

MICROMORPHOLOGY AND HISTOCHEMISTRY OF TRICHOMES OF ENDEMIC *NEPETA RTANJENSIS* (LAMIACEAE)

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Abstract

Micromorphological and anatomical analyses of the indumentum of *Nepeta rtanjensis*, a Serbian/local endemic species, were performed by means of light and electron microscopy and described in detail. The leaves and stems bear numerous glandular trichomes, both peltate and capitate, as well as non-glandular unbranched trichomes. A basal epidermal cell, a short stalk cell and a large round head of four secretory cells constitute one peltate trichome. Capitate trichomes are short- and long-stalked and can be divided into two types, based on the number of glandular head cells, and further into a few subtypes according to the length and number of cells in stalk. Capitate trichomes of smaller length with one-cellular or with a bicellular head have been previously reported in different *Nepeta* species; however, according to our best knowledge, no existing study has reported the presence of long capitate trichomes on vegetative organs in any other species of this genus.

Key words: Micromorphological, Histochemistry, Trichomes, *Nepeta rtanjensis*

Introduction

The *Nepeta* genus contains about 280 species (Hakimzadeh *et al.*, 2016) native to the Mediterranean basin and widely distributed in Central and Southern Europe, South-west and Central Asia and North Africa, but also naturalized in North and Central America and Canary Islands. *Nepeta rtanjensis* Diklić et Milojević is an endemic species, growing only on a few localities on the Rtanj Mountain in the southeastern Serbia (Diklić, 1999), and its distribution is separated from the other *Nepeta* species in the region. *N. rtanjensis* belongs to the family Lamiaceae, comprising many plants which are used as herbal teas, medicinal plants in the folk medicine, as spices, raw materials in cosmetic industry, or as ornamental plants. Aboveground plant parts representatives of this family are characterized by the presence of glandular trichomes, which are the sites of synthesis of secondary metabolites and according to Lange (2015) there seems to be a correlation between the chemical composition of secretory cells and their structure are in correlation. Various *Nepeta* species have been used in the traditional medicine usually to treat nervous ailments, rheumatic pains and high blood pressure, since they have diuretic, diaphoretic, antitussive, anti-asthmatic, febrifuge, emmenagogue, sedative and antispasmodic (Formisano *et al.*, 2011; Joharchi & Amiri 2012; Sharma & Canoo, 2013). There are a lot of data about chemical components of the essential oil of *N. rtanjensis* and their biological activity (Ljaljević-Grbić, *et al.*, 2011a; Ljaljević-Grbić *et al.*, 2011b; Ljaljević-Grbić *et al.*, 2015; Mišić *et al.*, 2015), but there is not much data focused on the micromorphology and anatomical features of this species. Very few research papers focused on the anatomical and morphological investigation of *Nepeta* species. However, there are published papers dealing with micromorphology of leaves of *N. racemosa* (Hallahan *et al.*, 2000a), *N. cataria* (Kolalite, 1998; Herron, 2003), *N. congesta* (Kaya *et al.*, 2007), *N. wuana* (Dong *et al.*, 2015),

N. menthoides (Kahkeshani *et al.*, 2014), but for species *N. rtanjensis* this information is limited. The aim of this research was to study the micromorphological and histochemical properties of glandular trichomes on vegetative parts of *N. rtanjensis* with two objectives: to enrich the current knowledge of this species, and to evaluate the practical usefulness of this feature for systematic purposes.

Material and Methods

Plant sampling: The fresh, fully developed plants (stem and leaves) of *N. rtanjensis* were collected from its natural habitat, the mountain of Rtanj in eastern Serbia (N 43°43.657' E 21°54.257'). Since the species is listed in the Regulation of Strictly Protected Wild Species of Plants, Animals and Mushrooms within the Law on Nature Protection of the Republic of Serbia (Official Gazette No 36/09 and 88/2010), a special permission for collecting a small amount of plant material was obtained from the Department of Nature Protection of Serbia. The plant material was authenticated by Prof. Zora Dajić Stevanović and a herbarium voucher was deposited in the Department of Applied Botany, Faculty of Agriculture, University of Belgrade (FOA NR 34).

Analysis of indumentum (micromorphological investigations): Leaf and stem surface features were studied using light and scanning electron microscopy (SEM). A bright field light microscope with reflected light (Stereomicroscope Nikon SWL18, Japan) was used for epidermis observation with magnification up to 270 times, and for more detailed examination SEM was used. For the purposes of SEM, a two-sided adhesive tape was used to fix small pieces of dried leaves and stem samples on aluminum stubs and coated with gold at 30 mAin BAL-TEC SCD 005 Sputter Coater. After that, samples have been observed in the JEOLJSM-6460 LV electron microscope with acceleration

voltage setup at 10–15 kV. In addition, abaxial and adaxial epidermal peels, as well as cleared leaf and stem samples, were observed in bright-field light microscopy in transmitted light (Leica DM2000, Germany).

Analysis of indumentum (histochemical investigations):

The anatomical investigation was performed using fresh specimens or specimens fixed in FAA and kept in 70% ethanol. Fixed tissue samples were processed by a standard paraffin wax method in automatic tissue processor Leica TP1020, sections were made with a sliding microtome (Leica SM2000 R) at a thickness of 5–10 μm , and stained with general dyes for plant histology: Safranin O and Alcian blue. Fresh plant material was sectioned by hand using commercial razorblades and observed in water, or stained by specific histochemical dyes and observed by bright-field light microscope (Leica DM2000 with camera DFC320) or by epi-fluorescence microscope Leica DMLS equipped with HBO 50 W mercury vapor lamp and filter cube A (band-pass filter 340–380 nm) and filter cube I3 (band-pass filter 450–490 nm) (Pljevljakušić *et al.*, 2012; Chwil *et al.*, 2016). With the aim of determining and localizing certain chemical compounds, the following reagents were used: Toluidin blue as a polychromatic dye; Ditmar's and Wagner's reagent for detecting alkaloids; Nadi reagent for essential oils; Acid fuchsin for proteins; Vanillin-hydrochloric acid, ferric sulphate and ferric chloride for phenolic compounds; antimony trichloride for steroids and antimony trichloride for flavonoids (Yeung *et al.*, 2015). Adequate control staining procedures were performed.

For capturing the microslides and for the analysis of images, the Leica IM1000 software was used. Thirty (30) measurements for each type of trichome were taken to determine the trichome micromorphology. The general trichome classification and the terminology are used according to Payne (1978) and Navarro & El Oualidi (2000).

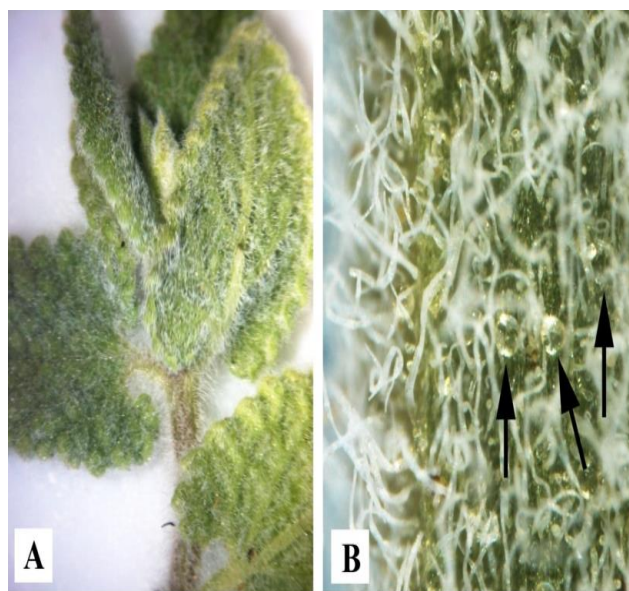


Fig 1. Very hairy surface of above ground parts of *Nepeta rtanjensis* observed in brightfield microscope in reflected light (A). Part of the adaxial leaf surface (B); peltate glandular trichomes are arrowed.

Results

The epidermis of leaf and stem of *N. rtanjensis* is rich in dense hairs, which could be observed by the naked eye as a silver cover of plant surface (Fig. 1A). The indumentum of *N. rtanjensis* leaves is composed of several types of glandular and non-glandular trichomes (Fig. 2).

Long non-glandular trichomes, as well as bright shiny globules of peltate glandular trichomes, could be observed by stereomicroscope (Fig. 1B), but were more noticeable when observed with SEM (Fig. 2), or in epidermal strips, or the leaf section observed with the bright-field light microscope.

The peltate trichomes occur on the upper and lower surface of the leaf (Fig. 3A). They have a basal cell supporting a short monocellular stalk which bears a head composed of four glandular cells arranged in a circle (Fig. 3B, C). A part of trichome above the head cell and below the cuticle is filled with secretion (Fig. 3D), resulting in a final diameter of these trichomes (average $63 \pm 6 \mu\text{m}$).

