SHORT REPORT

A C G publications

Rec. Nat. Prod. 8:3 (2014) 281-285

records of natural products

Seasonal Variation in Essential oil Composition of *Artemisia nilagirica* var. *septentrionalis* from Foot Hills of Western Himalaya

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(Received July 16, 2012; Revised September 23, 2013; Accepted February 16, 2014)

Abstract: Essential oils composition of the aerial parts of *Artemisia nilagirica* (Clarke) Pamp. var. *septentrionalis* Pamp. in different seasons viz. spring, summer, rainy, autumn and winter seasons under foot hills agroclimatic conditions of western Himalaya were analyzed and compared by GC–FID and GC–MS. Essential oils were mainly composed of monoterpenoids (59.0%-77.3%) and sesquiterpenoids (15.7%-31.6%). The major constituents identified were artemisia ketone (38.3%-61.2%), chrysanthenone (1.5%-7.7%), germacrene D (3.1%-6.8%), β-caryophyllene (1.9%-6.8%), germacra-4,5,10-trien-1-α-ol (1.9%-4.9%) and artemisia alcohol (1.4%-3.6%). Compositional analysis showed significant variations in the terpenoid compositions due to seasonal variations. Further, this is the first report on the seasonal variations in essential oil compositions of artemisia ketone rich chemotype of *A. nilagirica* var. *septentrionalis* from India.

Keywords: *Artemisia nilagirica*; essential oils; artemisia ketone; β-caryophyllene, germacrene D.

1. Plant Source

The genus *Artemisia* L., commonly known as wormwood, comprises more than 400 species widely distributed throughout the world. About 30 species of *Artemisia* are documented in the flora of western Himalaya. *Artemisia nilagirica* (Clarke) Pamp. var. *septentrionalis* Pamp. is a tall aromatic perennial shrub, distributed throughout the mountains regions of western Himalayas at an altitude of 1500-2200 m [1-3]. The aim of this study was to examine changes occurring in the essential oil yield and chemical composition of *A. nilagirica* var. *septentrionalis* grown in foot hills agroclimatic condition of Uttarakhand, India during different seasons.

Fresh samples of *A. nilagirica* var. *septentrionalis* were collected from the experimental field at CSIR-Central Institute of Medicinal and Aromatic Plants, Pantnagar (N 29°01.438'; E79°38.995; 243.84 m) throughout the year (2011). Plant materials were identified by one of the authors (AC) and a voucher specimen (CIM PANT#337) has been deposited at the Herbarium of the CSIR-CIMAP, Research Center, Pantnagar, Uttarakhand, India.

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 05/01/2014 EISSN: 1307-6167

2. Previous Studies

Investigation on essential oil composition of *A. nilagirica* from India showed extensive compositional diversity in essential oil constituents. The major constituents reported in *A. nilagirica* essential oil from north and south Indian origin were α-thujone, β-thujone, camphor, 1,8-cineole, borneol, linalool, artemisia alcohol, along with other mono and sesquiterpenoids [4-14]. To best of our knowledge, only two reports exist in literature on essential oil composition of *A. nilagirica* var. *septentrionalis* from Indian origin. In first report, caryophyllene oxide (28.6%), borneol (35.8%) and camphor (46.9%) were reported as major constituents [11]; while in another report camphor, β-caryophyllene, germacrene D, α-humulene and 1,8-cineole were reported as major constituents during different growth stages of *A. nilagirica* var. *septentrionalis* from northern plains of India [12].

3. Present Study

Essential oils were isolated from harvested samples (100 g, each, dried) by hydro-distillation using Clevenger-type apparatus. Essential oils were collected and dried over anhydrous Na_2SO_4 and were stored in sealed vials under refrigeration prior to analysis. Oil samples were analyzed by gas chromatography (GC) and GC-MS (mass spectrometry; MS) methods. GC was performed on a Nucon 5765 gas chromatograph equipped with Flame Ionization Detector (FID) and a DB-5 column (30 m × 0.25 mm i.d., 0.25 µm film coating). Temperature programming was from 60°C-210°C at 3°C/min; injector port and detector temperatures were 210°C and 230°C respectively. Hydrogen was used as the carrier gas at 1.0 mL/ min. GC-MS analyses of the essential oils were carried out on a Perkin- Elmer Turbomass Quadrupole Mass spectrometer fitted with PE-5 (Perkin-Elmer) fused silica capillary column (60 m × 0.32 mm; 0.25 µm film coating). The column temperature was programmed 70°C, initial hold time of 2 min, to 250°C at 3°C/min with final hold time of 3 min, using helium as carrier gas at a flow rate of 1.0 mL/min. The injector and ion source temperatures were 250°C. The injection volume was 0.02 µL neat (splitting mode) with split ratio 1:40. MS were taken at 70 eV (EI) source with mass scan range of *m/z* 40–400. Identification of constituents were done on the basis of retention index (RI, determined with reference to homologous series of n-alkanes C_8-C_{24}) under identical experimental condition, coinjection with standards (Aldrich and Fluka), mass spectra library search (NIST/EPA/NIH version 2.1 and Wiley registry of mass spectral data 7th edition) and by comparing with the mass spectral literature data [15]. The relative amounts of individual components were calculated based on GC peak areas without using correction factors.

