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Analysis of the diterpene rich essential oil of *Nepeta clarkei* Hooke. from Kashmir Himalayas by capillary GC-MS.

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Abstract: The essential oil of *Nepeta clarkei* obtained by hydrodistillation was analyzed by capillary Gas Chromatography-Mass Spectrometry (GC-MS). The analysis of the oil led to the identification of 20 chemical constituents accounting for 95.3% of the total oil composition. The oil composition was dominated by the presence of diterpenes constituting 74.2% of the total oil composition. The principal components were kaur-16-ene (36.6%), pimara-7,15-dien-3-one (19.7%), caryophyllene oxide (14.1%), methyl abietate (5.3%) and manoyl oxide (4.4%).
Key words: *Nepeta clarkei*, GC-MS, hydrodistillation, diterpenes, Kaur-16-ene.

INTRODUCTION

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

N. clarkei Hooke. is a perennial, herbaceous wild plant, characterized by paniculate inflorescence, verticillasters separate with diffused rhizomes. Literature survey reveals that some authors have studied the essential oil of *N. clarkei* from other parts of the country like Malari, Chamoli (India). These authors have reported strong antibacterial and antifungal activities of the *N. clarkei* essential oil [11]. In yet another study, some authors have isolated an unusual and rare terpene alkaloid named actinidine from the steam distilled essential oil of the aerial parts of *N.clarkei* collected from the Kumaon Himalayan region. They also reported that the isolated actinidine strongly inhibited the growth of mycotoxin producing and plant pathogenic fungi [12].

As part of our Institute's programme to screen the rich aromatic flora of the region for new essential oils and aroma chemicals, we report here for the first time the essential oil composition of *N. clarkei* growing wild in

high Himalayas of Kashmir (India) using capillary GC-MS analytical technique.

EXPERIMENTAL

Plant material

The plant material of *N. clarkei* was collected from the upper reaches of Gulmarg at an altitude of 2600m (Kashmir) and was identified by the department of plant taxonomy, University of Kashmir. A voucher specimen was deposited in the herbarium of the department (Voucher No. KASH- 2737).

Recovery of essential oils

Fresh 2000 Grams of the aerial parts of the plant were subjected to hydrodistillation in a conventional Clevenger type apparatus for four hours. The oil obtained was dried over anhydrous sodium sulphate and stored at 4 ^{0}C in a sealed vial prior to analysis. The yield of the oil was found to be 0.05% calculated on fresh weight basis.

GC-MS analysis

GC-MS analysis was carried on a Varian Gas Chromatograph coupled with a mass spectrometer-4000 series system fitted with a VF-5 ms fused silica capillary column (60mx0.25mm, film thickness 0.25µm). Injection volume 1µl with split ratio 60, helium as carrier gas at 1ml/min constant flow mode, injector temperature 230 ^{0}C, column temperature was programmed from 40 ^{0}C to 250 ^{0}C at 3 ^{0}C/min. Mass spectra: electron impact (EI^+) mode,70ev and ion source temperature 250 ^{0}C. Mass spectra were recorded over 50-500 a.m.u range. Identification of peaks was carried out by comparison of the mass spectra with those reported in NIST and WILEY electronic libraries and those published in literature [13]; by comparison of retention indices (relative to n-alkanes) with literature data[13]; by peak enrichment and co-injection with standard samples wherever possible. In addition, Mass Finder Computer Library was also used for identification.

RESULTS AND DISCUSSION

The various chemical constituents identified in the essential oil are reported in the Table and the compounds are listed in order of their elution from the VF-5 ms capillary column. The total ion chromatogram of the essential oil is shown in figure-1. Twenty chemical constituents were identified in the essential oil, and the oil composition was dominated by the presence of diterpenes accounting for 74.2% of the total oil composition. The diterpenes identified in the essential oil were classified into simple diterpenes (non-oxygenated) and oxygenated diterpenes. Kaur-16-ene was the only simple diterpene present constituting 36.6% of the total oil composition. Oxygenated diterpenes constituted 37.6% of the total composition and were represented by pimara-7,15-dien-3-one (19.7%), methyl abietate (5.3%), manoyloxide (4.4%), pimara-7,15-dien-3-ol (4.0%), m ethyl isopimarate (3.0%) and phytol (1.2%).Sesquiterp enoids constituted 20.3% of the total oil composition and the major component was caryophyllene oxide (14.1%). The chemical structures of some of the representative compounds present in the essential oil of *N. clarkei* are shown in figure-2.

