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Micromorphology of glandular trichomes of *Nepeta congesta* Fisch. & Mey. var. *congesta* (Lamiaceae) and chemical analysis of the essential oils

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Abstract

The micromorphology and distribution of foliar trichomes of *Nepeta congesta* var. *congesta* (Lamiaceae), a species endemic to Turkey, were investigated in order to evaluate the usefulness of this feature for systematic purpose. The aerial parts of *N. congesta* var. *congesta* bear an indumentum of glandular and non-glandular trichomes. Two types of glandular trichomes are identified. Peltate trichomes consist of a basal cell embedded in the epidermis, a stalk cell, and a four-celled secretory head. Capitate trichomes comprise either a unicellular head and uni- or bicellular stalk, or a bicellular head and unicellular stalk. Water-distilled essential oil of the aerial parts of *N. congesta* var. *congesta* was analysed by GC and GC/MS and the main components were found to be 1.8-cineole (29.9%), germacrene-D (20.3%) and sabinene (10.3%). © 2006 SAAB. Published by Elsevier B.V. All rights reserved.

Keywords: Essential oil; Glandular trichomes; Micromorphology; Nepeta congesta var. congesta; SEM

1. Introduction

Glandular trichomes are widely distributed over the aerial reproductive and vegetative organs of plants of the Lamiaceae, and their secretions contribute largely to its great importance. They are the primary secretory organs of these plants, and their structures can vary widely among species (Venkatachalam et al., 1984; Bosabalidis, 1990; Maleci and Servettaz, 1991; Maleci et al., 1992; Servattaz et al., 1992; Bourett et al., 1994; Serrato-Valenti et al., 1997; Kolalite, 1998; Ascensao et al., 1999; Rapisarda et al., 2001; Kaya et al., 2003). These glands, which are often microscopic, secrete various types of compounds. The essential oil produced by glandular trichomes may act to protect the aerial parts of the plant against herbivores and pathogens (Werker, 1993), and the biological activity of the secondary metabolites in the secreted products is of interest to the pesticide,

pharmaceutical, flavouring and fragrance industries (Wagner, 1991; Werker, 1993; Duke, 1994; Bisio et al., 1999).

In the Lamiaceae, glandular trichomes are generally classified as either capitate (clavate) or peltate (subsessile), based on morphological characteristics (Fahn, 2000). The compounds secreted by capitate glandular trichomes are mostly excreted to the surrounding environment, apparently through pores in the cuticle of the head cell(s). On contrast, in peltate glandular trichomes the secretions accumulate in a capacious subcuticular space formed by the separation of the head cell walls from the cuticle is physically ruptured. Thus, peltate glandular trichomes function as repositories for the specialized phytochemicals that they secrete (Siebert, 2004).

The genus *Nepeta* comprises about 250 species distributed in the central and southern parts of Europe, Asia and the Middle East (Tzakou et al., 2000). It is represented in Turkey by 33 species, 17 of them endemic (Hedge and Lamond, 1982).

Several *Nepeta* species are used in folk medicine as diuretic, diaphoretic, antitussive, antispasmodic, anti-asthmatic, febrifuge,

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emmenagogue and sedative agents (Topcu et al., 1996; Tzakou et al., 2000; Rapisarda et al., 2001).

Nepeta species contain monoterpenes, sesquiterpenes, cyclopentanoid iridoids derivatives and nepetalactones. The activity of nepetalactone and also of its isomers on the olfactory sense of domestic cats was demonstrated and they function also as insect attractants and repellants (Bicchi et al., 1984; Rapisarda et al., 2001).

Essential oils from 22 *Nepeta* species growing in Turkey have been studied by Baser et al. (2000). The major components of the essential oils in these species were $4a\alpha$ - 7α - $7a\alpha$ -nepetalactone (4 spp.), $4a\alpha$ - 7α - $7a\beta$ -nepetalactone (only in *N. racemosa* Lam.), caryophyllene oxide (7 spp.), 1,8-cineole/linalool (6 spp.), β pinene (only *N. phyllochlamys* PH. Davis), α -terpineol (only *N. viscida* Boiss), germacrene-D (only *N. sorgerae* Hedge and Lamond) and spathulenol (only *N. trachonitica* Post).