Essential oils from the aerial parts of *A. nilagirica* var. *septentrionalis* in different seasons viz. spring, summer, rainy, autumn and winter seasons under foot hill conditions of northern India; were analyzed by GC and GC-MS. A total of forty-nine constituents forming 91.8%-94.2% of the total oil compositions were identified. The average of essential oil yield on dry weight basis and relative percentage of the identified constituents of essential oils from the fresh aerial parts of *A. nilagirica* var. *septentrionalis* in spring (March–April), summer (May–June), rainy (July–September), autumn (October–November), and winter (December–February) seasons are summarized in Table 1 in order of their elution from a DB-5 column. Essential oil yield (v/w %) was found to vary from 0.45% to 0.70% during different seasons. The highest essential oil yield was found in rainy season (0.70%) followed by summer (0.68%) and spring (0.58%) season; while accumulation of essential oil was lowest (0.45%) in autumn and winter seasons.

The essential oils of *A. nilagirica* var. *septentrionalis* were mainly composed of monoterpenoids (59.0%-77.3%) represented by artemisia ketone (38.3%-61.2%), chrysanthenone (1.5%-7.7%), artemisia alcohol (1.4%-3.6%), perillene (0.5%-2.1%), bornyl acetate (1.0%-2.3%) and carvone (1.8%-1.6%). The major sesquiterpenoids identified in essential oils were germacrene D (3.1%-6.8%), β-caryophyllene (1.9%-6.8%), germacra-4,5,10-trien-1-α-ol (1.9%-4.9%), δ-cadinene (0.9%-2.4%), β-longipinene (0.2%-1.3%) and α-muurolol (0.4%-1.2%). Chemical compositions of *A. nilagirica* var. *septentrionalis* in different seasons summarized in Table 1 clearly indicate that the essential oil composition varies significantly due to seasonal changes.

Table 1. Seasonal variation in yield and composition of *A. nilagirica* var. *septentrionalis*

