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Chromatographic studies on *Artemisia nilagiria* Leaf Volatile oil

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ABSTRACT

The leaves of Artemisia nilagirica (C.B. Clarke) Pamp., collected from Ooty, Tamil Nadu, India was hydro distilled and analysed by GC-MS. A total of 45 compounds were separated from the oil, accounting for 100 % of the oil. The essential oil was dominated by α-thujone (65.4 %) followed by β-thujone (13.63 %). The percentage of other compounds viz., camphor, thujol, caryophyllene, (-)-germacra-1(10),4(15),5-triene, caryophyllene oxide varied from 1-2.5. The percentage of all other compounds lied below 1 %.

KEY WORDS: *Mugwort, Macippattiri, Karppoorathulasi, Damanaka, Afsantine-Hindi, A. vulgaris.*

INTRODUCTION

Artemisia nilagirica (C. B. Clarke) Pamp. syn. *A. vulgaris* Linn. var. *nilagirica* C. B .Clarke; *A vulgaris* sensu Hook.f., p.p., non Linn., belongs to Asteraceae family. It is distributed in the hilly regions of India especially at Mount Abu in Rajasthan, Western Ghats and from Konkan Southward to Kerala. It is known as Indian Wormwood, Fleabane, Dungwort, Mugwort and Wild Wormwood in English. In Siddha system of medicine, the plant is called as Macippattiri, Makkippu (1); it is named as Damanaka, Pushpachaamara, Gandhotkata in Ayurvedic system of medicine (2); it is called as Afsantine-Hindi in Unani system of medicine (3). *A. nilagirica* is a medicinal herb used as an alternate to cinchona in fevers (2). It is administered as decoction for a week to children affected with measles. Leaves are haemostatic and relieve burning sensation in conjunctivitis (4). The leaf is emmenogogue, menstrual regulator, nervine tonic, stomachic (in anorexia and dyspepsia), anthelmintic, choleretic, diaphoretic. It is useful as an emmenogogue (5). Its essential oil has good larvicidal action. The essential oil distilled from air-dried leaves shows antibacterial and antifungal activity in 1:1000 dilutions (6). Plants at lower altitude showed more percentage of cineol, thujone, thujyl and citral whereas from higher altitude, terpenes are higher in percentage. The highest amount of cineol was reported to be 30% (7). The major chemical of the volatile oil of A. *nilagirica* leaf differs depending on geographical variations (8-11). Croatian oils were reported to yield higher amount and contain more of hydrocarbon than French oils (12). Germacrene D (25%), caryophyllene (20%), α-zingiberene (15%) and borneol (11%) represented as major out of 22 components identified in Pennsylvanian *A. nilagirica* leaf oil (13). Plant from Kodaikanal area of Nilagiri Hills, Tamil Nadu which is about 250 km away from Ooty showed α-thujone (56.3%), β-thujone (7.49%) as dominant out of 21 compounds (14). In this study, author intends to reinvestigate the volatile oil constituents of *A. nilagirica* leaf.

MATERIALS AND METHODS

Plant Material

Leaves of *A. nilagirica* were collected from Ooty, Tamil Nadu, India (2200 m above sea level) in the month of January 2012. The plant was identified by Botanical Survey of India, Coimbatore. The leaves were air-dried and coarsely powdered.

Extraction of Essential Oil

100 g of crushed leaves were subjected to hydro distillation for 3 h using a Clevenger apparatus to give the colourless essential oil (0.4 ml/100 g). It was stored under refrigeration until analysis.

Gas Chromatography-Mass Spectroscopy analysis of the essential oil

The essential oil was analysed by GC-MS using an Agilent 6890 GC with Agilent 5973 mass selective detector [operated in the EI mode (electron energy = 70 eV), scan range was 45-400 amu and scan rate = 3.99 scans/sec], and an Agilent ChemStation data system. The GC column was made up of HP-5 MS fused silica capillary with a (5 %) phenyl-polymethylsiloxane stationary phase, film thickness of 0.25 µm. One microliter of the essential oil was injected into GC. The injector temperature was maintained at 250°C. The flame ionization detector was maintained at 280°C. Nitrogen gas was used as carrier gas and its pressure was maintained at 10 psi. The oven temperature was set at 60–280°C with a gradual increment of 10°C/min. The injected oil was eluted in the DB-5 MS column of 30 m length and 0.25 mm inner diameter and the eluted constituents were detected by flame ionization detector and the GC chromatogram was recorded.