Apart from this type of large, conspicuous peltate secretory trichomes, capitate glandular trichomes could also be recognized. They are multicellular and uniseriate, usually formed by one basal cell embedded in the epidermis, with a unicellular to multicellular stalk of variable length, and a unicellular or bicellular secretory head. Based on the number of secretory cells in head, above mentioned trichomes can be divided into two subtypes. Subtype I trichomes are short-stalked capitate with one basal cell, one stalk cell, and a bicellular secretory head (Fig. 4A). The average length of these trichomes is $31 \pm 3 \mu\text{m}$, and the diameter of secretory head is $20 \pm 2 \mu\text{m}$. There is no subcuticular space between secretory cells and cuticle. This subtype of trichome is the most abundant on veins. Subtype II are capitate trichomes with unicellular secretory head, and variable number of stalk cells and stalk length (Fig. 4B–E). The short-stalked capitate trichomes are characterized by a single basal cell, one or two stalk cells, and a single head cell. The mean length was $70 \pm 13 \mu\text{m}$ (Fig. 4B, C). Capitate trichomes with long stalks have one basal cell in their structure, an elongated multicellular stalk composed of three (trichome length $140 \pm 31 \mu\text{m}$) (Fig. 4D) or four cells (trichome length $287 \pm 113 \mu\text{m}$) and unicellular spherical glandular head (Fig. 4E). The morphology of these cells in the lower part resembles non-glandular trichomes, but they do not possess cuticular ornamentation on the surface typical for non-glandular trichomes. In all subtypes of capitates trichomes, the diameter of the secretory head is on average 16–18 μm (Table 1). A transversal section of leaf blade with different types of trichomes is shown in Fig. 5.

The same types of trichomes observed in *N. rtanjensis* lamina are also observed on the leaf petioles and on the stem epidermis (Fig. 6). In Table 1 list of the observed categories of trichomes and their respective distributions on vegetative plant parts is presented.

Non-glandular trichomes are composed of one basal epidermal cell and a few elongated cells. These trichomes are uniseriate and unbranched. Their length varies, usually between 400 and 600 μm , but could be up to 1000 μm long, and the shape of apex varies from rounded, sharp (the most abundant type) or curved ("hook" shaped). Non-glandular trichomes usually have a rough surface with visible warty ornamentation (Fig. 6).

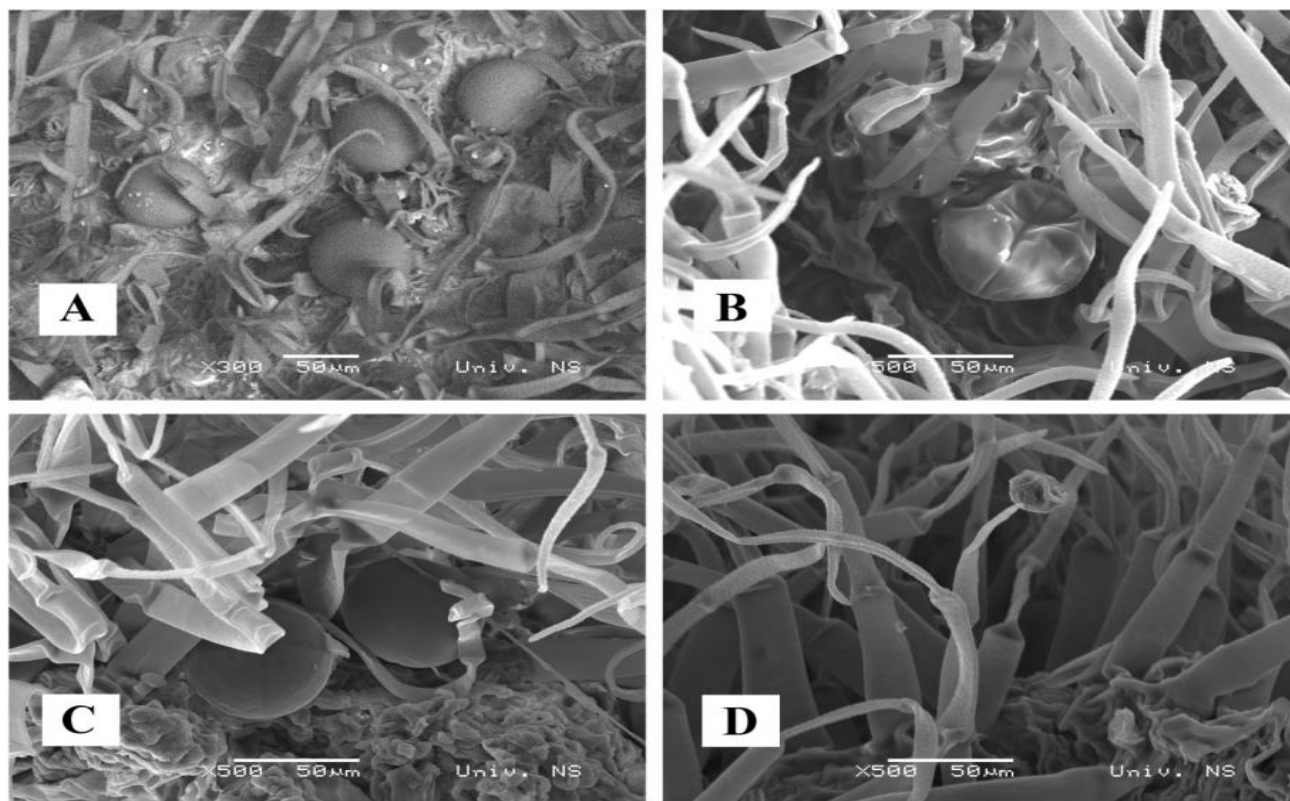


Fig 2. Dense indumentum on the adaxial (A, B) and abaxial (C, D) leaf surface of *Nepeta rtanjensis* observed by scanning electron microscope. Long non-glandular trichomes and glandular peltate trichomes visible in adaxial leaf surface (A). Furrows on the cuticle in peltate trichomes indicating position of four secretory cells (B). Long non-glandular trichomes and peltate trichomes in abaxial leaf surface (C) and long-stalked capitate trichome (D).

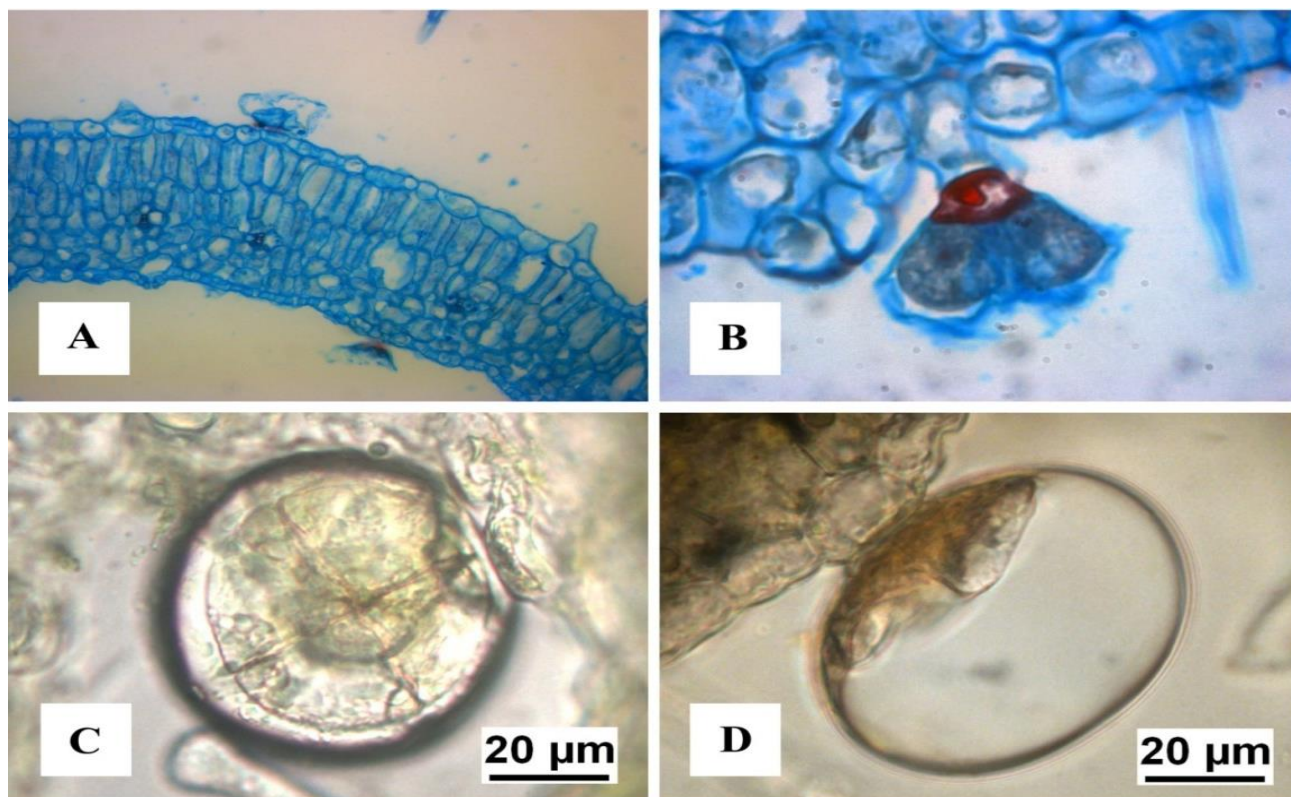


Fig 3. Peltate trichomes in abaxial and adaxial epidermis in the embedded leaf section of *Nepeta rtanjensis* (A). Detail of transversal section throughout the peltate trichome with the ruptured cuticle (B). Epidermal strips in a view from top indicating four secretory cells in the secretory head of peltate trichome (C) and in lateral view (D) showing the space filled with secretion below the cuticle and above the secretory cells.

