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MICROMORPHOLOGICAL AND PHYTOCHEMICAL STUDIES IN TWO NEW ENDEMIC *NEPETA* (LAMIACEAE) SPECIES IN YUGOSLAVIA

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Abstract

Within the *Nepeta sibthorpii*-complex of the section *Pycnonepeta* Bentham, two newly described endemic species from Yugoslavia viz., *N. rtanjensis* Diklic & Milojevic and *N. ernesti-mayeri* Diklic & Nikolic were examined for nutlet sculpturing and leaf indumentum (SEM) and leaf flavonoid glycosides. Significance of microcharacters and flavonoid glycosides composition as taxonomic markers in these two species are discussed.

Introduction

In taxonomic studies very little attention has been given to micromorphological characters of nutlets compared with other gross taxonomic features. Nutlet, leaf indumentum and phytochemical charcters are seldom mentioned in taxonomic revisions or major floras which is surprising in view of stability of many of their external and internal features (Davis & Heywood, 1963). Various workers have shown that micromorphology and chemotaxonomy can be used to separate genera, species and even varieties (Corner, 1951; Murley, 1951; McClure, 1957; Ball & Heywood, 1962; Harborne & Turner, 1984). In spite of taxonomic value of netlet characters found in several genera in Lamiaceae (Wojciechowska, 1961, 1966, 1972; Hedge, 1968, 1970; Hussain, 1983) the micromorphology and flavonoid chemistry as taxonomic markers have not been studied extensively in the genus *Nepeta*. (Seshadri & Sharma, 1973).

Nepeta L., is a polymorphic genus with about 150 species; mainly distributed in the temperate region. The centre of diversity is mainly in the Mediterranean Basin. They usually form a part of the vegetation in dry, rocky and hilly disturbed ground. Fig. 1 shows the distribution of *Nepeta sibthorpii* complex of the section *Picnonepeta* Bentham, which includes two new species *N. rtanjensis* and *N. ernesti-mayeri* from Yugoslavia (Dicklic & Milojevic, 1976; Diklic & Nikolic, 1977). In present study the micromorphological characters and leaf flavonoids as taxonomic markers in *Nepeta rtanjensis* and *N. ernesti-mayeri* from Yugoslavia is described.

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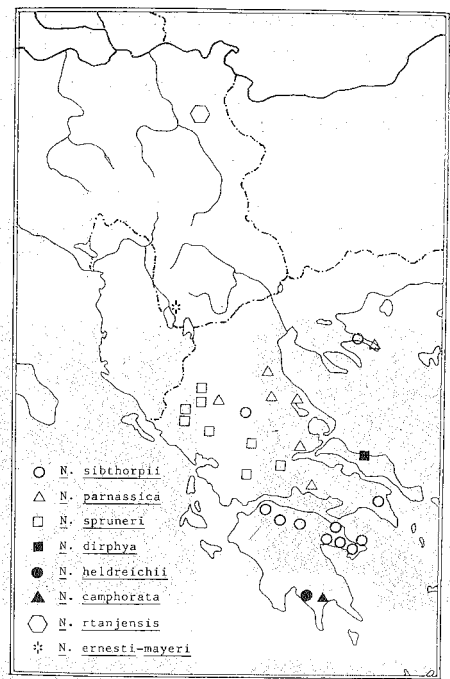

Fig. 1. Distribution Map of *Nepeta sibthorpii* - Complex.

Materials and Methods

Mature nutlets and leaf samples were collected from different localities of Yugoslavia (Fig. 1), for SEM examination and flavonoid analysis. Voucher specimens have been deposited in the Natural History Museum Herbarium, Belgrade BEO, Yugoslavia. Nutlet and leaf samples were placed on clean stubs and coated with 30 nm of gold in a Polaron E5150 Sputter Coater and examined in SEM T20 (JEOL) at different magnifications. Flavonoid glycosides were analysed by 2D-PC, against authentic markers on cellulose TLC and UV spectroscopy as described previously (Husain & Markham, 1981; Husain *et al.*, 1982).