TLC/HPTLC study

The essential oil was applied on TLC aluminium plate (Merck) precoated with silica gel $60F_{254}$ plates of 0.2 mm thickness using the Linomat IV (CAMAG, Muttenz, Switzerland) applicator as 6 mm bands. The plate was developed in the mobile phase of Toluene : Ethyl acetate (10:0.5, v/v) up to 8 cm height from the application point in the presaturated CAMAG TLC twin trough chamber. The developed plate was then air dried, dipped with vanillin-sulphuric acid reagent, heated in an air circulating hot oven at 105°C, till the color appearance of the spots and immediately scanned using CAMAG TLC scanner 030618 attached with WINCATS software at λ 650 using the slit dimension of 5x0.45 mm and tungsten lamp. The plate was then photo documented using the CAMAG visualizer.

RESULTS AND DISCUSSION

The GC-MS analysis showed 45 peaks within 30 minutes of elution time. The mass fragments were compared with the Wiley and NIST libraries and also identified in comparison with the retention indices. Forty compounds were identified and 5 were not identified. The major constituents α and β-thujone contributed to 79.03 % of the total oil. In the earlier report from India, the contribution of these compounds was found to be 63.79 % in the total of 21 compounds. Though the major compounds in the present study remained similar with the earlier study, the concentration and number of other compounds differed from each other.

