

Phytochemical analysis and antioxidant activity essential oil of *Anaphalis contorta* from uttrakhand himalayas

Abstract

Background: *Anaphalis contorta* shows antiasthmatic, anticoughing, expectorant and antiphlogistic activity. The leaves of *Anaphalis Contorta* were collected in the month of September 2015 from Kosi Katarmal near Almora, location of Kumaun Himalayas.

Methods: The plant *Anaphalis contorta* including leaves, stem flower, were extracted by hydro distillation method for 6 hours using Clevenger apparatus. The hydro-distilled essential oil of *Anaphalis contorta* has been examined by means of gas chromatography-mass spectrometry (GC-MS). Take 1.0ml E. Oil of various code samples and mixed with in 4.0ml DMSO (Dimethyl sulfoxide). The prepared extract was used for the determination of antioxidant activity (i.e., DPPH assay & ABTS assay) in among samples.

Results: Fifty-two constituents have been identified representing 89.64% of the total oil. The major constituents were E-caryophyllene (16.33%), β -himachalene (9.15%), δ -cadinene (9.07%), α -pinene (5.58%). The oil was rich in sesquiterpene hydrocarbon (53.71%), followed by oxygenated sesquiterpene (21.49%). Among sesquiterpene hydrocarbons, made its significant percentage.

Conclusion: The results data obtained in the present study suggest that some essential oils possess strong medicinal activities, which can be utilized for treatment of certain diseases.

Keywords: (E)-caryophyllene, *anaphalis contorta*, essential oil, GC-MS, antioxidant activity

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Abbreviations: DMSO, dimethylsulfoxide; DPPH, 2,2-diphenyl-1-picrylhydrazyl; GC-MS, gas chromatography/mass spectrometry; GC-FID, gas chromatography/flame ionization detector; RI, retention index

Introduction

Anaphalis contorta Hook is an erect herb. The stem is branched from the base, branches are often decumbent. The leaves are linear, crowded, shortly lobed at the base, margins recurved sometimes. The heads are in dense terminal corymbs and in involucre bracts are broad, obtuse, erect in flower, spreading in fruit; outer ones often pale purple. *A. contorta* is widely distributed in temperate region from Kashmir to Sikkim and Afghanistan to South West China at a height of 1500-4500m.¹⁻³

Anaphalis is a genus of flowering plants within the Asteraceae family, whose members are commonly known by the name pearl or yearly everlasting. *Anaphalis* is the largest genus within the Asian Gnaphalieae and it is well diversified in the Himalayas and the Qinghai-Tibet Plateau region.⁴ It comprises approximately 110 species, mostly distributed in Asia. Various medicinal plants have been used for years in day to day life to treat diseases all over the world. The use of traditional medicine and medicinal plants in developing countries, to meet some of their primary health care needs, has been widely documented. The family Asteraceae is one of the largest plant families on which the wound healing activity studies have been focused. The fresh leaves of this plant and some other *Anaphalis* species are bruised and applied to the cut wounds under a rag bandage.⁵ When taken before meals, the leaves stimulate the appetite and as a result, it is administered to convalescents for its sedative and tonic properties.⁶ It shows antiasthmatic, anticoughing, expectorant and antiphlogistic

activity. The oil of *Anaphalis contorta* has been found to possess antibacterial and antifungal activity.⁷ Present investigation of aerial parts of the plant (leaves, pre-mature and mature seeds and fruits) were taken to isolate essential oils and aim of the present study was to analyze the composition of the oils of *A. contorta* and antioxidant properties.

Materials and methods

Plant material

The leaves of *Anaphalis Contorta* were collected in the month of September 2015 from Kosi Katarmal near Almora, location of Kumaun Himalayas, The plant was authenticated by Botanical Survey of India (BSI).

Isolation of essential oil

The plant *Anaphalis contorta* including leaves, stem flower, were extracted by hydro distillation method for 6 hours using Clevenger apparatus.⁸ The oil was dried with anhydrous sodium Sulphate and stored at room temperature in a sealed vial until analysis was performed. The percentage oil yield was calculated based on the dry weight of the leaf. The oil yield was (0.7%).