Compounds	RI_{Exp}	RI_{Lit}	Content (%)[a]					Mean (±SD)
			I	**II**	**III**	**IV**	**V**	
α-Thujene	922	924	0.3	-	0.1	t	0.1	0.11 (0.11)
α-Pinene	936	932	t	0.3	0.1	0.3	0.1	0.17 (0.12)
Camphene	953	946	t	0.1	0.1	0.3	0.1	0.13 (0.10)
Sabinene	973	969	0.1	0.6	t	0.1	0.2	0.21 (0.22)
1-Octen-3-ol	976	974	0.8	0.6	1.2	1.0	1.0	0.92 (0.22)
β-Pinene	978	974	0.2	t	t	0.1	0.1	0.09 (0.06)
Yomigi alcohol	996	999	0.8	0.7	0.6	0.7	1.0	0.76 (0.15)
(3*E*)-Hexenyl acetate	998	1001	t	t	t	t	t	0.03 (0.0)
(3*Z*)-Hexenyl acetate	1002	1004	t	t	t	t	t	0.03 (0.0)
α-Tepinene	1011	1014	0.1	0.1	t	0.2	t	0.09 (0.06)
p-Cymene	1018	1020	0.1	t	t	t	t	0.04 (0.03)
β-Phellandrene	1023	1025	0.3	t	t	t	0.1	0.10 (0.11)
1,8-Cineole	1026	1026	0.9	3.2	3.0	3.4	1.5	2.40 (1.12)
Artemisia ketone	1054	1056	**60.7**	**55.2**	**45.0**	**38.3**	**61.2**	52.08 (10.08)
cis-Sabinene hydrate	1063	1065	0.4	1.0	1.1	0.4	0.3	0.64 (0.37)
Artemisia alcohol	1079	1080	3.6	3.0	2.3	1.4	3.2	2.70 (0.86)
Perillene	1100	1102	2.1	1.2	0.8	1.1	0.5	1.14 (0.60)
2,6-Dimethyl phenol	1104	1106	1.9	0.2	0.3	0.2	1.5	0.82 (0.81)
Chrysanthenone	1126	1126	**1.5**	**6.5**	**7.7**	**6.7**	**3.8**	5.24 (2.54)
(*Z*)-Epoxy ocimene	1127	1128	0.2	0.1	0.1	0.2	0.1	0.14 (0.05)
(*E*)-Epoxy ocimene	1134	1137	0.1	0.1	0.2	0.2	0.1	0.14 (0.05)
Myrcenone	1142	1145	0.2	0.9	0.3	0.7	0.3	0.48 (0.30)
Borneol	1167	1165	0.5	0.8	0.5	1.0	0.3	0.62 (0.27)
Myrtenol	1199	1194	0.3	0.4	0.5	0.4	0.4	0.40 (0.07)
Carvone	1238	1239	1.2	1.6	1.2	1.2	1.4	1.32 (0.17)
Bornyl acetate	1288	1284	1.2	1.5	2.3	2.3	1.0	1.66 (0.61)
α-Cubebene	1351	1347	0.2	0.2	0.2	0.1	0.2	0.18 (0.04)
α-Copaene	1379	1374	0.4	0.3	0.5	0.6	0.2	0.40 (0.15)
β-Longipinene	1404	1400	0.8	1.3	1.1	1.0	0.2	0.88 (0.42)
Longifolene	1409	1407	0.2	0.2	0.1	0.2	0.2	0.18 (0.04)
β-Caryophyllene	1424	1417	**3.3**	**2.3**	**4.3**	**6.8**	**1.9**	3.72 (1.95)
β-Copaene	1433	1430	0.2	t	t	t	t	0.06 (0.07)
(*Z*)-β-Farnesene	1438	1440	0.1	t	t	t	0.1	0.05 (0.03)
α-Humulene	1456	1452	0.4	0.4	0.6	1.2	0.8	0.68 (0.33)
(*E*)-β-Farnesene	1458	1454	t	0.3	0.2	0.1	0.3	0.19 (0.12)
Germacrene D	1485	1484	**3.1**	**4.2**	**6.8**	**6.8**	**3.1**	4.80 (1.88)
β-Selinene	1490	1489	0.7	0.4	0.8	1.1	0.5	0.70 (0.27)
(*Z*)-β-Guaine	1493	1492	0.3	0.2	1.0	1.1	0.7	0.66 (0.40)
β-Himachallene	1494	1500	0.4	0.2	0.2	0.3	0.3	0.28 (0.08)
(*E*)-β-Guaine	1498	1502	0.2	0.3	0.2	0.1	0.4	0.24 (0.11)
γ-Cadinene	1519	1513	0.3	t	0.2	0.1	0.1	0.15 (0.10)
δ-Cadinene	1525	1522	1.1	1.3	2.1	2.4	0.9	1.56 (0.65)
Spathulenol	1581	1577	0.2	0.3	0.5	0.6	0.6	0.44 (0.18)
Caryophyllene oxide	1583	1582	0.7	0.2	0.4	0.7	0.4	0.48 (0.21)
10-*epi*- γ-Eudesmol	1616	1622	0.4	0.1	0.4	0.5	0.5	0.38 (0.16)
epi- α-Cadinol	1639	1638	0.6	0.6	0.8	0.9	0.5	0.68 (0.16)
α-Muurolol	1642	1644	0.4	0.6	0.9	1.2	0.7	0.76 (0.30)
α-Eudesmol	1650	1652	0.2	0.4	0.5	0.9	0.5	0.50 (0.25)
Germacra-4,5,10-trien-1-α-ol	1678	1685	1.9	1.9	3.7	4.9	2.8	3.04 (1.27)
Class composition								
Monoterpene hydrocarbons			*1.1*	*1.1*	*0.3*	*1.0*	*0.7*	0.84 (0.34)
Oxygenated monoterpenes			*73.7*	*76.2*	*65.6*	*58*	*75.1*	69.72 (7.76)
Sesquiterpene hydrocarbons			*11.7*	*11.6*	*18.3*	*21.9*	*9.9*	14.68 (5.15)
Oxygenated sesquiterpenes			*4.4*	*4.1*	*7.2*	*9.7*	*6.0*	6.28 (2.28)
Other compounds			*2.7*	*0.8*	*1.5*	*1.2*	*2.5*	1.74 (0.82)
Total identified			**93.6**	**93.8**	**92.9**	**91.8**	**94.2**	**93.26 (0.94)**
Essential oil yield (%)[b]			***0.58***	***0.68***	***0.70***	***0.45***	***0.45***	***0.57 (0.12)***