Table 1. Types of glandular trichomes and their distribution on *N. rтанjensis* plants.

	No of secretory cells		Length of trichome (µm)	Length of stalk (µm)	Diameter of secretory head (µm)	
Peltate	4	Lamina	46.7±6.8	9.2±2.3	62.9±6.3	Fig. 3
		Leaf petiole	47.7±1.4		72.1±3.3	
		Stem	43.4±6.7	6.4±1.6	66.5±6.4	
Capitate subtype I	2	Lamina	31.5±3.0	9.8±3.3	20.2±2.1	Fig. 4A
		Leaf petiole	36.6±3.1	12.6±2.4	22.9±1.2	
		Stem	34.2±3.5	11.4±2.0	22.5±1.9	
Capitate subtype IIa	1	Lamina	57.2± 10.4	33.0± 9.9	17.0± 2.5	Fig. 4B
		Leaf petiole	49.5±7.3	30.8±10.6	15.7±1.6	
		Stem	56.3±8.1	35.1±8.0	16.1±2.4	
Capitate subtype IIb	1	Lamina	69.9±13.5	46.6±11.8	16.0±2.1	Fig. 4C
		Leaf petiole	86.0±25.8	64.4±25.5	16.6±1.5	
		Stem	71.5±15.5	49.9±14.3	15.2±3.6	
Capitate subtype IIc	1	Lamina	140.1±31.3	112.5±32.4	17.2±2.2	Fig. 4D
		Leaf petiole	179.4±63.5	155.5±65.3	18.0±2.4	
		Stem	187.8±121.8	161.3±103.2	24.0±18.3	
Capitate subtype IId	1	Lamina	287.0±113.1	266.4±115.1	17.7±3.9	Fig. 4E
		Leaf petiole	494.5±132.2	462.3±126.8	20.5±2.7	
		Stem	322.2±128.3	295.6±126.6	17.1±2.5	

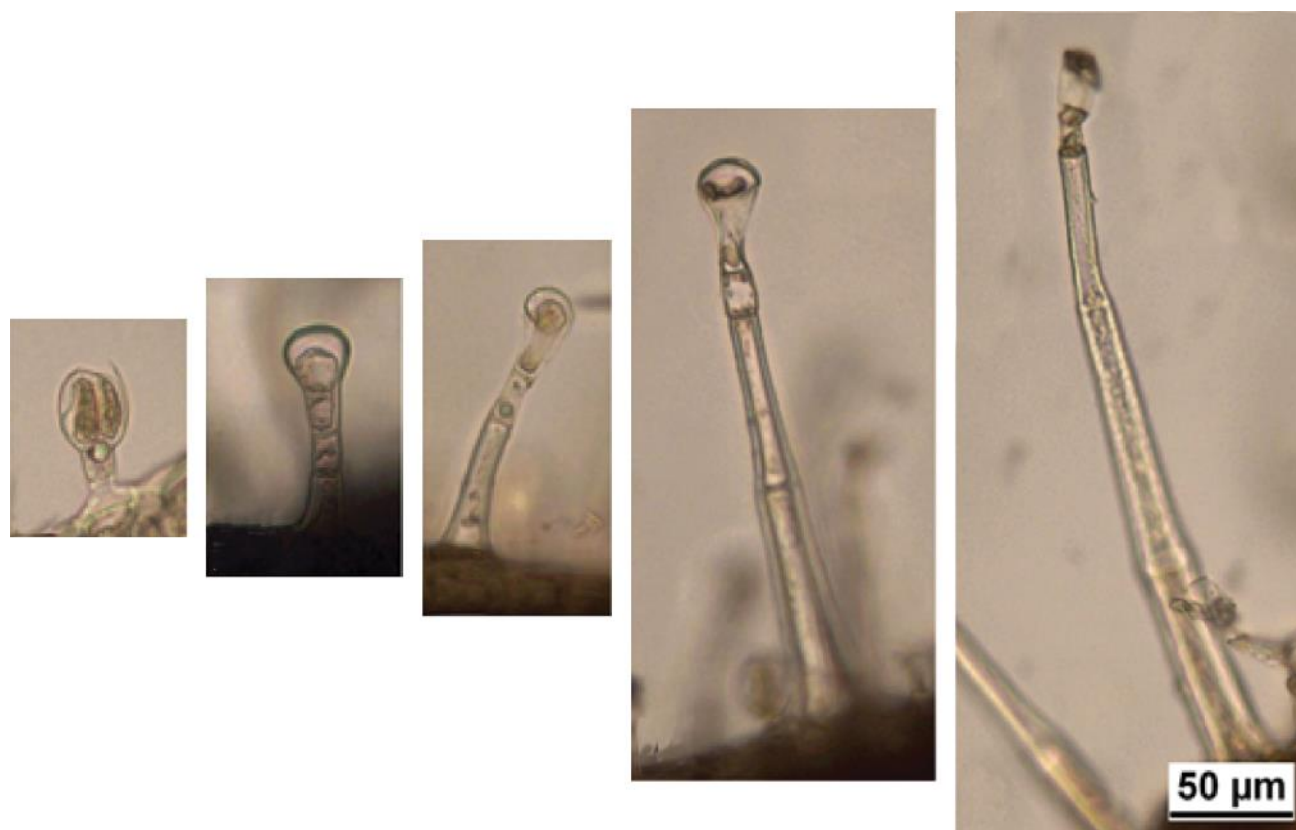


Fig. 4. Types of capitates trichomes on the leaf blade, leaf petiole and stem of *Nepeta rтанjensis* (relative size of trichomes corresponds to real values).

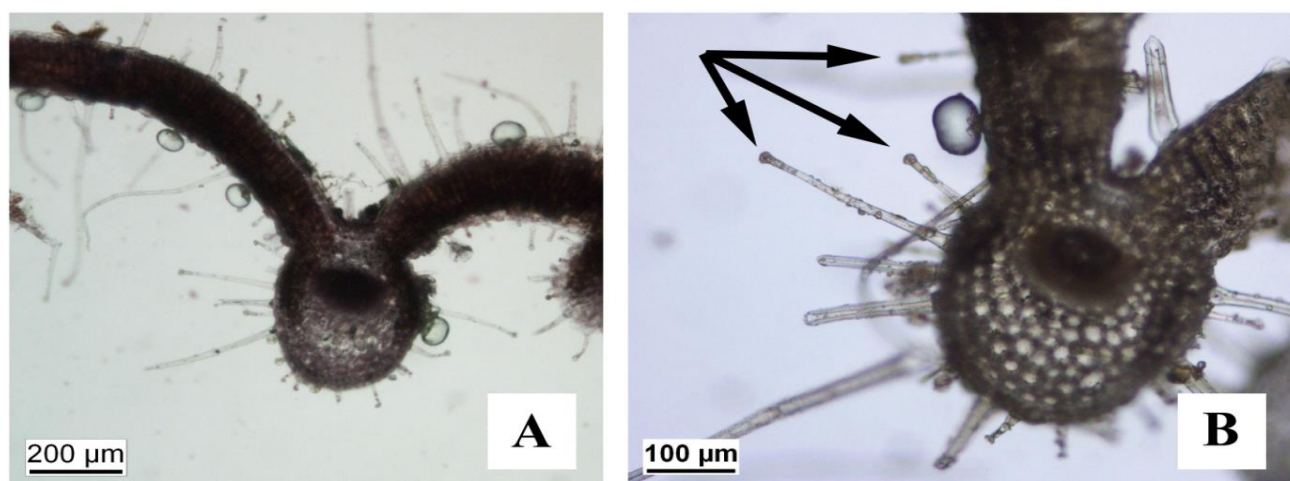


Fig. 5 Transversal section of *Nepeta rtanjensis* leaf blade (A) and section with the position of the main vein (B) showing different types of trichomes. Arrows showing long capitate trichomes.