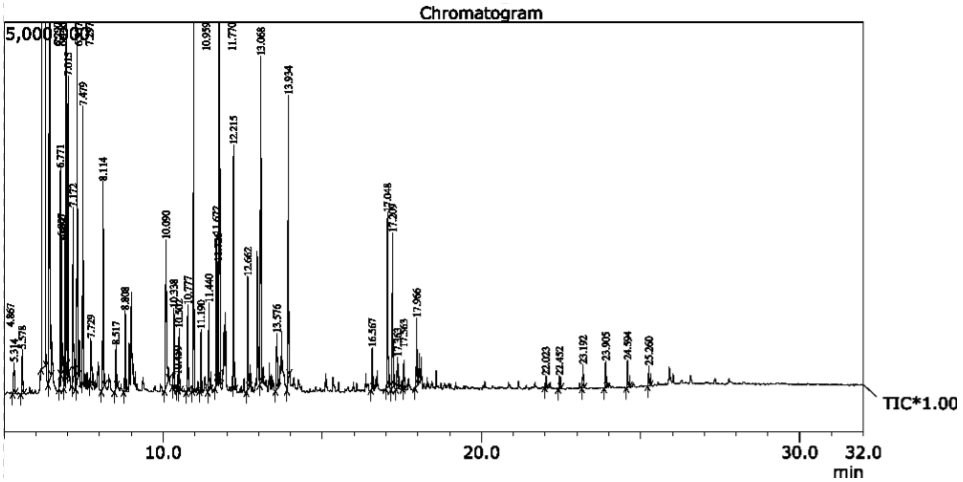

Figure 1. GC profile of *A. nilagirica* leaf volatile oil

Table 1: GC-MS data of *A. nilagirica* leaf volatile oil

Sl.No	Compounds	Retention time (min)	Area %	SI	Identification Method
1.	(+)-Sabinene	3.77	0.31	94	a
2.	4-Thujanol	4.867	0.19	94	a
3.	α-Thujone	5.314	65.40	92	a
4.	Unidentified	5.578	0.43	90	-
	γ-Terpinene	6.290	0.14	96	b, c
5.	1,8-Cineole	6.446	0.28	97	b, c
6.	β-Thujone	6.771	13.63	97	b, c
7.	Unidentified	6.807	0.70	-	-
8.	Unidentified	6.916	0.88	-	-
9.	3-Thujanol	6.957	1.17	93	a, b, c
10.	(+)-Camphor	7.015	1.19	94	a, b, c
11.	(S)-cis-Verbenol	7.172	0.43	97	b, c
12.	(-)-Terpinen-4-ol	7.297	0.95	95	a, b, c
13.	α -Terpineneol	7.479	0.22	96	a, b, c
14.	(-)-Lavandulol	7.725	0.62	96	a, b, c
15.	(+)- Carvone	8.117	0.15	92	a, b, c
16.	cis-Carveol	8.517	0.73	93	a, b, c
17.	Copaene	8.808	0.27	91	b, c
18.	Solanone	10.09	0.25	92	b, c
19.	Butyl benzoate	10.388	0.22	95	a, b, c
20.	Eugenol	10.459	0.67	91	a, b, c
21.	β-Copaene	10.502	0.04	82	a, b, c
22.	Guia-3,9-diene	10.777	0.29	96	a, b, c
23.	β-Sesquiphellandrene	10.959	0.21	93	a, b, c
24.	δ-Cadinene	11.190	0.76	94	a, b, c
25.	Caryophyllene	11.440	1.23	97	a, b, c
26.	Caryophyllene oxide	11.672	1.10	95	a, b, c
27.	(-)-Germacra-1(10),4(15),5-triene	11.726	2.41	96	a, b, c
28.	α-Curcumene	11.770	0.32	96	a, b, c
29.	β-Himachalene	12.215	0.49	92	a, b, c
30.	Caryophyllenol	12.662	0.41	82	a, b, c
31.	α-Humulene	13.068	0.29	96	a, b, c
32.	Cubenol	13.576	0.26	89	a, b, c
33.	Beta-Eudesmol	13.934	0.96	97	a, b, c
34.	Diisobutyl phthalate	16.567	0.26	97	a, b, c
35.	Eicosane	17.048	0.05	92	b, c
36.	n-Butyl phthalate	17.209	0.67	98	a, b, c

37.	Butyl 2-ethylhexyl phthalate	17.363	0.12	92	b, c
38.	Butyl octyl phthalate	17.563	0.58	93	a, b, c
39.	Unidentified	17.966	0.25	93	-
40.	Diisooctyl phthalate	22.023	0.04	93	b, c
41.	Neopentyl undecyl phthalate	22.452	0.16	87	b, c
42.	Tetratriacontane	23.192	0.12	95	a, b, c
43.	Hexatriacontane	23.905	0.08	96	b, c
44.	Unidentified	24.594	0.08	96	-
45.	Tetratetracontane	25.260	0.11	95	b, c

The TLC profile (Fig 2.) showed 5 spots at 0.31 (Blue), 0.38 (Blue), 0.57 (Bluish purple), 0.69 (Purple) and 0.85(Purple). The R_f value are given in Table 2.

The same plate on scanning at 650 nm gave 8 peaks in which 4 peaks are minor. The peak at R_f 0.38 contributed 70.52 % of the total composition of the oil. This peak appeared as a large peak in respect of the spots at R_f 0.31 and 0.38. The other peaks at R_f 0.78 contributed 13.67%; 0.63 contributed 9.60% and 0.68 contributed 3.55%. The other minor peaks contribution is only 2.66%. The HPTLC finger print and the 3D chromatogram are shown in Fig. 3 & 4.

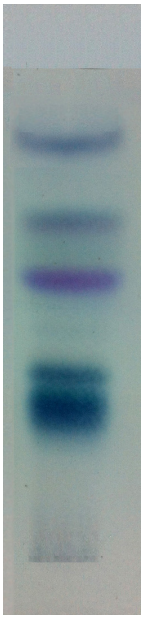

Figure 2. TLC profile of *A. nilagirica* leaf volatile oil in white light after derivatization

Table 2: R_f details of *A. nilagirica* leaf volatile oil

Sl. No	R_f value	Colour of the spot
1	0.31	Blue
2	0.38	Blue
3	0.57	Purple
4	0.69	Bluish purple
5	0.85	Purple

