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Essential Oil Composition of *Nepeta raphanorhiza* Benth growing in Kashmir valley

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Abstract: The essential oil composition of the aerial parts of *Nepeta raphanorhiza* is reported for the first time. Capillary GC-FID & GC-MS analysis of the essential oil led to the identification of 16 components accounting for 97.5% of the total oil. Sesquiterpene hydrocarbons dominated the oil composition accounting for 65.3 % followed by monoterpene hydrocarbons constituting 19.5 % of the total oil composition. The major c omponents were (Z) -*β*- farnesene (49.2%), *δ*-3-carene (12.3%), *α*-bisabolene (9.4%) and germacrene-D-4-ol (5. 8%).

Keywords: *Nepeta raphanorhiza*; Lamiaceae; GC-FID; GC-MS; (*Z*)-*β*- farnesene; *δ*-3-carene; *α*- bisabolene; ger macrene-D-4-ol.

1. Plant Source

Nepeta is a multiregional genus of the family Lamiaceae comprising about 250 species distributed mainly in Southwest & central Asia, Europe, North Africa & North America. About 30 species occur in India, mostly distributed in temperate Himalayas & a few on foothills & plains [1, 2]. Several *Nepeta* species are used in traditional system of medicine to treat dysentery, kidney and liver diseases, and teeth troubles. They are also used as diuretic, diaphoretic, vulnerary, antispasmodic, antiasthmatic, tonic, febrifuge and sedative agents [3,4,5,6]. Several *Nepeta* species are also reported to reduce serum lipids and anti-inflammatory effects [7, 8]. Most *Nepeta* species are rich in essential oils characterized by biologically active iridoids/monoterpene nepetalactones, possessing diverse biological activities like feline attractant, canine attractant and insect repellant [9, 10].

Nepeta raphanorhiza Benth locally known as "Kanz-gogal" is a low growing perennial herb with tubers growing up to 20 mm in diameters, stem prostrate ranging 10-25 cm. Leaves are green,

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broad triangular, ovate. It is mainly found in Afghanistan, Pakistan & Northwest India. Its rhizome is edible and is eaten by the locals.

The plant material of *Nepeta raphanorhiza* was collected from Pulwama region of Kashmir valley during April 2010 and identified in the Centre for Plant Taxonomy, Department of Botany, University of Kashmir, Srinagar (India). A voucher specimen was deposited in the herbarium of the department (voucher No. KASH-1043).

2. Previous Studies

Literature survey reveals that no phytochemical investigations from any part of the globe have been previously carried out on the essential oil of *Nepeta raphanorhiza.*As such the objective behind this research work was to characterize for the first time the essential oil components of *Nepeta raphanorhiza* growing in Kashmir valley using a combination of capillary GC-FID and GC-MS analytical techniques.

3. Present Study

The fresh plant material was finely chopped and then subjected to hydro-distillation using Clevenger-type apparatus for 3 hours. The oil yield was found to be 0.1% as calculated on fresh weight basis (v/w). The oil was dried over anhydrous Na_2SO_4 and stored in a sealed vial at - 4^0C until gas chromatographic analysis.

GC-MS analysis was carried on a Varian Gas Chromatograph series 3800 fitted with a VF-5 m s fused silica capillary column (60m x 0.25mm, film thickness 0.25µm) coupled with a 4000 series ma ss detector under the following conditions: injection volume 0.5 µl with split ratio 1:60, helium as carri er gas at 1.0 mL/min constant flow mode, injector temperature 230 ^{O}C, oven temperature 60 ^{0}C to 280 ^{0}C at 3 ^{0}C/min. Mass spectra: electron impact (EI+) mode,70 ev and ion source temperature 250 ^{0}C. M ass spectra were recorded over 50-500 a.m.u range**.** GC/FID was carried out on Perkin Elmer auto system XL Gas Chromatograph 8500 series equipped with flame ionization detector (FID) and head space analyzer using a fused silica capillary RTX-5 column (30m x 0.32 mm, film thick- ness 0.25µm) coated with dimethyl polysiloxane .Oven temperature was programmed from 60 to 280 ^{0} C at 3 ^{0}C/min, with injector temperature 230 ^{0}C and detector temperature 250 ^{0}C. Injection volume 1µl, nitrogen was used as a carrier gas (1.0 mL/min).