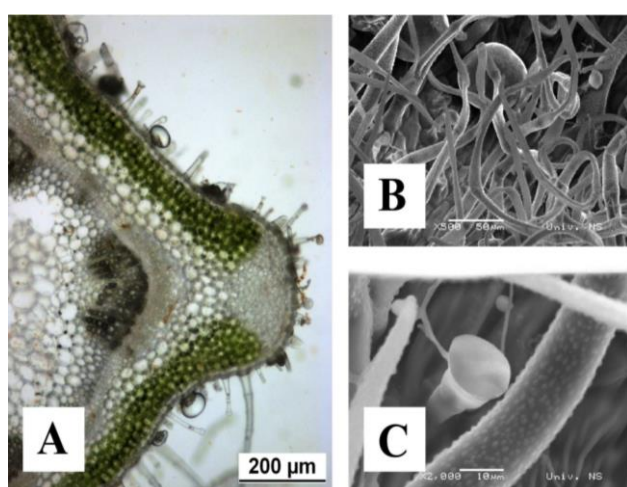


Fig 6. Indumentum on the stem of *Nepeta rtanjensis* observed by light microscope (A) and by scanning electron microscope (B, C). SEM micrographs showing abundant non-glandular trichomes, peltate trichomes and long capitate trichomes (B), short capitate trichome and prominent ornamentation on the non-glandular trichomes (C)

Components of secretory products in stem peltate trichomes show autofluorescence when observed by epi-fluorescent microscope without any staining (Fig. 7). Since none of applied histochemical tests showed the presence of phenolics in trichomes (data not shown), registered auto fluorescence of secreted compounds inside the peltate trichomes probably comes from some other chemical compounds. Flavonoids were detected in capitate trichomes on leaves (Fig. 8B, C) and in stem epidermal cells (Fig. 8E, F). Histochemical tests showed the presence of essential oils in peltate trichomes by Nadi reagent (Fig. 9A). In histochemical staining for detecting alkaloids, we have found that peltate trichomes with very similar outline features differ in reaction to the same reagents, which could indicate differences in their chemical content. The similar pattern of reaction was also detected in capitate trichomes (Fig. 9B). Histochemical tests for steroids, proteins and polysaccharides had negative results.

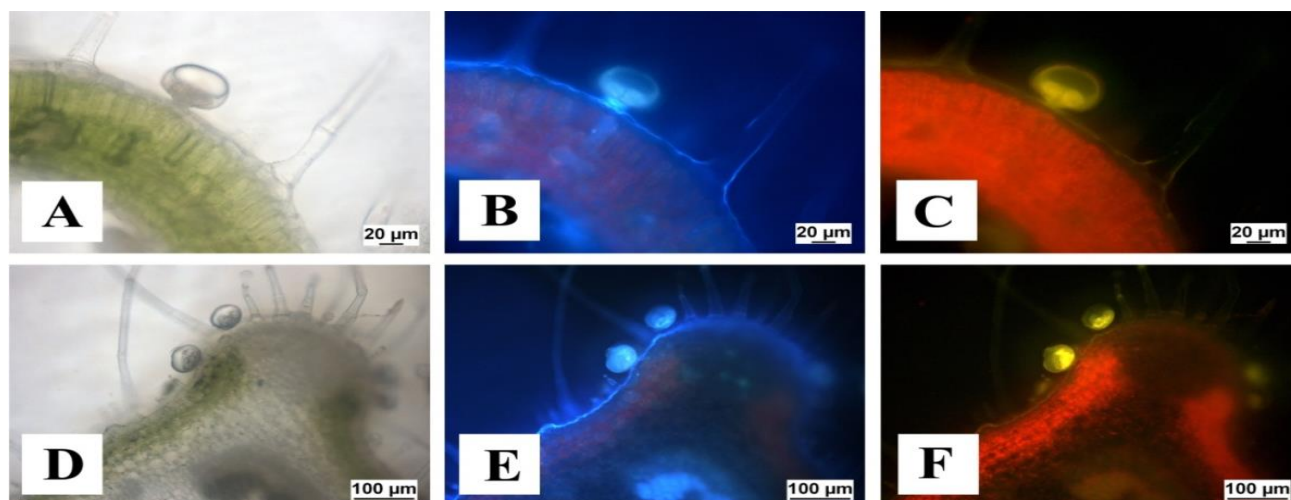


Fig 7. Transversal section of leaf (A, B, C) and stem (D, E, F) of *Nepeta rtanjensis* observed by bright field (A, D) and fluorescence microscopy without staining (B, C, E, F). Secretory cells in peltate trichomes in leaves (B, C) and secretory products accumulated below extended cuticle and secretory cells (E, F) showing autofluorescence.

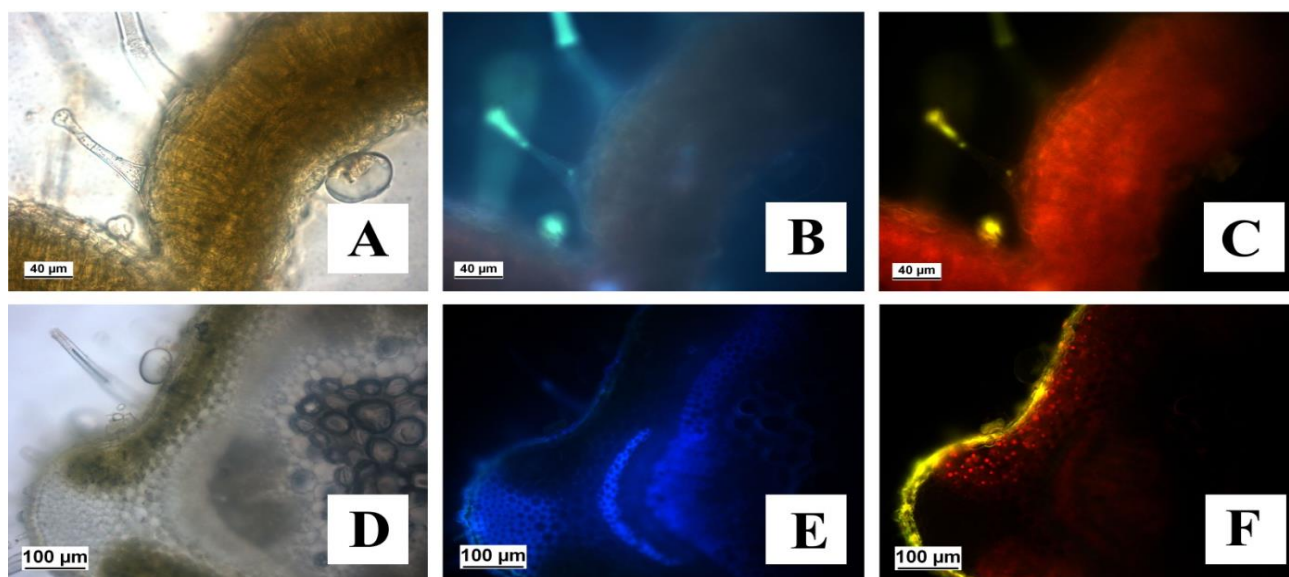


Fig. 8. Leaf section (A, B, C) and stem section (D, E, F) of *Nepeta rtanjensis* stained with AlCl_3 detecting flavonoids (flavonoids exhibiting the greenish-yellow fluorescence under the epi-fluorescence microscope). In leaves, flavonoids are localised in capitulate trichomes, but not in peltate trichomes (B, C) and in stem, flavonoids are detected in epidermal cells, but not in peltate or capitate trichomes of stem (E, F).

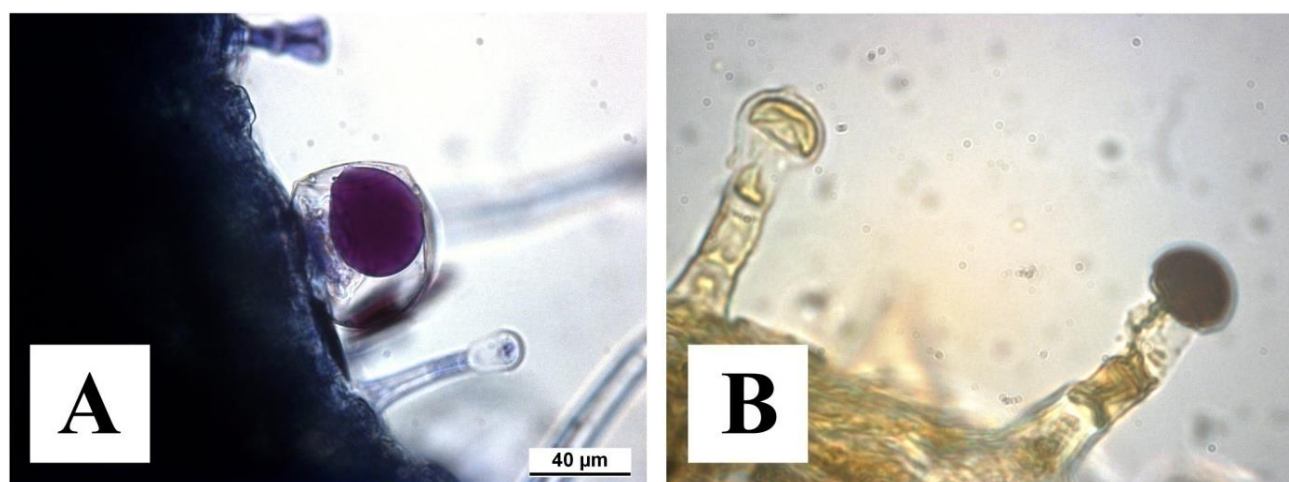


Fig. 9. Histochemical assays of secretory trichomes *Nepeta rtanjensis* leaf (A, B). Nadi reagent (A) revealed the presence of essential oil droplet within the cuticular extension of peltate trichomes (indicated by the violet-blue staining). Dittmar's reaction for detecting alkaloids (indicated by the dark blue staining) showing two morphologically similar capitates trichomes with different color, indicating differences in their secretory products (B).

