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CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *ANAPHALIS MARGARITACEA* FROM MUNSYARI, PITHORAGARH, INDIA

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ABSTRACT

Anaphalis margaritacea shows antiasthmatic, anticoughing, expectorant and antiphlogistic activity. The leaves of *Anaphalis margaritacea* were collected in the month of September 2015 from Kalamuni (Munsyari)near Pithoragarh, location of Kumaon Himalayas. The plant *Anaphalis margaritacea* including leaves, stem, and flowers were extracted by hydro distillation method for 6 hours using Clevenger apparatus. The hydro-distilled essential oil of *Anaphalis margaritacea* has been examined by means of gas chromatography-mass spectrometry (GC-MS).77 constituents have been identified representing 94.08% of the total oil. The main compounds in major amounts were E-caryophyllene (21.22%), δ-Cadinene(6.51%), Caryophyllene oxide (6.36%), Cubenol (4.08%), γ-Cadinene(2.32%), α-Pinene (2.22%) and Ledol (1.55%).

KEYWORDS: (E)-caryophyllene, *Anaphalis margaritacea*, Essential oil, GC-MS.

1. INTRODUCTION

As a part of an ongoing study of the essential oilcomposition of Munsyari forest plants, *Anaphalis margaritacea* was investigated. *A.margaritacea* belongs to Gnaphalieae (Family Asteraceae), a small tribe of 22 genera and 320 species, which occur worldwide (Ahmed et. al., 2004). *A.margaritacea* is used as an expectorant and astringent, especially in homeopathy. The whole plant is anodyne, antiseptic, astringent, expectorant and sedative and used internally, it is a good remedy for diarrhoea, dysentery and pulmonary infections. A poultice of the flowers or the whole plant is applied to burns, sores, ulcers, bruises, swellings and rheumatic joints (Anthony J Cichoke 2001). Chemical investigations of the flowering aerial parts of *A. margaritacea* have identified flavonoids (Wollenweber et al.,

1993), and diterpenes and hydroxylactones as active constituents with two diterpenes having antibacterial activity against *B. cereus, P. aeruginosa and E. coli* (Ahmed et al., 2004). The leaf and flower extracts of *A. margaritacea* display the good antibacterial activity (Haider et. al, 2014).

Present investigation of aerial parts of the plant (leaves, pre-mature and mature seeds and fruits) were taken to isolate the essential oil and aim of the present study was to analyze the composition of the oil of *A. margaritacea*.

2. MATERIALS AND METHODS

2.1 Plant Material

The leaves of *Anaphalis margaritacea* were collected in the month of September 2015 from September 2015 from Kalamuni (Munsyari) near Pithoragarh, India in theKumaon Himalayas. The plant was authenticated by Botanical Survey of India (BSI) and identification no. was 114844.

2.2 Isolation of essential oil

The plant *Anaphalis margaritacea* including leaves, stem, and flower was extracted by hydro-distillation method for 6 hours using Clevenger apparatus. The oil was dried over anhydrous sodium sulphate and stored at room temprature in a sealed vial until analysis was performed. The percentage oil yield was calculated based on the dry weight of the plant. The oil yield was from the dry arial part of pants 0.09%.

2.3 GC and GC/MS analyses and identification

The oil was analysed by using a Shimadzu 2010 auto system GC. The column temperature was programmed at 80°C(holding time for 2 minute) to 210°C(holding time 5 minute) at 3°Cmin^{-1} and then 210°C- 300°Cat 20°C$min^{-1}$with final hold time of 15 minute, using N_2 at 30.0 mL/min column head pressure as carrier gas, the injector temperature was 270°Cand detector (FID, Flame ionization detector) temperature 280°C.

The GC-MS used was Autosystem 2010 GC (Rtx- 5, 30m x 0.25mm, i.d. FID 0.25µm) coupled with Shimadzu QP 2010 plus with thermal desorption system TD 20 with (Rtx-5) fused silica capillary column (30 m x 0.25mm with film thickness 0.25µm). The column temperature was 80°C(holding time for 2 minute) to 210 °C(holding time 5 minute) at 3°Cmin^{-1} and then 210°C- 300°Cat 20°C$min^{-1}$with final hold time of 21 minute,using helium

as carrier gas. The injector temperature was 230°C and 0.2 µL in n-hexane, with split ratio of 1:30 MS were taken at 70 eV with a mass range of 40- 650 amu.

Identification of constituents were done on the basis of Retention Index (RI, determined with reference to homologous series of n-alkanes C8-C28, under identical experimental condition), MS library search (NIST and WILEY), and by comparison with MS literature data (Adams R.P. 2007). The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor. Retention indices (RI) were determined with reference to a homologous series of normal alkanes, by using the following formula (Kovats, 1958).

$$KI = 100[\, n + (N-n)x \,] \quad \frac{\log t^1_R \text{ (unknown)} - \log t^1_R (C_n)}{\log t^1_R (C_N) - \log t^1_R (C_n)}$$

t^1_R – the net retention time ($t_R - t_0$)

t_0 – the retention time of solvent (dead time)

t_R – the retention time of the compound.