In the present work, scanning electron microscopy (SEM) was used to determine the morphology and distribution of the glandular hairs of *Nepeta congesta* var. *congesta*, a Turkish endemic species, both to improve the present knowledge of the species and to evaluate the usefulness of this feature for systematic purpose. The chemical composition of its essential oil was also analysed.

2. Materials and methods

2.1. Plant material

Samples were collected during the flowering period (19 May 2004) from Eskisehir (in the vicinity of Oglakci village) pro-

vince of Turkey in Central Anatolia. Voucher specimens are deposited in the Herbarium of the Faculty of Pharmacy of Anadolu University in Eskischir, Turkey (ESSE 14355).

2.2. Scanning Electron Microscopy (SEM)

Fragments of leaves, stems and flowers were fixed with 3% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2 for 4 h at 4 °C. After washing in water the material was dehydrated through an acetone gradient and critical point dried. Specimens were mounted onto SEM stubs using double-sided adhesive tape and coated with gold.

2.3. Isolation of the essential oils

Air-dried aerial parts (100 g) were hydrodistilled for 3 h using a Clevenger type apparatus to obtain essential oil in 0.18% yield on dry weight basis.

2.4. Gas Chromatography (GC)

The essential oil was analysed using a Hewlett-Packard 6890 system. A polar Innowax FSC column (60 m×0.25 mm Ø, with 0.25 μ m film thickness) was used with Nitrogen (1 mL/min) as the carrier gas. Injection port temperature was at 250 °C. GC oven temperature was kept at 60 °C for 10 min and programmed to 220 °C for 10 min then raised to 240 °C at rate of 1 °C/min. Flame Ionizations Detector (FID) was used at 250 °C for quantitation.

Fig. 1. Glandular and non-glandular trichomes of N. congesta var. congesta in SEM. A3, B, C type hairs on the stem. Scale bars: ~500 µm.

Fig. 2. Glandular and non-glandular trichomes of N. congesta var. congesta in SEM. A3, B, C type hairs on the stem. Scale bars:~100 µm.

Fig. 3. Glandular and non-glandular trichomes of N. congesta var. congesta in SEM. A3, B, C type hairs on the stem. Scale bars:~ 50 µm.

Fig. 4. Glandular and non-glandular trichomes of *N. congesta* var. *congesta* in SEM. A1, A2, B, C types hairs and caryophyllaceous type stomata on the leaf abaxial surface. Scale bars:~200 μm.

Fig. 5. Glandular and non-glandular trichomes of *N. congesta* var. *congesta* in SEM. A1, A2, B, C types hairs and caryophyllaceous type stomata on the leaf abaxial surface. Scale bars:~200 μm.

Fig. 6. Glandular and non-glandular trichomes of *N. congesta* var. *congesta* in SEM. A1, A2, B, C types hairs and caryophyllaceous type stomata on the leaf abaxial surface. Scale bars:~200 µm.

Fig. 7. Glandular and non-glandular trichomes of *N. congesta* var. *congesta* in SEM. A type hairs and caryophyllaceous type stomata on the leaf adaxial surface. Scale bars:~ 100 µm.

Fig. 8. Glandular and non-glandular trichomes of *N. congesta* var. *congesta* in SEM. A type hairs and caryophyllaceous type stomata on the leaf adaxial surface. Scale bars:~100 µm.

Fig. 9. Glandular and non-glandular trichomes of *N. congesta* var. *congesta* in SEM. A type hairs and caryophyllaceous type stomata on the leaf adaxial surface. Scale bars:~100 µm.

Fig. 10. Glandular and non-glandular trichomes of N. congesta var. congesta in SEM. A, B, C type hairs calyx outer face. Scale bars:~100 µm.

Fig. 11. Glandular and non-glandular trichomes of N. congesta var. congesta in SEM. A, B, C type hairs calyx outer face. Scale bars:~100 µm.