Discussion

Trichomes of Lamiaceae are usually distinguished as glandular and non-glandular trichomes. Micromorphology of Nepetoideae has already been described in the genera: *Mentha* (Amelunxen, 1964); *Origanum* (Bosabalidis & Tsekos, 1982); *Thymus* (Jia *et al.*, 2013) *Salvia* (Bisio *et al.*, 1999, Janošević *et al.*, 2016).

As in other species within the Lamiaceae (Baran & Özdemir, 2013), leaf and stem surfaces of the *N. rtanjensis* are also covered with both glandular and non-glandular trichomes. Characteristics of trichomes of *Nepeta* species from available literature data are listed in Table 2. According to Bourett *et al.* (1994), each glandular trichome in *N. racemosa* originates from a single epidermal cell which undergoes serial anticlinal divisions, followed by periclinal divisions of the apical cell, resulting in structure consisting of a basal cell, a stalk made up from one or more

cells, and a secretory head. Based on the secretory head structure, glandular trichomes could be recognized as peltate, which consisted of up to 16 cells of the head or as capitata, which usually possess head of one or two secretory cells (Werker, 1993; Hallahan, 2000b; Kamatou *et al.*, 2006). Either way, secretory substances are stored in the subcuticular space, between the cuticula and cell wall. About peltate trichomes, the secreted material separates cuticle from the apical part of the cell wall (Handilou *et al.*, 1991) and the product of secretion remains accumulated in the subcuticular space. The secreted product continues to accumulate throughout the growth of the bearing organs (Werker, 1993, 2000); only after cuticle disruption, a plant secreted material can be present on the surface of the plant. It is considered that in capitata trichomes, the secretion is released through micropores – cuticle ruptures of head cells – and secreted compounds are exuded directly onto the surface of the trichomes.

Table 2. Comparative characteristics of trichomes in the *Nepeta* genus.

	Peltate	Capitate	Nonglandular	Ref.
<i>N. cataria</i>	Unicellular stalk and 10-16 cells in secretory head	Unicellular stalk and 4 cells in secretory head	Present	Kolalite, 1998
<i>N. cataria</i> var. <i>citriodora</i>	Unicellular or bicellular stalk and 10-16 cells in secretory head	Unicellular stalk and 4 cells in secretory head; bicellular stalk and 4-6 cells in secretory head	Present	Kolalite, 1998
<i>N. cataria</i>	One basal cell, stalk cells and 4 cells in secretory head	Unicellular stalk and bicellular secretory head		Coiner, 1973
<i>N. cataria</i>	Unicellular stalk and 4 cells in secretory head	Unicellular head and unicellular stalk	3-4 cells long and uniseriate	Sarkar <i>et al.</i> , 1995
<i>N. cataria</i>		Bicellular head	Elongated	Herron, 2003
<i>N. cataria</i>	Present	Present	Multicellular and uniseriated	Pădure, 2006b
<i>N. cataria</i>		Unicellular stalk and bicellular head; unicellular and bicellular stalk and unicellular head		Pădure, 2006a
<i>N. racemosa</i>	Present	Small capitate glands		Hallahan, 2000a
<i>N. racemosa</i>	4 cells in secretory head (on abaxial leaf surface)	Bicellular head (on the abaxial leaf surfaces)	Multicellular, uniseriate (on both leaf surfaces but more abundant on the abaxial face)	Bourett <i>et al.</i> , 1994
<i>N. baytopii</i>	Present	Bicellular stalk and unicellular head; unicellular stalk and unicellular head	Often 1-2 celled, but could be up to 5 cells long	Acar <i>et al.</i> , 2001
<i>N. sorgerae</i>		Bicellular stalk and unicellular head; unicellular stalk and unicellular head; unicellular stalk and bicellular head	Often 1-2 celled, but could be up to 8 cells long	Acar <i>et al.</i> , 2001
<i>N. wuana</i>	Present (on abaxial leaf surfaces)		Unicellular	Dong <i>et al.</i> , 2015
<i>N. menthoides</i>	Not present	Present	Unicellular and multicellular branched	Kahkeshani <i>et al.</i> , 2014
<i>N. congesta</i>	4 cells in secretory head (on abaxial surface)	Unicellular stalk and unicellular head; bicellular stalk and unicellular head; unicellular stalk and bicellular head	Unicellular or multicellular	Kaya <i>et al.</i> , 2007
<i>N. nuda</i>	Present (on abaxial leaf surface)	Single basal cell, a stalk cell, and unicellular head (on both leaf surfaces)	multicellular, composed of 4-7 cells in line, with the apical cell acute (denser on the adaxial leaf surface)	Kofidis & Bosabalidis, 2008
<i>N. nuda</i>	Present	Present	Unicellular short, very rare, usually absent; multicellular uniseriated (on both leaf surfaces)	Pădure, 2006b
<i>N. nuda</i>	Present	Unicellular stalk and bicellular head; unicellular or bicellular stalk and unicellular head		Pădure, 2006a
<i>N. sibthorpii</i>	Short stalk cell, 4 cells in secretory head (on adaxial leaf surface)	Unicellular or bicellular stalk and unicellular head (on all epidermises analyzed); unicellular stalk cell and bicellular head (on abaxial leaf surface)	Multicellular, uniseriate, with prominent ornamentation (on both leaf surfaces)	Rapisarda <i>et al.</i> , 2001
<i>N. ucranica</i>	Present	Present	Multicelled, uniseriated, short, very rare, usually absent	Pădure, 2006b
<i>N. parviflora</i>	Present on leaf, lacked in stem epidermis	Present	Multicellular composed of 2-16-cells (on both leaf surfaces), dense	Pădure, 2006b
<i>N. rtanjensis</i>	Present		Present	Husain <i>et al.</i> 1989
<i>N. rtanjensis</i>	4 cells in secretory head	Short capitate trichome: unicellular stalk and bicellular secretory head or unicellular stalk and unicellular secretory head; long trichome: two cellular stalks and unicellular secretory head or multicellular stalk (3-4 cells) and unicellular secretory head	Long, multicellular	Ljaljević-Grbić, 2006
<i>N. rtanjensis</i>	4 cells in secretory head (on both leaf surface)	Short stalked: unicellular or bicellular head; long stalked: unicellular head	Long, multicellular, on both leaf surfaces	Our findings

According to Metcalfe & Chalk (1972), the peltate trichomes found in *N. rtanjensis*, which consist of a four-cell head in one circle represent a very common feature in the *Nepeta* species, which has also been observed in other species of the Lamiaceae family, to name a few: subfamily Nepetoideae: *Ocimum basilicum* and *Salvia blepharophylla* (Werker, 1993; Bisio *et al.*, 1999), subfamily Ajugoideae: *Teucrium scorodonia* and *T. massiliense* (Antunes & Sevinate-Pinto, 1991; Maleci Bini *et al.*, 1992), and subfamily Lamioideae: *Phlomis russeliana* (Cali, 2016). Peltate trichomes similar to *N. rtanjensis* with unicellular stalk and four head secretory cells are registered in *N. cataria*, *N. sibthorpii*, *N. congesta* and *N. racemosa* (Coiner, 1973; Bourett *et al.*, 1994; Sarkar *et al.*, 1995; Rapisarda *et al.*, 2001; Kaya *et al.*, 2007). Only in *N. cataria* var. *citriodora* the bicellular stalk and 10-16 secretory head cell peltate trichomes were reported (Kolalite, 1998). In some Labiatae species, peltate trichomes are found solely on the lower surface of the leaf, for example in *Teucrium massiliense*, (Maleci Bini *et al.*, 1992) or in some *Nepeta* species as in *N. racemosa*, *N. congesta*, *N. wuana* and *N. nuda* (Bourett *et al.*, 1994, Kaya *et al.*, 2007, Dong *et al.*, 2015, Kofidis & Bosabalidis, 2008). However, in *N. sibthorpii* (Rapisarda *et al.*, 2001) peltate glands are present on the adaxial leaf surface, and in *N. rtanjensis* they are present on both leaf surfaces. Peltate trichomes were present only on leaves, but not on the stem as it was reported for *N. parviflora* (Pădure, 2006b), and according to our findings in *N. rtanjensis* they are present on the leaf lamina, petioles, and stem epidermis. Peltate trichomes have not been registered on leaves of *N. menthoides* (Kahkeshani *et al.*, 2014). According to Clark *et al.* (1997), the number of peltate trichomes per leaf could be high, estimated at 3000 in *N. racemosa*. Bourett *et al.* (1994) measured size of *N. racemosa* peltate trichomes on leaves in average 50 µm, while in *N. cataria* size was 35-60 µm (Kolalite, 1998), which is about the size of peltate trichomes in *N. rtanjensis* (60 µm in leaf blades).