RI_{Exp}: Retention Index determined with reference to homologous series of n-alkane (C_8–C_{24}) on DB-5 capillary column; RI_{Lit}: Retention index literature [15]; SD, standard deviation (±); MOD: [a]Content (%) of the individual constituents are average values of respective seasons; [b]Percentage calculated on dry weight basis (v/w %) are average values of respective seasons viz. **I**: Spring (March–April), **II**: Summer (May–June), **III**: Rainy (July–September), **IV**: Autumn (October–November), and **V**: Winter (December–February).

Monoterpenoids of were found to be at highest level during summer season (77.3%) followed by 75.8% in winter and 74.8% in spring season, while sesquiterpenoids reached their higher value in autumn season (31.6%) followed by rainy season (25.5%). Moreover, the amount of artemisia ketone, major constituents of essential oil throughout the year, was highest in winter season (61.2%) followed by 60.7% in spring season. In autumn; the content of artemisia ketone was found to be its lowest (38.3%). Chrysanthenone, second major constituent of essential oil, was found to be higher in rainy (7.7%) and autumn season (6.7%). Sesquiterpene hydrocarbons viz. germacrene D and β-caryophyllene were found higher in autumn and rainy seasons. Trend of accumulation of essential oil constituents showed no regular trend based on seasonal variation; nevertheless the essential oil obtained from different samples showed considerable variation in content and quantitative composition. This could be due to expression of different genes at different developmental stages of the plant and further by the environmental factors arising from seasonal variations. Several studies carried out in past on other aromatic plants have also shown that the essential oil composition may vary considerably throughout the year with seasonal change [16]. Earlier report showed camphor, β-caryophyllene, germacrene D, α-humulene, 1,8-cineole, caryophyllene oxide, borneol and thujones as major constituents from the essential oils of *A. nilagirica* from Indian origin [4-14]. Compositional analysis of essential oil of *A. nilagirica* from western Himalaya revealed camphor (9.7%), β-eudesmol (8.0%), 1,8-cineole (6.6%), borneol (5.3%) as major constituents [7]. While in another report on *A. nilagirica* from different altitudes of the Garhwal region showed α-thujone (36.99%), linalool (32.47%), and 4-nitrobenzonoic acid-4-methoxyphenyl ester (22.12%) as major constituents [8]. α-Thujone (36.35%), β-thujone (9.37%), germacrene D (6.32%), terpinen-4-ol (6.31%), β-caryophyllene (5.43%), and camphene (5.47%) were identified as the major constituents of *A. nilagirica* oil from northern hilly area of India [9]. The essential oil of the leaves of *A. nilagirica* from South India reports α-thujone (41.9%), borneol (10.8%), and β-thujone (9.1%) as major constituents [10]. Further, camphor (16.7–37.0%), β-caryophyllene (7.2–22.6%), germacrene D (3.6–11.9%), α-humulene (2.4–9.8%) and borneol (2.8–9.1%) were reported as major constituents in *A. nilagirica* var. *septentrionalis* from north Indian plains [11]. In contrary to these reports, in present analysis artemisia ketone (38.3%-61.2%) was found to be major constituents of essential oils throughout the year followed by chrysanthenone (1.5%-7.7%), germacrene D (3.1%-6.8%), β-caryophyllene (1.9%-6.8%) and germacra-4,5,10-trien-1-α-ol (1.9%-4.9%). Artemisia ketone and chrysanthenone was not noticed as major constituents in any of the earlier reports; and α- and β-thujones reported in most of earlier reports on oil composition of *A. nilagirica* were not noticed in present study. Therefore this is for the first time the seasonal variation in artemisia ketone rich new chemotypes of *A. nilagirica* var. *septentrionalis* was reported from foot hills of western Himalaya of India. *A. nilagirica* was reported to possess various biological activities and used in traditional medicine mainly on ground of its significant antifungal, antimycotic, anticonvulsant, antimalarial, and insecticidal activities [5-10, 17-19]. Therefore, the potential of present artemisia ketone rich chemotype of *A. nilagirica* var. *septentrionalis* need to be explored for its biological activities for industrial applications.