GC-FID and GC-MS analysis

Essential oil analysis was performed by using GC-MS and GC-FID was performed on a Shimadzu QP-2010 instrument, equipped with FID, in the same conditions, except hydrogen was used as the carrier gas. The percentage composition of the oil samples were computed from the GC peak areas without using correction for response factors. The oils were analyzed using a Shimadzu GC/MS Model QP 2010 Plus, equipped with a Rtx-5MS (30m \times 0.25mm; 0.25mm film

thickness) fused silica capillary column. Helium was used as carrier gas adjusted to 1.21 ml/min at 69.0 KPa; splitless injection of 1 µL, of a hexane solution; injector and interface temperature were 270°C; oven temperature programmed was 50–280°C at 3°C/min. EIMS: electron energy, 70 eV; ion source temperature was 230°C.

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.⁹ 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.

Extract preparation for antioxidant analysis

Take 1.0 ml E. Oil of various code samples and mixed with in 4.0 ml DMSO (Dimethyl sulfoxide). The prepared extract was used for the determination of antioxidant activity (i.e., DPPH assay & ABTS assay) in among samples.

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical, gallic acid, ascorbic acid, chlorogenic acid, caffeic acid, p-coumaric acid, 3-hydroxybenzoic acid, catechin and quercetin were procured from Sigma-Aldrich (Steinheim, Germany). Sodium carbonate, 2-(n-morpholino) ethanesulfonic acid (MES buffer), potassium persulphate, ferric chloride, sodium acetate, potassium acetate, aluminium chloride, glacial acetic acid and hydrochloric acid from Qualigens (Mumbai, India), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 2,4,6-tri-2-pyridyl-1,3,5-triazin (TPTZ), methanol and ethanol from Merck Company (Darmstadt, Germany).

diphenyl-1-picrylhydrazyl (DPPH) assay

Free radical DPPH scavenging assay was slightly modified [10] for the present study. DPPH (100 µM) was prepared in 80% (w/v) ethanol and 2.7 ml mixed with 0.9 ml of sample extract and allowed to

stand in the dark (22±10°C, 20 min). The reduction in the absorbance at 520 nm was recorded and results expressed in mM ascorbic acid equivalent per 100 g (mM AAE /100g).

Azinobis (3 benzylthiazole)-6- sulphonic acid (ABTS) assay

Total antioxidant activity was measured by improved ABTS (ethylbenzothiazoline 6-sulphonic acid) radical scavenging method.^{11,12} In brief, ABTS (7.0 µM) and potassium persulphate (2.45 µM) were added in amber coloured bottle for the production of ABTS cation (ABTS^{•+}) and kept in the dark (16 h, 22±1°C). ABTS^{•+} solution was diluted with 80% (v/v) ethanol till an absorbance of 0.700±0.05 at 734 nm is obtained. For sample analysis, 3.90 ml of diluted ABTS^{•+} solution was added to 0.10 ml of methanolic extract and mixed thoroughly. The reaction mixture was allowed to stand (22±10°C, 6 min, dark) and the absorbance was recorded at 734 nm with respect to blank. A standard curve of various concentrations of ascorbic acid is prepared in 80% v/v methanol for the equivalent quantification of antioxidant potential with respect to ascorbic acid. Results were expressed in mM ascorbic acid equivalent per 100 g (mM AAE /100g).

Results and discussion

Essential oil composition

The GC and GC-MS analysis of *Anaphalis contorta* resulted in the identification of fifty-two constituents, the oil yield was found to be 0.7% by weight. Both, the major as well as minor constituents were identified by their retention indices and comparison of their mass spectra. Total fifty-seven constituents were identified constituting 89.64% of the total oil. The main compounds in major amounts were E-caryophyllene (16.33%), β-Himachalene (9.15%), δ-cadinene (9.07%), α-pinene (5.58%). The oil was rich in sesquiterpene hydrocarbon (53.71%), followed by oxygenated sesquiterpene (21.49%). Among sesquiterpene hydrocarbons, made its significant percentage. This is the first report on the composition of essential oil from *Anaphalis contorta* Table (1&2).