Furthermore, capitate trichomes can be commonly found in Lamiaceae (Bisio *et al.*, 1999), however their structure and size vary significantly (Werker *et al.*, 1985a,b), and with that in mind they could be considered as an important taxonomic feature of the Lamiaceae (Metcalfe & Chalk, 1972; Kahraman *et al.*, 2010; Navarro & El Oualidi, 2000). Werker *et al.* (1985a) divided capitate hairs into three groups, according to their stalk size, glandular head morphology and the process of secretion; on the other hand, other researchers recognized only two groups of capitate trichomes: the short and the long (Ascensão & Pais, 1998; Bisio *et al.*, 1999; Al Sheef *et al.*, 2013). Short capitate trichomes occur more frequently and have been studied in detail (Modenesi *et al.*, 1984; Bisio *et al.*, 1999; Werker, 2000). The number of cells in stalk and the number of cells in the head of capitate trichomes typically vary between one and two. Metcalfe & Chalk (1972) claim that capitate trichomes with one-cell stalk and two-cell secretory head occur quite often in the *Nepeta* species, and have been previously reported in *N. sibthorpii*, *N. racemosa*, *N. sorgerae*, *N. congesta* and *N. nuda* (Bourett *et al.*, 1994; Acar *et al.*, 2001; Rapisarda *et al.*, 2001; Pădure, 2006a; Kaya *et al.*, 2007). Capitate trichomes with

unicellular head and one or two cells in the stalk are also common, and have been reported in *N. cataria*, *N. sorgerae*, *N. congesta*, *N. sibthorpii*, *N. baytopii* and *N. nuda* (Sarkar *et al.*, 1995; Acar *et al.*, 2001; Rapisarda *et al.*, 2001; Kaya *et al.*, 2007; Kofidis & Bosabalidis, 2008). Short and long capitate trichomes were found in *N. sorgerae*, *N. congesta* and *N. nuda* (Acar *et al.*, 2001; Pădure, 2006a; Kaya *et al.*, 2007) as well as in *N. rtanjensis* (Ljaljević Grbić, 2006). Majority of authors, who investigated leaves of *N. cataria*, reported capitate trichomes with unicellular and bicellular secretory head, however, Kolalite (1998) reported trichome with four celled head. The size of this capitate trichome in *N. cataria* was 25-30 microns, which was about the size of secretory cells in peltate trichome in *N. rtanjensis* without the cuticular extension, so there was a possibility that Kolalite (1998) instead capitate trichome described undeveloped peltate trichomes, since she was observed leaf primordies.

Long capitate trichomes have not been reported in any *Nepeta* species apart from *N. rtanjensis*, but were previously reported in few species of subfamily Nepetoideae (Werker *et al.*, 1985a; Ascensão & Pais, 1998; Bisio *et al.*, 1999). Long and short capitate trichomes consisting of an unicellular secretory head and multicellular stalk are reported in *Teucrium aitoloides* (El-Gazzar & Watson, 1970), *T. massiliense* (Maleci Bini *et al.*, 1992) and *Salvia officinalis* (Chakalova *et al.*, 1993). In *Teucrium massiliense* (Maleci Bini *et al.*, 1992), long-stalk capitate trichomes are not present on the leaves and the stem, but only on flower parts, and according to our investigation of *N. rtanjensis*, long stalked capitate trichomes are present only on vegetative plant parts, but are not present on floral parts.

Non-glandular trichomes are common in genera within the Lamiaceae family, and have been reported in *Micromeria*, *Calamintha*, *Satureja*, *Acinos* (Husain *et al.*, 1990), *Leucas* (El-Gazzar & Watson, 1970) and *Salvia* (Chakalova *et al.*, 1993). In some *Nepeta* species non-glandular trichomes could be very rare or almost absent, as for example in *N. ucranica* and *N. nuda* (Pădure, 2006b), short and unicellular in *N. wuana* (Dong *et al.*, 2015), both unicellular and multicellular in *N. menthoides*, *N. congesta*, *N. sorgerae*, *N. nuda*, *N. baytopii*, (Acar *et al.*, 2001; Kaya *et al.*, 2007; Kofidis & Bosabalidis, 2008; Kahkeshani *et al.*, 2014), or exclusively multicellular in *N. cataria*, *N. racemosa*, *N. sibthorpii*, *N. ucranica*, *N. parviflora*, and *N. rtanjensis* (Bourett *et al.*, 1994; Sarkar *et al.*, 1995; Rapisarda *et al.*, 2001; Pădure, 2006b; Pădure, 2006b; Ljaljević Grbić, 2006). Multicellular trichomes in some *Nepeta* species could be up to 16 cells long in the line. Non-glandular trichomes in *Nepeta* genus are usually uniseriate (Table 2), but branched multicellular non-glandular trichomes have also been reported (Kahkeshani *et al.*, 2014). Non-glandular trichomes of *N. rtanjensis* are characterized by the presence of micropapilar structures (cuticular ornamentation), which is usually less prominent on the basal cell. Similar cuticular patterns have also been reported in *Teucrium* sp. (Maleci Bini *et al.*, 1992) and on non-glandular trichomes of *N. cataria*. In *N. cataria*, these down-slanted projections known as cuticular warts are approximately 1 µm wide and 2-3 µm long, and according to Herron (2003) their function could be assisting in the transmission of essential oils from peltate glands.

In Serbia there are three *Nepeta* species, *N. rtanjensis*, *N. cataria*, and *N. nuda* (Diklić, 1999), but the species which are the most related to *N. rtanjensis* are *N. heldreichii*, *N. champhorata*, *N. spruneri*, *N. parnassica*, *N. dirphyta* and *N. sibthorpii* (Jamzad *et al.*, 2003). Unfortunately, based on available literature data, there are no micromorphological reports on *N. heldreichii*, *N. champhorata*, *N. dirphyta*, *N. spruneri*, and *N. parnassica*, but the micromorphology of *N. sibthorpii* (Rapisarda *et al.*, 2001), as well as the micromorphology of *N. cataria* (Coiner, 1973; Sarkar *et al.*, 1995; Kolalite, 1998; Herron, 2003; Pădure, 2006a,b) and of *N. nuda* (Kofidis & Bosabalidis, 2008; Pădure, 2006a,b) has been investigated. For *N. cataria* peltate trichomes with four cells in the secretory head (Coiner, 1973; Sarkar *et al.*, 1995) and capitate trichomes with one or two secretory cells and short unicellular or bicellular stalk are usually reported (Coiner, 1973; Sarkar *et al.*, 1995; Herron, 2003; Pădure, 2006a). Peltate trichomes had been previously discovered also in *N. nuda* (Pădure, 2006 a,b), although they were located only on the abaxial surface of the leaf (Kofidis & Bosabalidis, 2008), and capitate trichomes were with one head cell and unicellular or bicellular stalk (Pădure, 2006a; Kofidis & Bosabalidis, 2008) or with a bicellular secretory head and unicellular stalk (Kofidis & Bosabalidis, 2008). None of these authors reported long capitate trichomes on *N. cataria* or *N. nuda* leaves, so we could conclude that the presence of long capitate trichomes could be a good characteristic for differentiating *N. rtanjensis* from these two species which could be found in relatively nearby localities. The most similarity in micromorphological features is found between *N. rtanjensis* and *N. sibthorpii* based on research of Rapisarda *et al.* (2001): similar peltate trichome, short capitates trichomes with unicellular or bicellular head and long multicellular non-glandular trichomes with prominent ornamentation, present on both leaf surfaces.

Previous studies have confirmed the importance of trichome types differentiation and utilization in taxonomy and phylogeny of various genera within Lamiaceae family (Moon *et al.*, 2009; Salmakia *et al.*, 2009), and our results approved that this could be very true for *Nepeta* genus. Therefore, we suggest performing further detailed analysis of trichomes within this genus and their correlation with molecular analysis, since this data could be a useful tool for fast discrimination between species which do not differ clearly on the morphological level.