Fig. 12. Glandular and non-glandular trichomes of N. congesta var. congesta in SEM. A, B, C type hairs calyx outer face. Scale bars:~100 µm.

Fig. 13. Glandular and non-glandular trichomes of N. congesta var. congesta in SEM. A1, A2, B, C type hairs corolla outer face. Scale bars:~200 µm.

Fig. 14. Glandular and non-glandular trichomes of N. congesta var. congesta in SEM. A1, A2, B, C type hairs corolla outer face. Scale bars: ~100 µm.

Table 1 Trichome distribution on different plant parts in N. congesta var. congesta

Hair type	Stem	Leaves		Calyx		Corolla	
		Adaxial	Abaxial	Inner face	Outer face	Inner face	Outer face
A1	+	+	+	+++	+	-	++
A2	+	+++	+++	+	+++	_	+++
A3	+++	++	++	-	++	-	+
В	++	-	+++	-	++	-	++
C1	+	+	+	-	+	+	+
C2	+++	++	+++	++	+++	++	++

A. Short capitate trichomes. A1: head unicellular, stalk unicellular; A2: head unicellular, stalk bicellular; A3: heads bicellular, stalk unicellular. B. Peltate trichomes: short stalk cell and four secretory cells with a wide subcuticular space. C. Non-glandular trichomes. C1: unicellular; C2, multicellular. Symbols indicate: (-) absence of hairs, (+) few hairs, (++, +++) increasing presence of hairs.

2.5. Gas Chromatography Mass Spectrometry(GC/MS)

The essential oil was analysed using a Hewlett-Packard G1800A GCD system under the conditions described for GC but using helium (1 mL/min) as the carrier gas. Mass range

Table 2 The composition of the essential oil of N. congesta var. congesta

RRI	Compound	%
1032	α-Pinene	3.2
1076	Camphene	_
1118	β-Pinene	7.8
1132	Sabinene	10.3
1159	δ-3-Carene	tr
1174	Myrcene	3.2
1183	<i>p</i> -Mentha-1,7(8)-diene (= <i>Pseudolimonene</i>)	0.4
1188	α-Terpinene	0.2
1195	Dehydro-1,8-cineole	0.1
1203	Limonene	2.7
1213	1,8-Cineole	29.9
1225	(Z)-3-Hexenal	0.3
1246	(Z)-β-Ocimene	0.4
1255	γ-Terpinene	0.3
1266	(<i>E</i>)-β-Ocimene	2.4
1280	<i>p</i> -Cymene	tr
1290	Terpinolene	0.1
1303	Amyl isovalerate	tr
1304	1-octen-3-one	0.1
1327	(Z)-3-hexenyl acetate	_
1360	Hexanol	tr
1386	Octenyl acetate	tr
1400	Nonanal	tr
1429	Perillen	tr
1438	Hexyl 2-methyl butyrate	_
1452	1-Octen-3-ol	0.1
1474	trans-Sabinene hydrate	_
1482	(Z)-3-Hexenyl-2-methyl butyrate	0.1
1494	(Z)-3-Hexenyl isovalerate	tr
1495	Bicycloelemene	0.5
1497	α-Copaene	0.5
1528	α-Bourbonene	0.1
1535	β-Bourbonene	2.4
1549	β-Cubebene	0.1
1571	trans-p-Menth-2-en-1-ol	0.1