Results and Discussion

The nutlet surfaces of both species show striking similarities. They are broadly ovate, 1.8-0.2mm in length, 0.8-1.0mm in width and light brown in colour. The scar attachment does not pass on the dorsal part of the nutlet. The sculpturing pattern is loosely reticulate with pronounced bumps and tuberculate. The nutlet surface observed is shallow with not well marked undulations. Cells in *N. ernesti-mayeri* are rectangular, pentangular

and hexagonal in outline with varying thickness and tightly packed (Fig. 2A, B), whereas in *N. rtanjensis* (Fig. 3A, B), the sculpturing pattern is reticulate tuberculate with pronounced bumps and the cells are mainly hexagonal with outer cell walls forming the continuous channels (Fig. 3B). Posterior part of the nutlets in both species shows dents and well pronounced protrusions.

The indumentum show very similar pattern in *N. ernesti-mayeri,* at the adaxial (Fig. 2C) and abaxial (Fig. 2D, E) and in *N. rtanjensis* at the adaxial (Fig. 3C, D) and abaxial

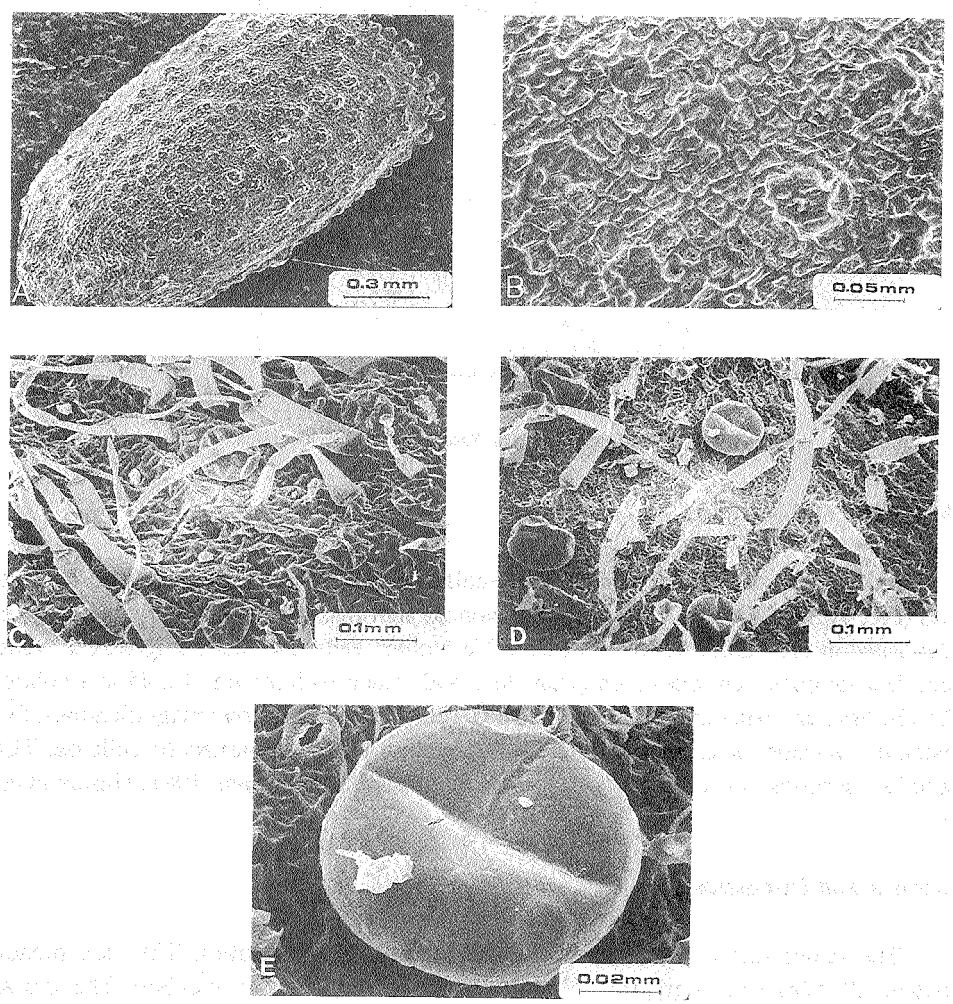

Fig. 2. Scanning electron micrographs of *Nepeta ernesti-mayeri*. A & B, Nutlet surface; C, Adaxial leaf surface; D & E, Abaxial leaf surface showing leaf gland.

(Fig. 3E, F) surfaces. Two types of trichomes are distinguished; one long, 0.2-1.5mm, multicellular with glandular apex and showing smooth surface but occasionally small granules are also observed (Fig. 3D). The second type of trichomes is short 0.01-0.08mm, papillate and very sparsely distributed. The density of long trichomes is very high, especially in *N. rtanjensis* (Fig. 3C, E). Sessile epidermal oil glands are densely distributed on both surfaces. Stomata are mainly present on abaxial surfaces and are of paracytic type.