Identification of the essential oil constituents was done on the basis of Retention Index (RI, determined with respect to homologous series of n-alkanes (C_9-C_{24}, Polyscience Corp., Niles IL) under the same experimental conditions), co-injection with standards (Sigma Aldrich and standard isolates), MS Library search (NIST 98 and WILEY), by comparing with the MS literature data [12,13].The relative percentages of the individual components were calculated based on GC peak area (FID response) without using correction factors.

The essential oil components identified in *N. raphanorhiza* are shown in Table 1. GC-FID and GC-MS analysis led to the identification of 16 components (supporting information) representing 97.5% of the total oil composition. The essential oil is dominated by the presence of sesquiterpene hydrocarbons constituting 65.3% followed by monoterpene hydrocarbons which constitute 19.5% of the total oil composition. The principal components of the sesquitrepene hydrocarbons were (*Z*)-*β*-farnesene (49.2%), *α*-bisabolene (9.4%), *β*-caryophyllene (3.2%) and *α*-humulene (2.0%), *δ*-3-carene was the main constituent of monoterpene hydrocarbons (12.3%). Some other chemical constituents with lesser quantity in the oil were sabinene (2.5%), germacrene-D-4-ol (5.8%), and caryophyllene oxide (3.4%). The characteristic feature of the essential oil composition is the low quantity of 4a*α*, 7*β*, 7aα-nepetalactone isomer.

Table 1. Chemical composition of the essential oil of *Nepeta raphanorhiza* growing in Kashmir.

RI[a]	RI[b]	Compound	% composition
937	932	α-Pinene	1.6
947	946	Camphene	0.4
972	969	Sabinene	2.5
976	974	β-Pinene	1.2
1011	1008	δ-3- Carene	12.3
1016	1014	α-Terpinene	1.5
1290	1287	Bornyl acetate	1.8
1394	1391	Nepeta lactone- (4aα,7β,7aα)	0.5
1419	1417	β-Caryophyllene	3.2
1443	1440	(*Z*)-β-Farnesene	49.2
1454	1452	α-Humulene	2.0
1486	1484	Germacrene D	0.8
1509	1506	α-Bisabolene	9.4
1525	1522	δ-Cadinene	0.7
1578	1574	Germacrene-D-4-ol	5.8
1587	1582	Caryophyllene oxide	3.4
1656	1652	α-Cadinol	1.0
		Class composition	
		Monoterpene hydrocarbons	19.5
		Oxygenated monoterpenes	2.3
		Sesquiterpene hydrocarbons	65.3
		Oxygenated sesquiterpenes	10.4
		Total	97.5

RI [a] ; Retention indices on RTX-5column (relative to n-alkanes).
RI [b]; Retention indices on DB-5 column (reported from literature).

The essential oils of several species of the genus *Nepeta* such as *N. sintenisii* Bornm. [14], *N. atlantica* Ball*, N. tuberosa* Linn. *, N. granatensis* Boiss*. N. cataria* Linn. [15], *N. cephalotes* Boiss. [16], *N. nuda* Linn*.* [17] and *N.coerulea* Aiton [18] have been examined and are characterized by the pre sence of one or more of the nepetalactone isomers viz, 4aα,7α,7aβ-nepetalactone, 4aα,7β,7aβ-nepeta-lactone and 4aα,7α,7aα-nepetalactone. These isomers have been labeled as the biochemical markers of the *Nepeta* essential oils and are very useful in chemotaxonomic studies. However, several of the Himalayan *Nepeta* species which are found in Jammu and Kashmir like *N.floccosa* Benth., *N.discolor* Royle ex Benth., *N.royleana* Steward [19], *N. leucophylla* Benth., *N. govaniana* (Wall. Ex Benth.) Ben th., *N. clarkie* Hook., *N. elliptica* (Royle ex Benth.) Benth., *N. erecta* (Royle ex Benth.). Benth. [20] and *N. laevigata* (D. Don) Hand. Mazz. [21] contain either low content of these nepetalactones or are absent. Comparing our results with other Himalayan *Nepeta* species, it implies that various marker constituents other than the nepetalactone isomers like 1,8-cineole, α-Pinene, β-Pinene, Iridodial β-monoenol acetate, Pregeijerene, Neral, geranial, β-Citronellol, β-Caryophyllene, Germacrene D, α-Bisabolol oxide B have been identified in the essential oils of these Himalayan *Nepeta* species. Thus the current study and the previous literature data indicate a considerable chemodiversity in the essential oils of Himalayan *Nepeta* species.

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