RRI	Compound	%
1589	β-Ylangene	0.8
1597	β-Copaene	0.6
1600	β-Elemene	0.9
1611	Terpinen-4-ol	0.4
1648	Myrtenal	0.1
1661	Alloaromadendrene	0.2
1682	δ-Terpineol	0.6
1706	α-Terpineol	0.6
1726	Germacrene D	20.3
1755	Bicyclogermacrene	3.6
1773	δ-Cadinene	0.5
1849	Calamenene	tr
1868	(E)-Geranyl acetone	tr
1945	1,5-Epoxy-salvial(4)14-ene	tr
1957	Cubebol	tr
1958	(E) - β -Ionone	_
2037	Salvial-4(14)-en-1-one	tr
2057	Ledol	tr
2069	Germacrene D-4β-ol	0.2
2080	Cubenol	tr
2098	Globulol	0.1
2104	Viridiflorol	0.1
2144	Rosifoliol	tr
2144	Spathulenol	0.5
2187	T-Cadinol	0.2
2209	T-Muurolol	0.1
2219	δ -Cadinol (=alpha-muurolol)	0.1
2239	Carvacrol	0.1
2247	trans-a-Bergamotol	0.1
2255	α-Cadinol	0.3
2300	Tricosane	tr
2392	Caryophylla-2(12),6-dien-5β-ol (=Caryophyllenol II)	0.2
2622	Phytol	0.2
	Total	96.1

RRI=Relative retention indices calculated against n-alkanes.

tr = Trace (<0.1%).

% calculated from FID data.

was recorded from m/z 35 to 425. Split ratio was adjusted at 50:1. MS were recorded at 70 eV. n-Alkanes were used as reference points in the calculation of relative retention indices (RRI). A library search was carried out using "Wiley and Adams-LIBR (TP) " and "Baser Library of Essential Oil Constituents.

3. Results

3.1. Morphology and distribution of the trichomes

The stem, leaves, calyx and corolla of N. congesta var. congesta bear glandular and non-glandular trichomes (Figs. 1-14). Distributions of the trichomes are given in Table 1. The glandular trichomes are of the peltate and capitate types that have been distinguished in other Nepeta species (Bourett et al., 1994; Kolalite, 1998; Rapisarda et al., 2001).

The trichomes may be divided into the following categories: A. Short capitate trichomes. A1: Head unicellular, stalk unicellular; A2: Head unicellular, stalk bicellular; A3: Head bicellular, stalk unicellular. B. Peltate trichomes: short stalk cell

and four secretory cells with wide subcuticular space. C. Nonglandular trichomes, uniseriate with cuticular micropappilae. C1: Unicellular. C2. Multicellular (2–5-celled).

The stems of *N. congesta* var. *congesta* bear glandular and non-glandular trichomes (A,B,C types). The non-glandular trichomes puberulous, \pm retrorse and uni- or multicellular. Multicellular hairs (especially 2–3-celled) are more common than unicellular hairs. Both glandular hair types (A and B-types) are present but short capitate hairs made up of a single stalk cell and a bicellular head (A3 type) and peltate hairs (B type) are most frequent (Figs. 1–3).

The leaves are simple, ovate-oblong to elliptic, $1.6-4.7 \times 0.6-2.4(-3)$ cm, truncate to cuneate, and crenate or the uppermost rarely entire. Both leaf surfaces bear non-glandular and glandular trichomes with glandular trichomes more common. The A2 type, comprising a bicellular stalk and unicellular head, is most frequent on both surfaces, but B type, peltate trichomes occur only on the abaxial surface and are densely distributed. Peltate trichomes are pale yellow-orange and easily distinguished under the stereoscope. Their heads are four-cellular in mature peltate hairs but two-cellular in young hairs (Figs. 6, 14). Secreted essential oil accumulates between the raised cuticle and the outer cell walls. Non-glandular trichomes are found on both surfaces. They are scarce on the adaxial surfaces, and are mainly restricted to the ribs on the abaxial surfaces.

Leaf epidermal cells are long and irregularly shaped, with sinuous anticlinal walls. The cuticle is smooth/striated on both the abaxial and adaxial surfaces. Leaves are amphiostomatic, with stomata of the caryophyllaceous type present in \pm equal densities on both surfaces (Figs. 4–9).

The calyx is 5-8(-11) cm long, ±campanulate, with glandular and non-glandular trichomes. A2-type glandular trichomes and C2-type non-glandular trichomes are more frequent on the outer (abaxial) surfaces. Multicellular (C2-type) trichomes occur in the throat of calyx, as well as short capitate hairs (A1-type), which are more frequent on the inner (adaxial) surface of the calyx tube. The morphology of both outer and inner epidermal cells is similar, and the cells are long and irregularly-shaped with sinuous anticlinal walls and striated cuticle (Figs. 10–12).