C_N – number of carbons in longer chain of alkane

C_n– number of carbons in shorter chain of alkane

n - is the number of carbon atoms in the smaller alkane

N - is the number of carbon atoms in the larger alkane

RESULTS AND DISCUSSION

The GC and GC-MS analyses of essential oil of *Anaphalis margaritacea* resulted in the identification of seventy seven compounds. The oil yield was found to be 0.09% by weight. Both, the major as well as minor constituents were identified by their retention indices and comparison of their mass spectra. Total seventy-seven compounds were identified constituting 94.08% of the total oil. The main compounds were E-caryophyllene (21.22%), δ-Cadinene(6.51%), Caryophyllene oxide (6.36 %), Cubenol (4.08%), γ-Cadinene(2.32 %), α-Pinene (2.22 %) and Ledol (1.55%). The main minor compounds were p-Dimethylbenzene (0.74%), β-Pinene(0.65 %), (E)-β- Ocimene (0.65%),β-Linalool (0.60%), Humulene epoxide II (0.90%), 1-Pentadecanal (0.91%) and geranyl-p-cymene (0.83%).

Table. 1: Essential oil composition of *Anaphalis margaritacea*.

S. N.	Compound	Area %	Mol. formula	Mol. Wt.	RI	Mode of identification
1.	Unidentified	0.74	C_8H_{10}	106	910	a, b
2.	β-Pinene	0.65	$C_{10}H_{16}$	136	978	a, b
3.	(E)-β- Ocimene	0.65	$C_{10}H_{16}$	136	1046	a, b
4.	β-Linalool	0.60	$C_{10}H_{18}O$	154	1082	a, b
5.	Humulene epoxide II	0.90	$C_{15}H_{24}O$	220	1613	a, b
6.	1-Pentadecanal	0.91	$C_{15}H_{30}O$	226	1701	a, b
7.	geranyl-p-cymene	0.83	$C_{20}H_{30}$	270	2006	a, b
8.	Unidentified	0.80	$C_{20}H_{40}O$	296	2114	a, b
9.	n-Hexacosane	0.89	$C_{26}H_{54}$	366	2600	a, b
10.	n-Hentriacontane	0.62	$C_{31}H_{64}$	436	3100	a, b
11.	Eremophilene	2.65	$C_{15}H_{24}$	204	1486	a, b
12.	α-Humulene	7.65	$C_{15}H_{24}$	204	1455	a, b
13.	Bis(2-Ethylhexyl) Phthalate	1.72	$C_{24}H_{38}O_4$	390	2499	a, b
14.	γ-Muurolene	2.94	$C_{15}H_{24}$	204	1478	a, b
15.	β-Selinene	4.95	$C_{15}H_{24}$	204	1492	a, b
16.	Aromadendrene	1.97	$C_{15}H_{24}$	204	1440	a, b
17.	α-Himachalene	0.07	$C_{15}H_{24}$	204	1450	a, b
18.	4,5-di-epi-aristolochene	0.07	$C_{15}H_{24}$	204	1472	a, b
19.	Larixol	0.08	$C_{20}H_{34}O_2$	306	2263	a, b
20.	(Z)- α-Bisabolene	0.09	$C_{15}H_{24}$	204	1503	a, b
21.	Methyl-nerate	0.10	$C_{11}H_{18}O_2$	182	1276	a, b
22.	α-Ocimene	0.12	$C_{10}H_{16}$	136	1042	a, b
23.	2,6-Dimethylundecane	0.12	$C_{13}H_{28}$	184	1210	a, b
24.	a-Calacorene	0.12	$C_{15}H_{20}$	200	1540	a, b
25.	Z,Z-2,15-Octadecedien-1-ol acetate	0.12	$C_{20}H_{36}O_2$	308	2193	a, b
26.	Hexadec-(11E)-en-1-ol	0.14	$C_{16}H_{32}O$	240	1869	a, b
27.	(-)-cis-Myrtanol	0.15	$C_{10}H_{18}O$	154	1180	a, b
28.	Benzylacetone	0.15	$C_{10}H_{12}O$	148	1214	a, b
29.	epi-Longipinanol	0.15	$C_{15}H_{26}O$	222	1558	a, b
30.	Globulol	0.16	$C_{15}H_{26}O_1$	222	1589	a, b
31.	Z-Lanceol acetate	0.16	$C_{17}H_{26}O_2$	262	1860	a, b
32.	(E)-p-2-Menthen-1-ol	0.17	$C_{10}H_{18}O$	154	1109	a, b
33.	1,10-Diepicubenol	0.17	$C_{15}H_{26}O$	222	1618	a, b
34.	Methyl isoamyl ketone	0.18	$C_7H_{14}O$	114	789	a, b
35.	α-Campholenal	0.18	$C_{10}H_{16}O$	152	1125	a, b
36.	Caryophylla-4(12),8(13)-dien-5-alpha-ol	0.19	$C_{15}H_{24}O$	220	1642	a, b
37.	5-Isopropyl-6-methyl-5-hepten-3-yn-2-ol	0.20	$C_{11}H_{18}O$	166	1192	a, b
38.	Bulnesol	0.20	$C_{15}H_{26}O_1$	222	1665	a, b
39.	n-Hexatriacontane	0.20	$C_{36}H_{74}$	506	3600	a, b
40.	Shellsol	0.21	C_9H_{20}	128	916	a, b
41.	(-)-Terpinen-4-ol	0.21	$C_{10}H_{18}O$	154	1137	a, b
42.	5-Propyldecane	0.22	$C_{13}H_{28}$	184	1249	a, b
43.	13-epi-Manoyl oxide	0.23	$C_{20}H_{34}O$	290	2023	a, b
44.	n-Decane	0.24	$C_{10}H_{22}$	142	993	a, b
45.	trans-Verbenol	0.24	$C_{10}H_{16}O$	152	1145	a, b

