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Chemical constituents of leaf essential oil of *Nepeta laevigata* (D. Don) Hand.-Mazz from Kumaun Himalaya

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ABSTRACT

The chemical profile of the hydro distilled essential oil obtained from the aerial parts of *Nepeta laevigata* from Kumaun Himalaya was analysed by capillary GC-FID and GC-MS. The essential oils of aerial parts led to the identification of 64 constituents accounting for 83.82% of the total oil composition. The 1, 8-cineole (9.08%), caryophyllene oxide (11.16%), manool (7.91%) and pimaradiene (4.60%) were the principle components.

Keywords: *Nepeta laevigata*, Lamiaceae, Essential Oil, Caryophyllene Oxide, GC-FID, GC-MS,

1. Introduction

Nepeta (Lamiaceae) is a genus of about 250 species of flowering herbaceous, small shrub, rarely tree, often with quadrangular stems, glandular and aromatic with opposite leaves placed successively at right angles to each other^[1]. *N. laevigata* is an erect 30-90 cm, flowers are blue-purple, in dense whorls crowded into long terminal spikes, leaves usually stalked, ovate acute to triangular-lanceolate, found at the height of 3000-3500 m near glaciers^[2]. Among 31 species reported in the Himalayan region six viz, *N. connata* Royle ex Benth, *N. leucophylla* Benth, *N. ciliaris* Benth, *N. distans* Royle ex Benth, *N. elliptica* Royle ex Benth, *N. spicata* Benth, are found in the Kumaun region^[3]. *Nepeta* species are used as antispasmodic, diuretic, febrifuge, diaphoretic, antimicrobial and antiseptic agents and also in the treatment of dysentery, tooth trouble and kidney and liver diseases. Diverse biological activities viz. feline attractant, canine attractant, insect repellent and arthropod defense are accredited to the presence of biologically active iridoids/monoterpene nepetalactones in *Nepeta* species^[4, 5, 6]. Our previous studies on *Nepeta* species showed, 7R- trans, trans-nepetalactone (80.0%) from the essential oil of *N. elliptica*^[7]. The essential oil from the aerial parts of *N. govaniiana* was shown to contain pregeijerene (38.4%), (+)-isoiridomyrmecin and 4 α , 7 α , 7 α -nepetalactone (13.9%) as major constituents^[8] β -Caryophyllene, linalool and germacrene D were reported as the major constituents from the essential oil of *N. spicata*^[9]. Coleon U-12 methyl ether, dehydro Coleon U-12 methyl ether and its 7-(1-methyl ethenyl) homologue were isolated from *N. elliptica*^[10]. Three new iridodial derivatives viz. iridodial β -monoenoil acetate, dihydro iridodial diacetate and iridodial dienol diacetate have been isolated from the essential oil of *N. leucophylla*^[11], 1, 8-cineole and sabinene were reported as the major constituents from *N. discolor*^[12]. Iridodial β -monoenoilacetate isolated from essential oil of *N. leucophylla* Benth. and actinidine isolated from essential oil of *N. clarkei* Hook f. were shown to have significant antibacterial and antifungal activities. The essential oils from six Himalayan *Nepeta* species, viz. *Nepeta leucophylla* Benth., *Nepeta discolor* Royle ex Benth., *Nepeta govaniiana* Benth., *Nepeta clarkei* Hook f., *Nepeta elliptica* Royle ex Benth. and *Nepeta erecta* Benth., were tested for their *in vitro* antimicrobial activity against six pathogenic bacterial and two fungal strains with some significant results^[13].

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2. Materials and methods

2.1 Plant Material

Nepeta laevigata was collected from Milam glacier (3400 mt) at flowering stage in Sept.2006. Identification of the plant material was done at the Botanical Survey of India, Dehradun. The voucher specimen (No.CHEM/DST/06/04) has been deposited in the Phytochemistry research laboratory, Kumaun University, Nainital.

2.2 Oil Isolation

The fresh plant materials (5.0 kg leaves) was subjected to steam distillation in a copper electric still fitted with spiral glass condensers for two hours obtaining 10 L water distillate. The distillates were saturated with NaCl and the oils were extracted with hexane and dichloromethane. The organic phase was then dried over anhydrous Na₂SO₄ and the solvent distilled in a thin film rotary vacuum evaporator at 30^o C. The oil yields 0.3% (v/w) respectively.

2.3 GC and GC/MS

The oils were analyzed by using a Nucon 5765 gas chromatograph (Rtx-5 column, 30 m × 0.32 mm, FID), split ratio 1: 48, N₂ flow of 4 kg/cm² and on Thermo Quest Trace GC 2000 interfaced with Finnigan MAT Polaris Q Ion Trap Mass spectrometer fitted with a Rtx-5 (Restek Corp.) fused silica capillary column (30 m × 0.25 mm; 0.25 μm film coating). The column temperature was programmed 60-210 °C at 3 °C / min using He as carrier gas at 1.0 mL/min. The injector temperature was 210 °C, injection size 0.1 μL prepared in hexane, split ratio 1:40. MS were taken at 70 eV

with a mass range of 40-450 amu. The identification was done on the basis of Retention Index (RI), MS Library search (NIST and WILEY), by comparing with the MS literature data [14], 1H-NMR and ¹³C-NMR data of major isolates. The NMR spectra were taken in CDCl₃ on a Bruker-Avance DRX 300 MHz at 25^o C. The percentage contents of constituents were determined on the basis of FID response on GC.