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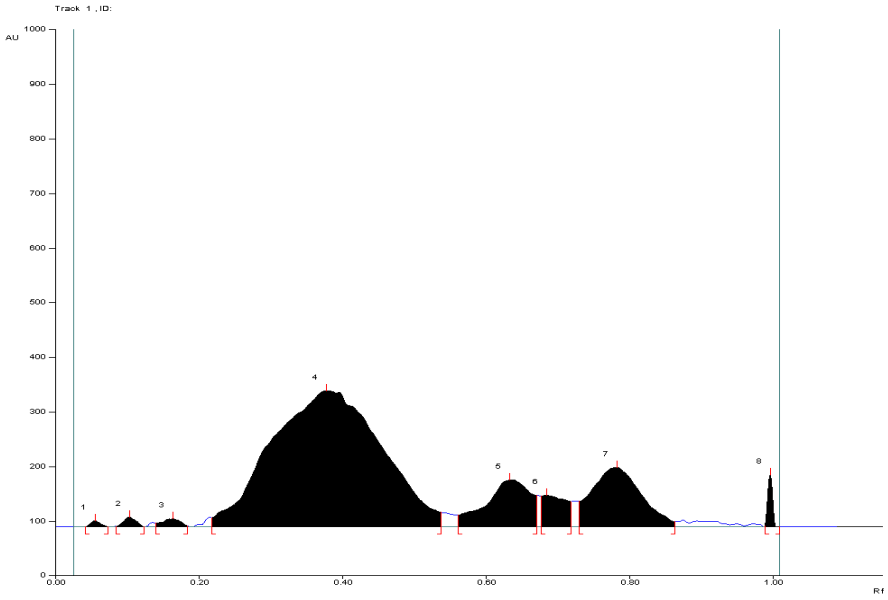

Figure 3. HPTLC chromatogram of *A. nilagirica* leaf volatile oilin white light after derivatization.

Table 3. Table showing R_f value and area of *A. nilagirica* leaf volatile oil

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.04	0.0	0.06	11.2	1.75	0.07	0.2	135.9	0.28
2	0.08	0.6	0.10	17.8	2.78	0.12	0.0	259.6	0.53
3	0.14	6.3	0.16	14.1	2.21	0.18	1.9	336.1	0.68
4	0.22	16.7	0.38	249.1	38.97	0.54	26.2	34672.3	70.52
5	0.56	21.1	0.63	85.4	13.37	0.67	57.3	4722.6	9.60
6	0.68	56.2	0.68	58.0	9.07	0.72	46.1	1747.2	3.55
7	0.73	46.6	0.78	108.6	16.99	0.86	9.6	6719.2	13.67
8	0.99	0.9	1.00	95.0	14.86	1.01	0.0	575.6	1.17

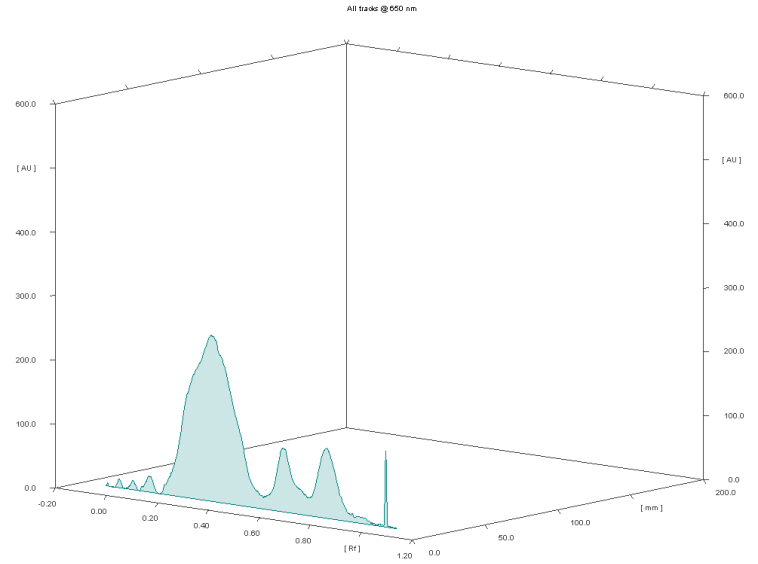

Figure 4. HPTLC 3D chromatogram of *A. nilagirica* leaf volatile oil

CONCLUSION

The composition of the essential of the *A. nilagirica* leaf depends on the different geographical regions. However the Indian variety remains predominant with α and β-thujone and only varies in the number and concentration of the other minor compounds.

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