The corolla is 6-8(-9) cm long, and white to creamcoloured. Non-glandular and glandular trichomes are found on the outer (abaxial) surfaces, especially lower lips, but glandular hairs are more common. Short capitate hairs (A2-type) are most frequent. C-type hairs were observed on the inner surface of both upper and lower lips. The outer epidermal cells are orbicular-polygonal, with entire or ±undulate anticlinal walls and a smooth cuticle (Figs. 13, 14).

3.2. Essential oil composition

Anaylsis of the essential oil from the aerial parts yielded 0.18% of oil in which the main components were identified as 1,8-cineole (29.9%), germacrene-D (20.3%) and sabinene (10.3%) (Table 2, Fig. 15). Sixty-eight compounds were identified, accounting for 96.1% of the oil. Monoterpene hydrocarbons account for 31%, oxygenated monoterpenes



Fig. 15. Structure of the main components of N. congesta var. congesta essential oil.

31.9%, sesquiterpene hydrocarbons 30.5%, oxygenated sesquiterpenes 2.0%, diterpenes 0.2% and others 0.5%.

4. Discussion

As in plants of most Lamiaceae species, *N. congesta* var. *congesta* has both glandular and non-glandular trichomes, with two types of glandular trichomes: peltate and capitate types.

Capitate hairs are widespread in the Lamiaceae, but they vary greatly in structure and size. The capitate hairs of N. congesta var. congesta varied in the number of both stalk and head cells, and three subtypes are present. Subtypes A2, which comprise a bicellular stalk and unicellular head; and A3, which comprise a unicellular stalk cell and bicellular head of glandular hairs, and which are very common in Nepeta species (Metcalfe and Chalk, 1950), were more frequent than the A1 type, which are composed of a unicellular head and unicellular stalk. In Nepeta racemosa L. Bourett et al. (1994) observed small capitate glands with two head cells, similar to type A3 observed in N. congesta var. congesta. Peltate hairs are widespread on the abaxial surface of the leaves. The heads of these peltate trichomes are made up of four secretory cells, which is very common in Nepeta species (Metcalfe and Chalk, 1950), and also observed in other species in the family, including *Ocimum basilicum* (Werker et al. 1993) and Salvia blepharophylla Brandegee ex Epling (Bisio et al., 1999). Non-glandular hairs were observed in all parts of the plant and multicellular trichomes (especially 2-3 celled) were more common than unicellular hairs.

Trichome types previously observed in *N. sibthorpii* Bentham (Rapisarda et al., 2001) *N. racemosa* L. (Bourett et al., 1994) and *N. cataria* L. (Kolalite, 1998) resemble those of *N. congesta* var. *congesta*. Anatomically, the peltate glands of *N. cataria* and *N. cataria* var. *citriodora* were characterized by numerous leucoplasts sheathed by smooth reticular tubules and smooth endoplasmic reticulum, and were thought to synthesize terpenes (Kolalite, 1998).

The main chemical constituents of the oils of *N. congesta* var. *congesta* are 1,8-cineole (29.9%), germacrene-D (20.3%) and sabinene (10.3%). *Nepeta* species can be divided into two groups: those containing nepetalactone (Bicchi et al., 1984; Pooter et al., 1987; Baser et al., 2000; Tzakou et al., 2000; Habibi and Sedaghat, 2002), and those lacking nepetalactone (Baser et al., 2000). $4a\alpha$ - 7α - $7a\alpha$ -Nepetalactone is the most frequently encountered nepetalactone in *Nepeta* oils. In *Nepeta* species that contain no nepetalactone, caryophyllene oxide or

1,8-cineole/linalool is found as the main constituents in their essential oils. From this analysis it is clear that *N. congesta* var. *congesta* belongs to the nepetalactone-absent group. *N. congesta* var. *cryptantha* (Boiss.) Hedge and Lamond also belong to this group since its main component is 1,8-cineole (40%) (Baser et al., 2000).

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