, 252 and 322 nm. IR: OH at 3500cm<sup>-1</sup>,  $\alpha$ -pyrone at 1720 cm<sup>-1</sup> and bands corresponding to the aromatic residue.

The obtained data suggested that the isolated coumarin II might be umbelliferone which was verified by direct comparison (TLC, m.p. and mixed m.p.). Compound III ( $R_f$  0.2), obtained by further elution with 2% MeOH in benzene and crystallized from methanol as colourless needles (20mg), m.p. 268-70°C, gave blue fluorescence under UV and light brown when sprayed with I<sub>2</sub>/KI. Its spectral data are: UV,  $\lambda_{max}$  260 and 350, I.R. it gave bands at 3350 cm<sup>-1</sup> for aromatic OH, 1720 ( $\alpha$ -pyrone C=O) and 1630, 1582, 1515, 965cm<sup>-1</sup> for the aromatic residue. Also, MS gave molecular ion at m/e 178. From the obtained data and by direct comparison (m.p and mixed m.p., TLC), the compound was identified as aesculetin.

The fat fraction was evaporated to dryness and then treated with 5% alcoholic KOH, refluxed for 2 h, and then extracted with ether; the ether layer was dried and evaporated to give 2g brown gum, which was subjected to column chromatography using silica gel. Elution of the column with benzene afforded colourless needles crystallized from methanol, and identified as  $\beta$ -amyrin, m.p and mixed m.p 197-98°. Further elution of the column with 0.5% MeOH gave a mixture of sterols which was analyzed by GLC under the conditions for sterols (McNair and Bonelli 1968). Quantitative analysis was carried out according to normalization method using peak area technique. The results are shown in Table 1.

Table 1

Sterols	wt. %	Retention time
Squalene	5.6	53.25
Cholesterol	14.3	61.83
Campesterol	10	68.61
Stigmasterol	18.95	71.81
$\beta$ -Sitosterol	44.16	75.10

*N.B.* All the measured values are not mean values.

*D. Study of the volatile oil contents.* The essential oil content was determined using 100 g of fresh vegetable material, 200 ml water and 100 ml of glycerin (as antifoaming agent), subjected to a continuous extractive distillation technique for 3 h using modified Likens and Nickerson apparatus (Macleod and Cave 1975), following the methodology reported in Literature (Guenther 1952).

	<i>Achillea</i>	<i>Achillea</i>
Plant organ	leaves	stems
v wt <sup>-1</sup> %	0.9	0.2

**Table 2**  
Results of Gas/ Mass analysis of the volatile oil of *A. millefolium*

Relative Ret.time	Compounds	% in Leaves	% in Stems	M.wt.	Fragmentation	
					B.P	Other fragments
0125	Octane	0.28	0.14	114	43	98,85,71,57
0165	Unidentified	0.34	-	122	43	101,59
0180	Unidentified	0.22	-	126	41	111,98,83,69
0183	3-Hexene-1-ol	0.49	-	130	67	100,82,55,41
0217	Nonane	0.58	-	126		104,97,83,69
0261	Thujene	0.2	-	136	93	121,105,77
0283	$\alpha$ -Pinene	0.4	-	136	93	121,105,77
0360	Sabinene	20.7	0.20	136	93	121,107,77
0368	Octen-3-ol	0.74	-	128	57	109,99,85,69
0401	Hexene 1-ol acetate	1.34	-	142	43	82,67,55
0406	$\alpha$ -Terpinene	0.60	-	136	121	105,93,77,65
0414	Cymene	0.66	-	136	119	103,91,77,65
0421	1,8 Cineol	3.25	-	154	43	139,125,111,81,69,55
0440	Ocimine (cis)	0.20	-	136	93	121,105,79
0448	Ocimine (trans)	0.41	-	136	91	121,105,79
0458	$\gamma$ -Terpinene	1.23	-	136	93	121,105,77
0489	Menthenol (cis)	1.70	0.061	154	71	139,121,111
0500	Nonanone	0.43	-	152	57	113,95,85,72,43
0529	Menthenol (trans)	0.77	0.067	154	43	139,121,111,93,81
0562	Pinene hydrate	0.19	-	154	43	139,121
0577	Camphor	0.28	-	152	95	137,119,108,81,67
0620	Borneol	0.58	0.046	154	95	136,121,110,71,55
0640	Terpineol-4	2.09	0.18	154	71	136,125,111,93,55
0652	$\alpha$ -Terpineol	0.48	0.1	154	59	136,121,107,93,81
0693	Decanal	0.30	-	218	82	103,67,57,41
0806	$\delta$ -Elemene	0.22	0.44	204	121	189,161,147,136
0821	$\alpha$ -Cubebene	5.77	-	204	119	161,133,105,91
0843	Eugenol	0.57	-	164	164	149,131,121,103
0847	Yalangen	0.21	-	204	119	189,161,147,133
0850	Patchoulene	0.89	-	204	122	189,175,161,147
0857	Bourbounene	0.27	-	204	81	133,123,105,91 0861
0861	$\beta$ -Elemene	0.45	-	204	93	189,175,101,147
0884	Jasmone	0.59	-	164	79	149,145,122,107,91
0890	Cedrene	-	1.24	204	119	189,161,133,105
0897	$\beta$ -Caryophyllene	1.79	0.44	204	93	175,161,147,105
0936	Humulene	0.27	1.36	204	93	189,161,147,107
0972	$\beta$ -Gurjunene	2.10	-	204	161	189,161,147,105
0975	$\alpha$ -Cadinene	0.95	-	204	161	147,133,119,105
1000	Germacrene D	7.43	28.5	204	161	147,133,119,105
1004	Germacrene B	6.03	8.44	204	121	189,161,133,105
1007	$\alpha$ -Farnesene	0.20	0.10	204	93	189,161,133,107
1008	$\gamma$ -Cadinene	0.32	-	204	161	189,176,148,133
1011	$\delta$ -Cadinene	2.30	2.66	204	161	189,176,134,119
1054	Nerolidol (cis)	0.50	-	220	69	189,177,149,135

(Cont'd....)