Capitate trichomes typically synthesize non-volatile or poorly volatile compounds, such as polysaccharides (Modenesi *et al.*, 1984; Werker *et al.*, 1985a, Werker, 1993; Fahn, 2000), whereas the volatile essential oil is produced and stored in peltate trichomes (Fahn, 2000, Naidoo *et al.*, 2013) with mainly terpenes as constituents (Clark *et al.*, 1997; Turner *et al.*, 2000). In *N. cataria*, various secondary metabolites like nepetalactone, actinidine alkaloids, and acyclic monoterpene citronellol are synthesized and stored in peltate trichomes (Herron, 2003). According to our results, peltate trichomes on leaves of *N. rtanjensis* stained positively with Nadi, indicated the presence of terpenoids. Terpenoids (nepetalactones and cyclopentanoid iridoids derivatives) and terpenes are present in *Nepeta* species (Kaya *et al.*, 2007). Considering the presence of nepetalactone in plants belonging to the genus *Nepeta*, two

subgroups of the genus can be observed: those that contain this component and those that do not contain (Baser *et al.*, 2000; Kaya *et al.* 2007). In *N. cataria*, *N. citriodora*, and *N. mussin* the principal constituents of the essential oil are methylcyclopentane monoterpene (Regnier *et al.*, 1967), in *N. congesta* var. *congesta* cyclic monoterpene, 1,8-cineole (Kaya *et al.*, 2007), and in *N. racemosa* and *N. cataria* bicyclic monoterpene, iridoids (nepetalactones). In *N. rtanjensis*, flavonoids were shown mainly in capitate trichomes, and alkaloids in both trichome types. In various *Nepeta* species phytochemical studies have already shown the presence of flavonoids (rutin) and different phenolic acids, rosmarinic acid, chlorogenic acid, etc. (Modnicki *et al.*, 2007). The rosmarinic acid is almost exclusively found in Nepetoideae, and this compound has been reported in a large portion of the species and genera studied within this subfamily (Harley *et al.*, 2004).

Conclusions

Micromorphological features have been examined in only about 4% of all species within the *Nepeta* genus. Considering that leaves, glandular and non-glandular trichomes are present and distributed clearly in diverse schedule within different *Nepeta* species, these researches should be performed in more detail and correlated with chemical composition and molecular data, with the aim of making a tool for fast and reliable determination on the species or subspecies level, which is especially important for taxa which do not differ significantly on the morphological level. Different chemical contents shown by histochemical staining within trichomes with similar morphological features in *N. rtanjensis* could be the starting point for molecular investigation of mechanisms involved in regulating gene expression in secretory cells.

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References

- Acar, M., T. Ozcan, F. Satil and T. Dirmenci. 2001. A comparative anatomical study on two endemic *Nepeta* L. species (*N. baytopii* and *N. sorgerae*). *Biodivers. Conserv.*, 4(3): 58-70.
- Al Sheef, B.N., S. Duletić-Laušević, D. Janošević, S. Budimir, M. Marin, A. Alimpić, A.A.M. Giweli and P.D. Marin. 2013. Micromorphology and ultrastructure of trichomes of Libyan *Salvia fruticosa* Mill. *Arch. Biol. Sci.*, 65(1): 239-246.
- Amelunxen, F. 1964. Elektronenmikroskopische Untersuchungen an den Drüsenhaaren von *Mentha piperita* L. *Planta Med.*, 12(02): 121-139.

- Antunes T. and I. Sevinate-Pinto. 1991. Glandular trichomes of *Teucrium scorodonia* L. Morphology and histochemistry. *Flora*, 185: 65-70.
- Ascensão, L. and M.S. Pais. 1998. The leaf capitate trichomes of *Leonotis leonurus*: histochemistry, ultrastructure and secretion. *Ann. Bot.*, 81: 263-271.
- Baran, P. and C. Özdemir. 2013. Morphological, anatomical and cytological studies on endemic *Lamium pisidicum*. *Pak. J. Bot.*, 45(1): 73-85.
- Baser, K.H.C., N. Kirimer, M. Kurkcuoğlu and B. Demirci. 2000. Essential oils of *Nepeta* species growing in Turkey. *Chem. Nat. Compd.*, 36: 356-359.
- Bisio, A., A. Corallo, P. Gastaldo, G. Romussi, G. Ciarallo, N. Fontana, N.D. Tommasi and P. Profumo. 1999. Glandular hairs and secreted material in *Salvia blepharophylla* Brandege ex Epling grown in Italy. *Ann. Bot.*, 83: 441-452.
- Bosabalidis, A.M. and I. Tsekos. 1982. Glandular scale development and essential oil secretion in *Origanum dictamnus* L. *Planta*, 156: 496-504.
- Bourett, T.M., R.J. Howard, D.P. O'Keefe and D.L. Hallahan. 1994. Gland development on leaf surfaces of *Nepeta racemosa*. *Int. J. Plant Sci.*, 155: 623-632.
- Cali, I.O. 2016. Anatomy and trichome characteristics of endemic taxon *Phlomis russeliana* (sims.) bentham and their systematic implications. *Bangladesh J. Bot.*, 45(2): 297-304.
- Chakalova, E.S., E.M. Genova and N.T. Thuy. 1993. Comparative anatomical investigations on leaves of *Salvia officinalis* L. and *S. tomentosa* Mill. *Fitologija*, 46: 3-11.
- Chwil, M., R. Nurzyńska-Wierdak, S. Chwil, R. Matraszek and J. Neugebauerová. 2016. Histochemistry and micromorphological diversity of glandular trichomes in *Melissa officinalis* L. leaf epidermis. *Acta Sci. Pol., Hortorum Cultus*, 15(3): 153-172.
- Clark, L.J., J.G.C. Hamilton, J.V. Chapman, M.J.C. Rhodes and D.L. Hallahan. 1997. Analysis of monoterpene in glandular trichomes of the catmint *Nepeta racemosa*. *Plant J.*, 11: 1387-1393.
- Coiner, D.E. 1973. *The morphology, development and histochemistry of the secretory trichomes of Nepeta cataria* L. PhD thesis Faculty of the Graduate College of the Oklahoma State University
- Diklić, N. 1999. *Nepeta rtanjensis* Diklić & Milojević. In: (Ed.): Stevanović, V. *The Red Data Book of Flora of Serbia 1. Extinct and Critically Endangered Taxa*, Beograd, Serbia, pp. 153-155 (488).
- Dong, H.J., Z. Jamzad and C.L. Xiang. 2015. *Nepeta wuana* (Nepetinae, Nepetoideae, Lamiaceae), a New Species From Shanxi, China. *Iran. J. Bot.*, 21 (1), 13-18.
- El-Gazzar, A. and L. Watson. 1970. Some economic implications of the taxonomy of Lamiaceae essential oils and rusts. *New Phytol.*, 69(2): 487-492.
- Fahn, A. 2000. Structure and function of secretory cells. In: (Eds.): Hallahan, D.L., J.C. Gray and J.A. Callow. (Eds.) *Advances in botanical research. Plant trichomes*. Academic Press, San Diego, pp. 37-75.
- Formisano, C., D. Rigano and F. Senatore. 2011. Chemical constituents and biological activities of *Nepeta* species. *Chem. Biodivers*; 8: 1783-818.
- Hakimzadeh, M.A., M.H. Hakimi and H. Sodaiezadeh. 2016. Effects of seed source and soil texture on germination and survival of *Nepeta asterotricha* as a medicinal plant. *J. Range. Sci.*, 6(3): 242-252.
- Hallahan, D.L., J.C. Gray and J.A. Callow (Eds). 2000a. *Advances in botanical research: Plant trichomes* Academic Press, San Diego.
- Hallahan, D.L. 2000b. Monoterpenoid biosynthesis in glandular trichomes of Lamiaceae plants. In: (Eds.): Hallahan, D.L., J.C. Gray and J.A. Callow. *Advances in botanical research: Plant trichomes*. Academic Press, San Diego. pp. 77-120.
- Handilou, E., S. Kokkini, A.M. Bosabalidis and J.-M. Bessière. 1991. Glandular trichomes and essential oil constituents of *Calaminta menthifolia* (Lamiaceae). *Plant Syst. Evol.*, 177: 17-2.
- Harley, R.M., S. Atkins, A.L. Budantsev, P.D. Cantino, B.J. Conn, R. Grayer, M.M. Harley, R. De Kok, T. Krestovskaya, R. Morales, A.J. Paton, O. Ryding and T. Upson. 2004. Lamiaceae. In: (Ed.): Kadereit, J.W. *The families and genera of vascular plants, Flowering plants. Dicotyledons, Lamiales (except Acanthaceae including Avicenniaceae)*, vol 7. Springer, Berlin, pp. 167-275.
- Herron, S. 2003. Catnip, *Nepeta cataria*, a Morphological comparison of mutant and wild type specimens to gain an ethnobotanical perspective. *Econ. Bot.*, 57(1): 135-142.
- Husain, S.Z., P.D. Marin, N. Diklic and B. Patcovic. 1989. Micromorphological and phytochemical studies in two new endemic *Nepeta* (Lamiaceae) species in Yugoslavia. *Pak. J. Bot.*, 21(2): 210.
- Husain, S.Z., P.D. Marin, Č. Šilić, M. Qaiser and B. Patcović. 1990. A micromorphological study of some representative genera in the tribe Saturejeae (Lamiaceae). *Bot. J. Linn. Soc.*, 103(1): 59-80.
- Jamzad, Z., M.W. Chase, M. Ingrouille, M.S.J. Simmonds and A. Jalili. 2003. Phylogenetic relationships in *Nepeta* L. (Lamiaceae) and related genera based on ITS sequence data. *Taxon*, 52: 21-32.
- Janošević, D., S. Budimir, A. Alimpić, P. Marin, N. Al Sheef, A. Giweli and S. Duletić-Laušević. 2016. Micromorphology and histochemistry of leaf trichomes of *Salvia aegyptiaca* (Lamiaceae). *Arch. Biol. Sci.*, 68(2): 291-301.
- Jia, P., H. Liu, T. Gao and H. Xin. 2013. Glandular trichomes and essential oil of *Thymus quinquecostatus*. *Sci. World J.*, 2013.
- Joharchi, M.R. and M.S. Amiri. 2012. Taxonomic evaluation of misidentification of crude herbal drugs marketed in Iran. *Avicenna. J. Phytomed.*, 2(2): 105.
- Kahkeshani, N., M. Khanavi and A. Hadjiakhoondi. 2014. Quality Control of *Nepeta menthoides* Boiss & Buhse using Micromorphological Analysis and Phytochemical Screening. *J. Med. Plants*, 13(49): 34-38.
- Kahraman, A., F. Celep and M. Dogan. 2010. Anatomy, trichome morphology and palynology of *Salvia chrysophylla* Stapf (Lamiaceae). *S. Afr. J. Bot.*, 76: 187-195.
- Kamatou, G.P.P., R.L. Van Zyl, S.F. Van Vuuren, A.M. Viljoen, A.C. Figueiredo, J.G. Barroso, L.G. Pedro and P.M. Tilney. 2006. Chemical composition, leaf trichome types and biological activities of the essential oils of four related *Salvia* species indigenous to southern Africa. *J. Essent. Oil Res.*, 18: 72-79.
- Kaya, A., B. Demirci and K.H.C. Baser. 2007. Micromorphology of glandular trichomes of *Nepeta congesta* Fisch. & Mey. var. *congesta* (Lamiaceae) and chemical analysis of the essential oils. *S. Afr. J. Bot.*, 73: 29-34.
- Kofidis, G. and A.M. Bosabalidis. 2008. Effects of altitude and season on glandular hairs and leaf structural traits of *Nepeta nuda*, L. *Bot. Stud.*, 49: 363-372.
- Kolalite, M.R. 1998. Comparative analysis of ultrastructure of glandular trichomes in two *Nepeta cataria* chemotypes (*N. cataria* and *N. catena* var. *citriodora*). *Nord. J. Bot.*, 18: 589-598.
- Lange, B.M. 2015. The evolution of plant secretory structures and emergence of terpenoid chemical diversity. *Annu. Rev. Plant Biol.*, 66: 139-15.
- Ljaljević-Grbić, M. 2006. *Ispitivanje interakcije rtanjske metvice (Nepeta rtanjensis Diklić et Milojević) i odabranih mikromiceta*. PhD thesis, Faculty of Biology, Belgrade, Serbia, pp 131 (in Serbian).

