

CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF *Nepeta laevigata* AND *Nepeta elliptica* FROM INDIA

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The genus *Nepeta* (Lamiaceae), also called Glechoma and Cataria, is a multiregional genus and consists of about 250 species of perennial herbs distributed in central and southern parts of Europe, Asia, and the Middle East [1–3]. These plants are commonly known as catmint [4], and about 30 species occur in India. Many *Nepeta* species have been reported to be biologically active and are used in folk medicine because of their spasmodic, diuretic, antiseptic, antitussive, antiasthmatic, and febrifuge activities [5–9]. Several *Nepeta* species are also reported to reduce serum lipids and to possess anti-inflammatory effects [10, 11]. 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 repellent, arthropod defense [12, 13], antibacterial, antifungal, and antiviral activities [14].

N. laevigata and *N. elliptica* are distributed through Afghanistan, China, Nepal, Pakistan, and India. In India, the two plant species are largely confined to Himachal Pradesh, Jammu and Kashmir, and Uttar Pradesh. *Nepeta laevigata*, also known as *Betonica laevigata*, is a perennial aromatic herb that grows to a height of 80 cm, is white pubescent, and has petiole 2–12 mm and leaf blade ovate to triangular, while *N. elliptica* is a small ascending or flexuous herb, 30–60 cm high.

Both *N. laevigata* and *N. elliptica* are used in traditional medicine. *Nepeta laevigata*, is reported to be used locally in fevers and for sore throat, while, as an infusion of the seeds, *N. elliptica* is used in dysentery.

According to our finding, there is no report on the chemical composition of the essential oil of *N. laevigata* and *N. elliptica* growing in J & K, India, so the aim of the present work was to compare the chemical composition of these two *Nepeta* species.

The chemical constituents of the volatile oils were analyzed by capillary GC-FID and GC-MS. The components of the oils of the air-dried aerial parts of *N. laevigata* and *N. elliptica* are listed in Table 1 with their percentages and relative retention indices (RRI). The different chemical constituents of the essential oils are listed in order of their elution from an RTX-5 column. As shown, 24 components belonging to different class of compounds were identified in the oil of *N. laevigata*, making up 86.7% of the total oil. β -Citronellol (16.5%), germacrene D (19.4%), β -caryophyllene (10.8%), α -bisabolol oxide B (12.4%), β -bourbonene (4.5%), α -humulene (3.5%), spathulenol (3.9%), and α -bisabolol (5.3%) were the major ones. Other constituents such as $4\alpha,7\alpha,7\alpha$ -nepetalactone (2.0%), *allo*-aromadendrene (1.1%), and caryophyllene oxide (3.2%) were present in small amounts. In addition, some other constituents such as β -pinene, 1,8-cineole, linalool, geraniol, citronellyl acetate, etc. were present in trace amounts. The essential oil composition is dominated by the presence of sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and oxygenated monoterpenes constituting 40.9%, 25.1%, and 20.7%, respectively, of the total oil composition. Of the various nepetalactone isomers, viz., $4\alpha,7\alpha,7\alpha\beta$ -nepetalactone, $4\alpha,7\beta,7\alpha\beta$ -nepetalactone, and $4\alpha,7\alpha,7\alpha$ -nepetalactone, which have been labeled as the biochemical markers of the *Nepeta* essential oils and are very useful in chemotaxonomic studies, only one nepetalactone isomer viz., $4\alpha,7\alpha,7\alpha$ -nepetalactone, as a minor constituent, was present in the essential oil of *Nepeta laevigata*. β -Caryophyllene, which is one of the major constituents of the essential oil, has been reported in some *Nepeta* species such as *N. depauperata* [15], *N. flavida* [16], and *N. nuda* [17] as the major component. Likewise, germacrene-D, the other major constituent of the oil sample, has also been reported in various other *Nepeta* species such as *Nepeta macrosiphon* [18] and *Nepeta sintensii* [19]. In addition, the other major components such as β -bourbonene and spathulenol have been reported in *N. depauperata* [15], *N. macrosiphon* [18], and *N. sintensii* [19].

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TABLE 1. The List of Detected Compounds in the Essential Oils of *Nepeta laevigata* (1) and *Nepeta elliptica* (2) along with Their Relative Percentages

Compound ^a	RRI	1, % ^b	2, % ^b	Compound ^a	RRI	1, % ^b	2, % ^b
(Z)-3-Hexen-1-ol	767	0.3	–	Zingiberene	1492	–	2.8
β -Pinene	973	0.2	0.2	Bicyclogermacrene	1498	1.0	13.1
1,8-Cineole	1024	0.5	–	β -Bisabolene	1502	–	3.2
Linalool	1094	0.3	–	γ -Cadinene	1513	–	3.3
Citronellal	1146	0.2	–	δ -Cadinene	1520	0.4	–
α -Terpineol	1184	0.4	1.7	Elemol	1552	–	3.5
β -Citronellol	1220	16.5	–	Germacrene B	1559	–	0.7
Geraniol	1250	0.5	–	Spathulenol	1575	3.9	2.6
Geranial	1263	–	0.4	Caryophyllene oxide	1582	3.2	0.4
Citronellyl acetate	1352	0.3	–	<i>epi</i> - α -Cadinol	1636	0.2	5.7
4 α ,7 α ,7 α -Nepetalactone	1355	2.0	–	α -Bisabolol oxide B	1657	12.4	–
α -Copaene	1376	0.2	–	α -Bisabolol	1682	5.3	–
β -Bourbonene	1385	4.5	–	α -Bisabolol oxide A	1746	0.1	–
4 α ,7 β ,7 α -Nepetalactone	1389	–	0.3	Monoterpene hydrocarbon		0.2	0.2
β -Elemene	1395	–	23.4	Oxygenated monoterpene		20.7	2.4
Longifolene	1406	–	0.7	Sesquiterpene hydrocarbon		40.9	68.8
β -Caryophyllene	1416	10.8	5.5	Oxygenated sesquiterpene		25.1	12.2
α -Humulene	1455	3.5	11.8	Others		0.3	–
<i>allo</i> -Aromadendrene	1460	1.1	0.7	Total		86.7	83.6
Germacrene D	1481	19.4	3.6				