Survey revealed that the essential oil of *N. clarkei* has been studied from the Uttarakhand region of India and the main chemical constituents of the essential oil were reported to be β-sesquiphellandrene, germacrene D, α-guaiene and iridodial β-monoenol acetate diastereomers. The authors also reported a strong *in vitro* antimicrobial activity of the essential oil against six pathogenic bacteria and two fungal strains [11]. In another study from the Kumaon Himalayan region of India, an unusual, rare terpene alkaloid named actinidine was reported from the steam distilled essential oil of the aerial parts of *N. clarkei* which was also reported to strongly inhibit the growth of mycotoxin producing and plant pathogenic fungi [12].

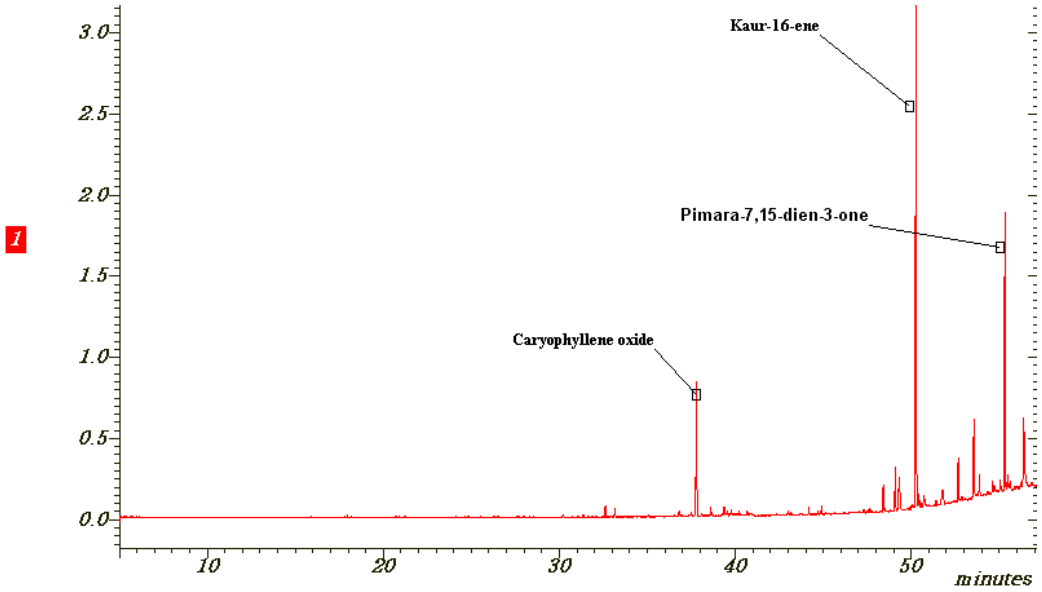
Comparing our results with the previously reported data, the essential oil composition of *N. clarkei* in the current study which was dominated by diterpenes showed remarkable qualitative and quantitative compositional differences with the previously reported chemical constituents of the essential oil of *N. clarkei*. These compositional differences in the essential oils can be attributed to different agro climatic, geographical conditions and harvesting period.

Table : Essential oil composition of the aerial parts of *N. clarkei* growing wild in the upper reaches of Gulmarg (Kashmir).