Results of the leaf flavonoids of two species showed the presence of 10 phenolic compounds on 2D chromatogram (Table 1 & 2). The presence and absence of each spot

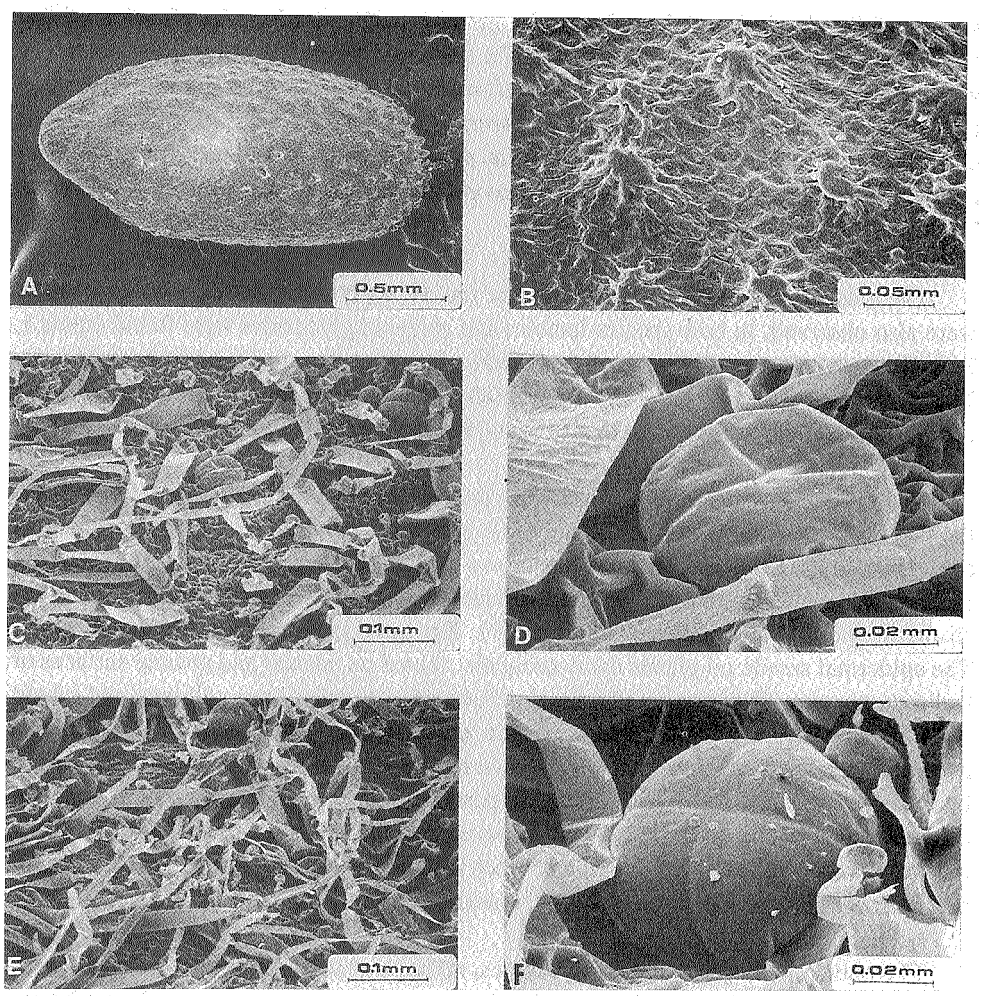

Fig. 3. Scanning Electron Micrographs of *Nepeta rtanjensis*: 'A & B, Nutlet surface; C & D, Adaxial leaf surface; E & F, Abaxial leaf surface.

Table. 1. Flavonoid glycoside spots found in two *Nepeta* species.

	Spot No.									
Taxa	1	2	3	4	5	6	7	8	9	10
N. rtanjensis	+	+	+	(+)	+	+	+	+	+	+
N. ernesti-mayeri	+	+	+	+	+	+	—	+	+	+

Key: + = present, — = absent, (+) = trace

was carefully checked and the relevant information with tentative identification of flavonoid glycosides is presented in Table 2. Six flavonoid glycosides were fully identified, whilst the components of spots 6, 7, 9 and 10 were partially characterised and marked as unknown. The colours, characteristics of glycosides developed on chromatograms were examined under UV light. The Rf values in different solvents and the UV absorption maxima in pure methanol and in the presence of standard inorganic reagents are given in Table 2.