–: not detected.

^aCompounds listed in order of elution from RTX-5 column.

^bPercentage was calculated from the GC-FID peak area without the use of correction factors.

RRI: relative retention indices to C₆–C₂₆ *n*-alkanes on RTX-5 column.

However, 1,8-cineole and linalool, which were the most abundant components in many *Nepeta* species [20–22], were present in traces in the present work. α -Bisabolol oxide-B and α -bisabolol, which were present in sizable amounts in the present study, occur in very few *Nepeta* species. This is not unexpected since plants often manufacture different secondary metabolites when grown in different geographical locations.

As shown in Table 1, nineteen compounds were identified in the essential oil of *Nepeta elliptica*, accounting for 83.6% of the total oil. β -Elemene (23.4%), bicyclogermacrene (13.1%), α -humulene (11.8%), β -caryophyllene (5.5%), germacrene D (3.6%), β -bisabolene (3.2%), γ -cadinene (3.3%), elemol (3.5%), zingiberene (2.8%), and spathulenol (2.6%) were the major ones; the other constituents such as β -pinene, α -terpineol, geranial, 4 α , 7 β , 7 α -nepetalactone, longifolene, *allo*-aromadendrene, germacrene B, and caryophyllene oxide were present as minor constituents. The essential oil of *N. elliptica* is dominated by the presence of sesquiterpene hydrocarbons and oxygenated sesquiterpenes, accounting for 68.8% and 12.2%, respectively, of the total oil composition. Bicyclogermacrene, which is a major constituent of *N. elliptica*, has also been reported in other *Nepeta* species such as *Nepeta macrosiphon* [18]. Similarly elemol, β -bisabolene, and *epi*- α -cadinol, which are present in sizable amounts in our oil sample, have been reported in *Nepeta sintensii* [19], though in lesser percentages. On comparative analysis of the essential oils of the two *Nepeta* species concerned, it appears that both essential oils are dominated by sesquiterpenoids (including sesquiterpene hydrocarbon and oxygenated sesquiterpenes), accounting for 81% (*N. laevigata*) and 66% (*N. elliptica*) of the two essential oils, respectively.

Plant Material. The aerial parts (inflorescences) of *Nepeta laevigata* and *Nepeta elliptica*, growing wildly in the high Himalayas of J & K India, were collected in June 2009. Voucher specimens of the plant were deposited in the herbarium of the Centre for Plant Taxonomy, University of Kashmir, J & K India.

Extraction of the Essential Oil. The air-dried aerial parts of the plant were subjected to hydrodistillation using a Clevenger type apparatus for 3 hours. After drying over anhydrous Na₂SO₄, the oil samples were stored in glass vials at 4°C prior to analysis. The percentage of oil from *Nepeta laevigata* was found to be 0.8%, and that of *Nepeta elliptica* was 0.3%, calculated on a dry weight basis.

GC-FID and GC-MS. The oil samples were analyzed by a combination of capillary GC-FID and GC-MS. GC-FID analysis was carried out on a Perkin–Elmer autosystem XL gas chromatographic 8500 series equipped with flame ionization

detector (FID) and head space analyzer using a fused silica capillary column (30 m × 0.32 mm, film thickness 0.25 μm) coated with dimethylpolysiloxane RTX-5. Column temperature was programmed from 60 to 230°C, with injector temperature 230°C and detector temperature 250°C. The carrier gas nitrogen had flow rate 1 mL/min and injection volume 0.8 μL. GC/MS analysis was carried out on a Varian gas Chromatograph series 3800 fitted with a VF-5ms fused silica capillary column (60 m × 0.25 mm, film thickness 0.25 μm) coupled with a 4000 series mass detector under the following conditions: injection volume 1 μL with split ratio 60, helium as carrier gas at 1 mL/min constant flow mode, injector temperature 230°C, and oven temperature 40°C to 250°C at 3°C/min. The MS operating parameters were as follows: electron impact (EI⁺) mode 70 eV and ion source temperature 250°C. Mass spectra were recorded over the 50–500 a.m.u range.

Identification of the components was based on retention indices (RI) relative to *n*-alkanes (C₆–C₂₆) and computer matching with the Wiley and NIST libraries, as well as by comparison of the fragmentation pattern of the mass spectra with data published in the literature [23, 24]. The percentage composition of the individual components was computed from the GC-FID peak areas without the use of correction factors.

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