- Ljaljević-Grbić, M., M. Stupar, J. Vukojević and D. Grubišić. 2011a. Inhibitory effect of essential oil from *Nepeta rtanjensis* on fungal spore germination. *Cent. Eur. J. Biol.*, 6(4): 583-585.
- Ljaljević-Grbić, M., M. Stupar, J. Vukojević and D. Grubišić. 2011b. In vitro antifungal and demelanizing activity of *Nepeta rtanjensis* essential oil against the human pathogen *Bipolaris spicifera*. *Arch. Biol. Sci.*, 63(3): 897-905.
- Ljaljević-Grbić, M., M. Stupar, N. Unković, J. Vukojević, B. Stevanović and D. Grubišić. 2015. Diversity of microfungi associated with phyllosphere of endemic Serbian plant *Nepeta rtanjensis* Diklić & Milojević. *Braz. J. Bot.*, 38(3): 597-603.
- Maleci Bini, L., A. Pinetti and O. Servettaz. 1992. Micromorphological and phytochemical researches on *Teucrium massiliense* L. In: (Eds.): Harley, R.M. and T. Reynolds. *Advances in Lamiaceae Science*. Royal Botanic Gardens, pp. 349-355.
- Metcalfe, C.R. and L. Chalk. 1972. *Anatomy of the dicotyledons*. Vol. II. Oxford University Press, London, pp. 276.
- Mišić, D., B. Šiler, U. Gašić, S. Avramov, S. Živković, J. Nestorović-Živković, M. Šlutinović and Ž. Tešić. 2015. Simultaneous UHPLC/DAD/ (+/-) HESI-MS/MS Analysis of Phenolic Acids and *Nepeta* lactones in Methanol Extracts of *Nepeta* Species: A Possible Application in Chemotaxonomic Studies. *Phytochem. Anal.*, 26(1): 72-85.
- Modenesi, P., G. Serrato-Valenti and A. Bruni. 1984. Development and secretion of clubbed trichomes in *Thymus vulgaris* L. *Flora*, 175: 211-219.
- Modnicki, D., M. Tokar and B. Klimek. 2007. Flavonoids and phenolic acid on *Nepeta cataria* L. var. *citriodora*. *Acta Pol. Pharm. et Drug Research*, 64: 247-252.
- Moon, H.K., S.P. Hong, E. Smets and S. Huysmans. 2009. Phylogenetic significance of leaf micromorphology and anatomy in the tribe Mentheae (Nepetoideae: Lamiaceae). *Bot. Journ. Linn. Soc.*, 160: 211-231.
- Naidoo, Y., N. Kasim, S. Heneidak, A. Nicholas and G. Naidoo. 2013. Foliar secretory trichomes of *Ocimum obovatum* (Lamiaceae): micromorphological structure and histochemistry. *Plant. Syst. Evol.*, 299: 873.
- Navarro, T. and J. ElOualidi. 2000. Trichome morphology in *Teucrium* L. (Lamiaceae), a taxonomic review. *Anales Jard. Bot. Madrid*, 57(2): 277-297.
- Pădure, I.M. 2006a. Morpho-anatomy of Flower and Inflorescence in *Nepeta* L. (Lamiaceae, Nepetoideae). *An. Stiint. Univ. Al. I. Cuza Iasi, Sect. II a. Biol. Veget.*, 52(2): 19-29.
- Pădure, I.M. 2006b. *Nepeta* (Lamiaceae) morphology and anatomy in Romania and their taxonomic significance. Proceedings of IV BBC Conference: Plant, fungal and habitat diversity investigation and conservation. Sofia, Bulgaria, p.p. 34-38.
- Payne, W. 1978. A glossary of plant hair terminology. *Brittonia*, 30: 239-255.
- Pljevljakušić, D., D. Rančić, M. Ristić, Lj. Vujčić, D. Radanović and Z. Dajić-Stevanović. 2012. Rhizome and root yield of the cultivated *Arnica montana* L., chemical composition and histochemical localization of essential oil. *Ind. Crops Prod.*, 39: 177-189.
- Rapisarda, A., E.M. Galati, O. Tzakou and M. Flores. 2001. *Nepeta sibthropii* Benth (Lamiaceae) micromorphological analysis of leaves and flowers. *Farmaco*, 56(5-7): 413-415.
- Regnier, F.E., G.R. Waller and E.J. Eisenbraun. 1967. Studies on the composition of the essential oils of three *Nepeta* species. *Phytochemistry*, 6: 1281-1289.
- Salmakia, Y., S.H. Zarre, Z. Jamzad and C. Brauchler. 2009. Trichome micromorphology of Iranian *Stachys* (Lamiaceae) with emphasis on its systematic implication. *Flora*, 204 (5): 371 - 81.
- Sarkar, M., R. Rashmi and P.N. Vikramaditya. 1995. Pharmacognosy of *Nepeta cataria*. *Anc. Sci. Life*, 14(4): 225.
- Sharma, A. and D.S. Cannoo. 2013. Phytochemical composition of essential oils isolated from different species of genus *Nepeta* of Lamiaceae family: A review. *Pharmacophore*, 4(6): 181-211.
- Turner, G.W., J. Gershenzon, and R. B. Croteau. 2000. Development of peltate glandular trichomes of Peppermint. *Plant. Physiol.*, 124: 665-679.
- Werker, E. 1993. Function of essential oil-secreting glandular hairs in aromatic plants of the Lamiaceae—a review. *Flavour Fragr. J.*, 8: 249-255.
- Werker, E., U. Ravid and E. Putievsky. 1985a. Structure of glandular hairs and identification of the main components of their secreted material in some species of the Lamiaceae family. *Israel J. Bot.*, 34: 31-45.
- Werker, E., U. Ravid and E. Putievsky. 1985b. Glandular hairs and their secretions in the vegetative and reproductive organs of *Salvia sclarea* and *S. dominica*. *Israel. J. Bot.*, 34: 239-252.
- Werker, E. 2000. Trichome diversity and development. In: Hallahan, D.L., J.C. Gray and J.A. Callow. *Advances in botanical research. Plant trichomes*. Academic Press, San Diego, pp. 1-35.
- Yeung, C.T., C. Stasolla, M.J. Sumner and B.Q. Huang. (Eds.) 2015. *Plant Microtechniques and Protocols*. Springer International Publishing, Switzerland.

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