The most common leaf constituents were flavone glycosides, but flavonol glycosides were also observed. In both species luteolin and apigenin glycosides were found (spots 1, 2, 3, 8). Apigenin-6, 8-di-C-glucoside (Vicenin-20, Spot 8) was found in both species. This is the first report of a flavone c-glycoside in section *Picnonepeta*. Flavonols quercetin-3-0-galactoside (Spot 5) were also found in both species.

The present investigation confirms that on the basis of the nutlet micromorphology, the two endemic *Nepeta* species broadly fall within the genus. The other related genera e.g., *Melissa* L., *Clinopodium* L., *Calamintha* Miller, *Micromeria* Benth., *Hyssopus* L., *Satureja* L., and *Origanum* L., exhibit different sculpturing features (Husain, 1983; Seshadri & Sharma, 1973; Husain *et al.*, 1989). The same is true about leaf indumentum. The epidermal sessile oil glands are densely distributed on adaxial and abaxial surfaces of leaf indumentum. They consist of a basal cell, uniseriate stalk of one cell long and a head from several secretory cells, which contain essential oils (Fhan, 1974). Considerable pharmaceutical and culinary interest is shown because of the presence of essential oils in Lamiaceae in general and *Nepeta* genus in particular. The main constituents of essential oils are mono- and sesquiterpenes. In *N. rtanjensis* and *N. cataria* the essential oil nepetalactone is very characteristic. These and other monoterpenes are constant enough to warrant consideration as taxonomic markers (Husain, 1983).

Flavonoid chemistry in these two species, reflect a general pattern in Lamiaceae (Harborne & Williams 1971; Husain *et al.*, 1982). Both the species show very close relationship with each other since out of 10 flavonoid glycosides observed in both species, 9 are shared. Only spot number 7 is absent in *N. ernesti-mayeri*, whereas rutin (Spot 4) varies

Table 2. The characteristics of flavone glycosides found in *Nepeta rtanjensis* and *N. ernesti-mayeri*.

Spot No.	Colour in UV/UV+NH_3		Rf values x 100 in:				UV Spectral Analysis (Max nm)						Acid Hydrolysis		Tentative Identification
			BAW	15% HOAc	BEW	CAW 1:1	MeOh Band I Band II	MeOH + NaOAC Band II	MeOh+ H_3BO_3 Band II	MeOH+ NaOH Band II	MeOH+ $AlCl_3$ Band II	MeOH+ HCl Band II	Aglycone	Sugar	
1	D	Y	42	16	36	1	257 351	256	378	388	417	355	Luteolin	gluc	Luteolin-7-0-glucoside
2	D	Y	18	33	20	3	256 352	256	376	—	415	353	Luteolin	gluc	Luteolin 7, 4,-0-diglucoside
3	D	Y	60	10	—	25	265 338	265	362	—	—	—	Apigenin	gluc	Apigenin-7-0-glucoside
4	D	Y	44	55	—	—	260 364	267	381	402	421	352*	Quercetin	gluc + RHA	Quercetin-3-0-rutinoside (Rutin)
5	D	Y	60	40	—	34	265 269	267	382	—	—	—	Quercetin	gal	Quercetin-3-0-galactoside
6	D	lY	25	42	31	5	257 340	265	358	—	—	—	—	—	unknown
7	—	Y	30	27	—	—	—	—	—	—	—	—	—	—	unknown
8	DB_R	B_RO_R	30	61	—	—	—	—	—	—	—	—	Apigenin	gluc	Apigenin-6,8, DI-C-glucoside (vicennin-2)
9	DB_R	B_RO_R	18	62	—	—	—	—	—	—	—	—	—	—	unknown
10	D	dYG	81	7	—	—	—	—	—	—	—	—	—	—	unknown

Key: D = Dark, Y = Yellow; l : Light; OR = Orange; G = Green; d = Dull; BR = Brown.

quantitatively, and is only found in trace amounts in *N. rtanjensis*. This investigation provides more information and highlights the significance of the micromorphological and phytochemical characters as taxonomic markers in these two endemic species of *Nepeta*.

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