Table 1 Essential oil composition of the *Anaphalis contorta*

S.N.	Compound	Area %	Mol. Formula	Mol. Wt.	RI	Mode of Identification
1	4,5-Dimethyl-4-hexen-3-one	0.12	C ₈ H ₁₄ O	126	915	a, b
2	α-Pinene	5.58	C ₁₀ H ₁₆	136	933	a, b
3	β-Pinene	0.08	C ₁₀ H ₁₆	136	978	a, b
4	Myrcene	0.12	C ₁₀ H ₁₆	136	987	a, b
5	(4E)-4-Hexenyl acetate	0.12	C ₈ H ₁₄ O ₂	142	992	a, b
6	Limonene	0.2	C ₁₀ H ₁₆	136	1024	a, b
7	Ocimene	0.14	C ₁₀ H ₁₆	136	1029	a, b
8	(E)-β-ocimene	0.43	C ₁₀ H ₁₆	136	1046	a, b
9	Linalool	0.29	C ₁₀ H ₁₈ O	154	1101	a, b
10	Lavandulol	0.22	C ₁₀ H ₁₈ O	154	1150	a, b
11	Safranal	0.15	C ₁₀ H ₁₄ O	150	1182	a, b
12	Lavandulyl acetate	1.12	C ₁₂ H ₂₀ O ₂	196	1275	a, b
13	α- Cubebene	0.33	C ₁₅ H ₂₄	204	1349	a, b
14	α- Copaene	1.3	C ₁₅ H ₂₄	204	1375	a, b
15	α- ylangene	0.9	C ₁₅ H ₂₄	204	1376	a, b
16	Italicene	1.11	C ₁₅ H ₂₄	204	1408	a, b
17	E- Caryophyllene	16.33	C ₁₅ H ₂₄	204	1419	a, b
18	α- Humulen	1.12	C ₁₅ H ₂₄	204	1454	a, b
19	Sesquisabinene	0.14	C ₁₅ H ₂₄	204	1455	a, b
20	9-epi-(E)-β-Caryophyllene	0.68	C ₁₅ H ₂₄	204	1464	a, b

Table Continued...

S.N.	Compound	Area %	Mol. Formula	Mol.Wt.	RI	Mode of Identification
21	Cadina-1(6),4-diene	0.68	C ₁₅ H ₂₄	204	1472	a, b
22	Germacrene-D	0.24	C ₁₅ H ₂₄	204	1475	a, b
23	trans-β-Bergamotene	3.18	C ₁₅ H ₂₄	204	1480	a, b
24	trans-b-Bergamotene	0.65	C ₁₅ H ₂₄	204	1483	a, b
25	Viridiflorene	1.88	C ₁₅ H ₂₄	204	1491	a, b
26	β-Himachalene	9.15	C ₁₅ H ₂₄	204	1503	a, b
27	γ-Cadinene	2.37	C ₁₅ H ₂₄	204	1507	a, b
28	Cubebol	0.87	C ₁₅ H ₂₆ O	222	1514	a, b
29	δ-cadinene	9.07	C ₁₅ H ₂₄	204	1520	a, b
30	α-curcumene	0.65	C ₁₅ H ₂₂	202	1536	a, b
31	α-Cadinene	2.46	C ₁₅ H ₂₄	204	1538	a, b
32	β-Bulnesene	1.45	C ₁₅ H ₂₄	204	1558	a, b
33	β-Calacorene	0.26	C ₁₅ H ₂₀	200	1564	a, b
34	Isoshyobunone	0.15	C ₁₅ H ₂₄ O	220	1571	a, b
35	Axenol	0.58	C ₁₅ H ₂₆ O	222	1574	a, b
36	Globulol	0.69	C ₁₅ H ₂₆ O	222	1586	a, b
37	Caryophyllene oxide	2.38	C ₁₅ H ₂₄ O	220	1587	a, b
38	Copabornene	0.44	C ₁₅ H ₂₆ O	222	1613	a, b
39	1,10-Diepicubenol	0.58	C ₁₅ H ₂₆ O	222	1618	a, b
40	Epicubenol	2.02	C ₁₅ H ₂₆ O	222	1631	a, b
41	Epi-α-Cadinol	3.72	C ₁₅ H ₂₆ O	222	1640	a, b
42	Pogostol	4.98	C ₁₅ H ₂₆ O	222	1650	a, b
43	Epi-β-Bisabolol	2.66	C ₁₅ H ₂₆ O	222	1675	a, b
44	α-Bisabolol	0.83	C ₁₅ H ₂₆ O	222	1688	a, b
45	Murolan-3,9(11)-diene-10-peroxy	0.24	C ₁₅ H ₂₄ O ₂	236	1729	a, b
46	Cembrene	0.59	C ₂₀ H ₃₂	272	1939	a, b
47	Sclarene	0.33	C ₂₀ H ₃₂	272	1943	a, b
48	m-Camphorene	0.34	C ₂₀ H ₃₂	272	1946	a, b
49	Neocembrene	0.7	C ₂₀ H ₃₂	272	1960	a, b
50	n-Hexadecanoic acid	0.2	C ₁₆ H ₃₂ O ₂	256	1977	a, b
51	Manool	3.37	C ₂₀ H ₃₄ O	290	2062	a, b
52	Labd-7,13-dien-15-ol	1.45	C ₂₀ H ₃₄ O	290	2293	a, b
		89.64				