46.	α-Ylangene	0.26	$C_{15}H_{24}$	204	1371	a, b
47.	n-Tetracosane	0.26	$C_{24}H_{50}$	338	2400	a, b
48.	Terpinene	0.28	$C_{10}H_{16}$	136	1051	a, b
49.	Cubeban-11-ol	0.28	$C_{15}H_{26}O$	222	1599	a, b
50.	Hedycaryol	0.28	$C_{15}H_{26}O$	222	1692	a, b
51.	t-Cadina-1,4-diene	0.30	$C_{15}H_{24}$	204	1523	a, b
52.	γ-Gurjunene	0.32	$C_{15}H_{24}$	204	1474	a, b
53.	Phytone	0.33	$C_{18}H_{36}O$	268	1841	a, b
54.	α-Cadinene	0.34	$C_{15}H_{24}$	204	1534	a, b
55.	n-Nonane	0.35	C_9H_{20}	128	900	a, b
56.	Dodecane	0.39	$C_{12}H_{26}$	170	1200	a, b
57.	α-Muurolol	0.39	$C_{15}H_{26}O$	222	1650	a, b
58.	n-Decane	0.42	$C_{10}H_{22}$	142	1000	a, b
59.	2,4-Dimethylheptane	0.45	C_9H_{20}	128	788	a, b
60.	α-Fenchene	0.51	$C_{10}H_{16}$	136	948	a, b
61.	β-Bourbonene	0.58	$C_{15}H_{24}$	204	1386	a, b
62.	β-Gurjunene	0.59	$C_{15}H_{24}$	204	1413	a, b
63.	Farnesyl acetone	0.59	$C_{18}H_{30}O$	262	1902	a, b
64.	Methylheptane	1.07	C_8H_{18}	114	752	a, b
65.	Cadin-4-en-10-ol	1.09	$C_{15}H_{26}O$	222	1659	a, b
66.	Myrcene	1.14	$C_{10}H_{16}$	136	991	a, b
67.	α-Cubebene	1.22	$C_{15}H_{24}$	204	1349	a, b
68.	α-Copaene	1.26	$C_{15}H_{24}$	204	1379	a, b
69.	β-Copaene	1.50	$C_{15}H_{24}$	204	1430	a, b
70.	Limonene	1.51	$C_{10}H_{16}$	136	1030	a, b
71.	Ledol	1.55	$C_{15}H_{26}O_1$	222	1580	a, b
72.	Unidentified	2.22	C_9H_{20}	136	936	a, b
73.	γ-Cadinene	2.32	$C_{15}H_{24}$	204	1507	a, b
74.	Cubenol	4.08	$C_{15}H_{26}O_1$	222	1630	a, b
75.	Caryophyllene oxide	6.36	$C_{15}H_{24}O$	220	1587	a, b
76.	δ-Cadinene	6.51	$C_{15}H_{24}$	204	1520	a, b
77.	(E)-Caryophyllene	21.22	$C_{15}H_{24}$	204	1421	a, b
		94.08				

a=Retention Index (RI),b=MS (GC-MS)

The essential oil from *A. margaritacea* showed a qualitative and quantitative make-up of constituents. Clinically, this herb can be a good source of herbal medicine for the treatment of diseases indigenously. The study will also help to generate a database of species which can be exploited scientifically and judiciously in the future by local people and so that ecological balance is maintained. The results obtained in the present study suggest that the essential oil of *A. margaritacea* possesses medicinally active compounds.

This is the first report of chemical composition of essential oil of *Anaphalis margaritacea* from higher altitudes of Kumaon Himalayas. However, a report from China on A.

Margaritacea has shown 19.5% caryophyllene and 87.9% of sesquiterpenes which is almost similar to our report (21.2% caryophyllene) (Feng Shi lan, 2006).

CONCLUSION

The results data obtained in the present study suggest that the essential oil possesses strong medicinal activities and can be utilized for the treatment of diseases.

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AUTHOR CONTRIBUTIONS

The first author, Chandan Ram, who pursues his Ph.D under the supervision of Prof Pushpa Joshi carried out all the experimental work. Kundan Prasad, the third author, designed all the experiments, analyzed the data, and prepared the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

The following abbreviations are used in this manuscript.

GC-MS gas chromatography/mass spectrometry

GC-FID gas chromatography/flame ionization detector

RI: retention index.

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