S.no	compound	RT	% age	Methods of identification
1	Limonene	17.97	0.3	A, B
2	α- Thujone	21.24	0.1	A, B
3	Eugenol	30.22	0.4	A, B
4	β- Bourbonene	31.37	0.4	A, B
5	β- Caryophyllene	32.61	1.1	A, B,C
6	α- Bergamotene	32.61	0.4	A, B
7	(Z)-β-Farnesene	33.15	0.8	A, B
8	α- Humulene	33.77	0.3	A, B
9	(E,Z)-α-Bisabolene epoxide	37.42	1.0	A, B,C
10	Caryophyllene oxide	37.79	14.1	A, B,C
11	Isoaromadendrene epoxide	38.07	0.3	A, B
12	Viridiflorene	39.36	1.9	A, B,C
13	Hexahydrofarnesyl acetone	44.18	0.8	A, B
14	Manoyl oxide	49.30	4.4	A, B
15	Kaur-16-ene	50.26	36.6	A, B
16	Phytol	50.71	1.2	A, B
17	Methyl isopimarate	52.66	3.0	A, B
18	Methyl abietate	53.56	5.3	A, B
19	Pimara-7,15-dien-3-one	55.32	19.7	A, B
20	Pimara-7,15-dien-3-ol	56.38	4.0	A, B
	Sesquiterpene hydrocarbons		4.9	
	Oxygenated sesquiterpenes		15.4	
	Simple diterpenes		36.6	
	Oxygenated diterpenes		37.6	
	Others		0.8	
	Total (%)		**95.3**	

Compounds are listed in the order of their elution from VF-5 ms capillary column.
A = Kovat's Indices; B = Mass Spectra; C = Co-injection.

Fig-1: Total ion chromatogram of the essential oil of *N. clarkei* showing three main chemical constituents of the oil.



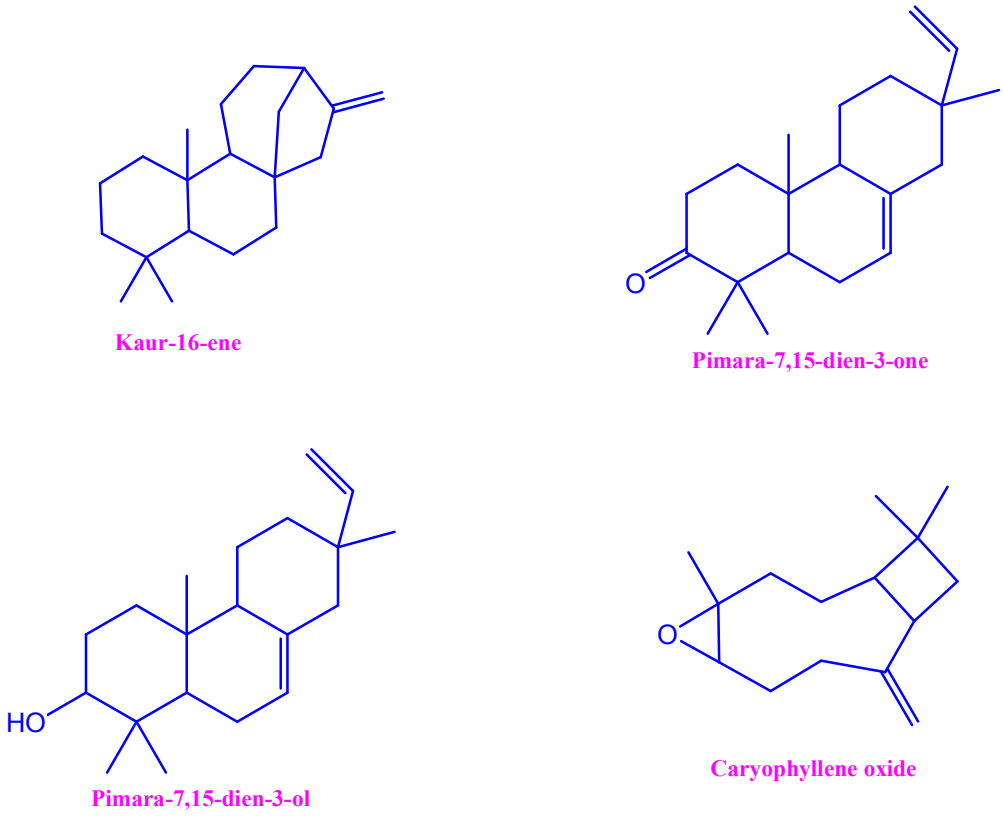


Fig-2: Structures of some of the main chemical constituents of the essential oil of *N. clarkei*

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