a=Retention Index (RI); b=MS (GC-MS).

Table 2 Essential oil of *Anaphalis contorta*, contain the following classes of chemical compounds

SN	Chemical Composition	Percentage
1	Monoterpene hydrocarbon	6.55%
2	Oxygenated monoterpene	0.90%
3	Sesquiterpene hydrocarbon	53.71%
4	Oxygenated sesquiterpene	21.49%
5	Diterpene hydrocarbon	1.8%
6	Oxygenated diterpene	4.82%

Table 3 Antioxidant activity (DPPH & ABTS assay) in *Anaphalis contorta*

Name of Plants	DPPH Activity (mMAAE/100g)	DPPH Activity (mMAAE/100g)
<i>Anaphalis contorta</i>	9.47±0.03	7.31±0.01

Antioxidant activity

The essential oil showed good DPPH and ABTS radical scavenging activity. Antioxidant activity of plants *A. contorta* analyzed (Table 3). The free radical scavenging activity (DPPH assay) was 9.47mMAE/100g recorded in *Anaphalis contorta* aromatic oil. Total antioxidant activity (ABTS assay) was found (7.31mMAAE/100g) in *A. contorta* aromatic oil. This activity is significant, especially since this essential oil are composed mainly of monoterpenes and sesquiterpene hydrocarbons and oxygenated ones which have a moderate activity compared to phenolics and vitamin C. Our findings

revealed that the percentage of oxygenated monoterpenes was 0.90% and monoterpenes hydrocarbons was 6.55%. This result might be related to the antioxidant activity of our essential oil. All the essential oils had low amounts of phenolic compounds but showed good antioxidant activity. The diversified mono- and sesquiterpenoids present in the complex mixture of essential oils might be responsible for the good antioxidant activity because of synergetic effects of the constituents. This can be evidenced by a report which says that antioxidant capacity is affected by other bioactive compounds and could involve synergistic effects.¹³

Conclusion

The essential oil from *Anaphalis contorta* showed a qualitative and quantitative make-up of constituents. The oils also showed good antioxidant activities. 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 data obtained in the present study suggest that some essential oils possess strong medicinal activities, which can be utilized for treatment of certain diseases.

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Author Contribution

The first author, Kavita Rawat persuade his Ph.D under the supervision of Prof Ganga Bisht carried out all the experiments work, Kundan Prasad the second author designed all the experiments and 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.

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