(Table 2 Continued)

Relative Ret. time	Compounds	% in Leaves	% in Stems	M. wt.	Fragmentation	
					B.P	Other fragments
1079	Nerolidol (trans)	3.28	0.88	222	69	220,161,148,136
1090	Germacrene D 4 ol	-	5.04	222	81	161,123,105,93
1091	Elemenone	6.40	-	218	119	175,161,147,106
1102	Cedranone	1.26	-	220	124	205,191,177,163,149,135
1132	Cubenol	0.43	-	222	148	203,179,135
1136	$\gamma$ -Eudesmol	1.37	5.36	222	119	175,149,133,105
1165	$\alpha$ -Eudesmol	8.18	19.71	222	148	204,135,109
1170	$\alpha$ -Cadinol	4.62	4.30	222	95	204,148,137,121
1176	Patchoulol	0.25	-	222	43	205,149,138,125
1194	Isocedranol	3.13	-	222	84	189,161,137,109
1199	Neocedranol	-	10.69	222	43	204,189,161,135
1217	Germacrene	0.42	-	218	93	175,148,136,121
1249	Farnesol (cis, trans)	0.23	6.37	222	43	179,121,82,69
1275	Farnesol (cis, cis)	0.21	0.12	222	43	179,119,82,68
1285	Farnesol (trans, cis)	-	0.12	222	43	179,95,82,68
1331	Hexadecanol	-	0.23	242	95	124,109,95,82,68
1360	Cedran-Diol (8s,14)	-	0.22	238	43	189,123,95,82,68
1369	Cedran-Diol (8s,13)	-	0.23	238	43	220,161,123,68
1420	Phytol	0.08	0.1	296	71	123,109,81,55,43
1425	Dibutyl phthalate	0.09	-	278	149	
1460	Abietriene	-	0.06	270	40	213,185,157,101
1543	Octadecanol	-	0.33	270	41	125,111,97,85,69
1567	Isophytol	0.10	0.17	296	71	123,109,81,55,43
1578	Henicosane	-	0.18	296	57	113,99,85,71,40
1699	Docosane	0.07	0.61	310	40	105,97,85,71,57
1722	Abietol	0.06	-	288	93	203,175,149,135
1753	Tetracosane	-	0.62	388	123	147,112,101,83
1774	Neobietol	0.18	-	288	40	241,187,148,135,93
1778	Hexanedioic acid	-	0.12	370		199,147,112,101,83
1860	Pentacosane	-	0.7	352	57	85,71

Components were identified by their retention times which were compared with those of reference compounds and/or with recently published materials (Adams 1989) (Table 2).

### Results and Discussion

From the coumarin fraction of the aerial parts of *A. millefolium*, three coumarins were isolated for the first time and identified as scopoletin (I), umbelliferone (II) and aesculetin (III) based on direct comparison with authentic samples (TLC, m.p.'s and mixed m.p.'s and by the spectral data). From the unsaponifiable matter, squalene was detected for the first time.

Phytochemical investigation of the volatile oil from leaves and stems of *A. millefolium* was carried out by means of GC/MS

analysis (Table 2). The oil from leaves revealed the presence of 62 components, 60 components were identified representing 97% of the total components, they consisted of 56% sesquiterpenes and 44% monoterpenes. Sabinene (20.7%) was the major constituent; also,  $\alpha$ -eudesmol (8.18%), germacrene D (7.43%), elemenone (6.40%), germacrene B (6.03%),  $\alpha$ -cubenone (5.77%) and  $\alpha$ -cadinol (4.62%) were observed.

In the previous study, the oil from leaves collected during vegetative phase monoterpenes was less than (3%) whereas sesquiterpenes amounted to (92%); major component of the oil was germacrene-D (65%) (Fegueriedo *et al* 1992).

The volatile oil from *A. millefolium* stems revealed the presence of 35 components, all of which were identified. The oil from

stem consists mainly of 91% sesquiterpenes; germacrene D (28.5%),  $\alpha$ -eudesmol (19.71%) and neocedranol (10.69%) were the major constituents. Also, germacrene B (8.44%), germacrene D-4-ol (5.04%),  $\gamma$ -eudesmol (5.36%) and farnesol (cis, trans) (6.37%) were observed.

Hydrocarbon (*viz.* octane, nonane); hydrocarbon alcohols (*viz.* 3-hexane-1-ol, octane-3-ol); terpene alcohols (*viz.* menthenol cis-trans terpinol-4) were detected in *A. millefolium* for the first time but as a minor constituents. *A. millefolium* is rich in sesquiterpene oil; germacrene B was found in two investigated samples.  $\alpha$ -eudesmol,  $\gamma$ -eudesmol were detected in a higher concentration in leaves than stems. Farnesol (cis-trans) was detected in stems higher than in leaves.  $\alpha$ -cadinol,  $\delta$ -cadinene were detected in the two investigated samples. Neocedranol, germacrene D-4-ol were detected in stems at high concentration.

All of the above compounds were identified for the first time in *A. millefolium*. A number of minor constituents of the essential oil have been detected in small amount.

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