3. Results and discussion

The GC and GC-MS analysis of leaf oil of *N. laevigata* resulted in the identification of 64 constituents. The identified constituents of the oil are listed in Table 1 in order of their elution in Rtx-5 column. The major constituents in leaf oil were caryophyllene oxide (15.16 %), 1, 8-cineole (11.08%), (7.91 %) β-caryophyllene (5.71%) and pimaradiene (4.65 %), whereas the essential oil composition of *Nepeta laevigata* from Jammu and Kashmir showed the presence of β-citronellol (16.5 %), germacrene D (19.4 %), β-caryophyllene oxide (10.8 %), α-bisabolol oxide B (12.4 %), β-bourbonene (4.5 %), α-humulene (3.5 %), spathulenol (3.9 %) and α-bisabolol (5.3 %) as major compounds. In literature, the iridoid monoterpenes nepetalactones frequently appear as the main constituents of *Nepeta* essential oils which are responsible for various biological activities. Nepetalactones are also absent in previous report on essential oil from *N. laevigata* from Jammu and Kashmir [15]. The essential oil composition of our collection is totally different from previous report. This is first report of an essential oil of *N. laevigata* from Kumaun Himalaya.

Table 1: Chemical Constituents of Leaf Essential Oil of *Nepeta laevigata*

S. No.	Compounds	RI	% Composition (FID)	Mode of identification
1	α-thujene	932	1.11	a,b
2	α-pinene	939	2.77	a,b
3	camphene	954	1.15	a,b
4	sabinene	978	2.87	a,b
5	β-pinene	981	0.54	a,b
6	β-myrcene	994	0.23	a,b
7	α-phellandrene	1006	0.14	a,b
8	α-terpinene	1064	1.10	a,b
9	p-cymene	1026	t	a,b
10	β-phellandrene	1037	0.30	a,b
11	1, 8-cineole	1038	11.08	a,b
12	(Z)-β-ocimene	1041	t	a,b
13	(E)-β-ocimene	1050	1.10	a,b
14	γ-terpinene	1065	2.74	a,b
15	(Z)-sabinene hydrate	1069	2.04	a,b
16	terpinolene	1086	t	a,b
17	(E)-sabinene hydrate	1069	2.06	a,b
18	linalool	1101	t	a,b
19	(Z)-p-menth-2-en-1-ol	1120	0.10	a,b
20	dihydrolinalool	1130	t	a,b
21	(E)-p-menth-2-en-1-ol	1145	1.10	a,b
22	benzyl acetate	1157	0.71	a,b
23	pinocarvone	1160	0.25	a,b
24	terpinen-4-ol	1175	2.54	a,b
25	p-cymen-8-ol	1177	0.23	a,b

26	α -terpineol	1189	0.48	a,b
27	thymol methyl ether	1192	0.21	a,b
28	carvacrol methyl ether	1195	0.26	a,b
29	thymol	1340	t	a,b
30	carvacrol	1355	t	a,b
31	δ -elemene	1339	t	a,b
32	α -copaene	1378	0.37	a,b
33	β -elemene	1389	0.10	a,b
34	β -caryophyllene	1418	5.71	a,b
35	(Z)- β -farnesene	1440	0.21	a,b
36	α -humulene	1457	t	a,b
37	(E)- β -farnesene	1459	t	a,b
38	germacrene D	1482	0.67	a,b
39	β -selinene	1489	1.24	a,b
40	α -selinene	1498	0.60	a,b
41	α -muurolene	1499	0.21	a,b
42	γ -cadinene	1524	0.44	a,b
43.	germacrene D-4-ol	1574	2.05	a,b
44.	caryophyllene oxide	1581	15.16	a,b,c
45.	globulol	1590	t	a,b
46.	humulene epoxide II	1606	0.14	a,b
47.	10- <i>epi</i> - γ -eudesmol	1619	0.36	a,b
48.	γ -eudesmol	1630	t	a,b
49.	<i>epi</i> - α -cadinol	1640	1.81	a,b
50.	α -muurolol	1644	t	a,b
51.	cubenol	1645	0.30	a,b
52.	β -eudesmol	1650	0.10	a,b
53.	α -cadinol	1653	t	a,b
54.	bulnesol	1666	t	a,b
55.	β -bisabolol	1671	t	a,b
56.	<i>epi</i> - β -bisabolol	1674	0.16	a,b
57.	laurenene	1876	0.22	a,b
58.	<i>epi</i> -laurenene	1901	0.19	a,b
59.	isopimara-9 (11),15-diene	1903	0.62	a,b
60.	pimaradiene	1945	4.65	a,b
61.	sandaracopimara-8 (14)-15 diene	1965	2.02	a,b
62.	isokaurene	2038	2.64	a,b
63.	kaurene	2042	1.17	a,b
64.	manool	2054	7.91	a,b
Monoterpene hydrocarbons			26.90	
Oxygenated monoterpenes			9.66	
Sesquiterpene hydrocarbons			9.55	
Oxygenated sesquiterpenes			25.08	
Diterpene hydrocarbon			11.51	
Oxygenated diterpene			7.91	
Total			89.61	

a=Retention Index (RI) on Rtx-5 capillary column, b=MS (GC-MS), c=¹H NMR ¹³C NMR data, Compounds >3.0% represented in bold face, t= trace (<0.1%).

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