References

[1] R. Costa, M. Rosa De Fina, M.R. Valentino, A. Rustaiyan, P. Dugo, G. Dugo and L. Mondello (2009). An investigation on the volatile composition of some *Artemisia* species from Iran, *Flav. Frag. J.* **24**, 75-82.

[2] A.R. Ahameethunisa and W. Hopper (2010). Antibacterial activity of *Artemisia nilagirica* leaf extracts against clinical and phytopathogenic bacteria, *BMC Complement. Altern. Med.* **10**, 2-6.

[3] P.K. Hajra, R.R. Rao, D.K. Singh and B.P. Uniyal (1995) Flora of India (Asteraceae-Heliantheae). Publication Director, Botanical Survey of India, Calcutta, India.

[4] R. Badoni, D.K. Semwal and U. Rawat (2010). Composition variation in essential oils of *Artemisia nilagirica* and *Artemisia capillaris* growing in India. *J. Appl. Nat. Sci.* **2**, 30-33.

[5] A. Vijayalakshmi, R. Tripathi and V. Ravichandiran (2010). Characterization and evaluation of antidermatophytic activity of the essential oil from *Artemisia nilagirica* leaves growing wild in nilgiris, *International J. Pharm. Pharmaceut. Sci.* **2**, 93-97.

[6] A. Vijayalakshmi, V. Ravichandiran, K.V. Durga Prasad, A. Kiran Kumar, K. Rakesh, K. Ramesh Naidu and K. Shravya Vardhan (2011), Anticonvulsant activity of *Artemisia nilagiric*a leaves, *J. Pharm. Res*. **4**, 2881-2883.

[7] G.C. Uniyal, A.K. Singh, N.C. Shah and A.A. Naqvi (1985). Volatile constituents of *Artemisia nilagirica*, *Planta Med.* **51**, 457-458.
[8] R. Badoni, D.K. Semwal and U. Rawat (2009), Altitudinal variation in the volatile constituents of *Artemisia nilagirica*, *International J. Essent. Oil Therapeutics* **3**, 66-68.
[9] S.C. Sati, N. Sati, V. Ahluwalia, S. Walia and O.P. Sati (2013). Chemical composition and antifungal activity of *Artemisia nilagirica* essential oil growing in northern hilly areas of India, *Nat. Prod. Res.* **27**, 45-48.
[10] P.M. Shafi, M.K.G. Nambiar, R.A. Clery, Y.R. Sharma and S.S. Veena (2004). Composition and antifungal activity of the oil of *Artemisia nilagirica* (Clarke) Pamp, *J. Essent. Oil Res.* **16**, 377-379.
[11] F. Haider, A.A. Naqvi and G.D. Bagchi (2007). Oil constituents of *Artemisia nilagirica* var. *septentrionalis* during different growth phases at subtropical conditions of north Indian plains, *J. Essent. Oil Res.* **19**, 5-7.
[12] F. Haider, N. Kumar, A.A. Naqvi and G.D. Bagchi (2010). Oil constituents of *Artemisia nilagirica* var. *septentrionalis* growing at different altitudes, *Nat. Prod. Commun.* **5**, 1959-1960.
[13] V.K. Saxena and G.C. Samaiya (1984), Chemical study of the stem of *Artemisia nilagirica*, *Acta Ciencia Indica, Chemistry **10***, 272-5.
[14] L. Leeja and E. John Thoppil (2004), Essential oil composition and mosquito larvicidal activity of *Artemisia nilagirica* (C.B. Clarke) Pamp. from South India, *J. Phytological Res*. **17**, 155-158.
[15] R.P. Adams (2007). Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation, Carol Stream, IL, USA.
[16] R.S. Verma, R.C. Padalia, V.K. Arya and A. Chauhan (2012). Aroma profiles of the curry leaf, *Murraya koenigii* (L.) Spreng. chemotypes: variability in north India during the year, *Indust. Crop Prod*. **36**, 343-349.
[17] N. Kishore, N.K. Dubey and J.P.N. Chansouria (2001). Antimycotic activity of the essential oil of *Artemisia nilagirica*, *Flav. Frag. J.* **16**, 61-63.
[18] P. Tripathi, N.K. Dubey, R. Banerji and J.P.N. Chansouria (2004), Evaluation of some essential oils as botanical fungitoxicants in management of post-harvest rotting of citrus fruits, *World J. Microbiol. Biotechnol*. **20**, 317-321.
[19] A. Dikshit and A. Husain (1984), Antifungal action of some essential oils against animal pathogens, *Fitoterapia* **55**, 171-176.