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**MORPHOLOGICAL AND CHEMICAL VARIABILITY OF THE  
POPULATIONS OF THE *ALYSSUM MARKGRAFII* SCHULZ  
(*BRASSICACEAE*)**

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Janačković P., Tešević V. (1995): *Morphological and chemical variability of the populations of *Alyssum markgrafii* Schulz (Brassicaceae)*. – Glasnik Instituta za botaniku i botaničke bašte Univerziteta u Beogradu, Tom XXIX, 187 - 198.

Morphological and chemical variability of populations of *Alyssum markgrafii* Schulz on the territory of the Republic of Serbia was analyzed using the scanning electronic microscope (SEM) and high performance liquid chromatography (HPLC). Achieved results proved that there was some variability in length and width of leaves among populations analyzed. Also, variability of the height of the plants matched literary data and the number of trichomes on the square millimeter at both sides of the leaf, especially the back side of the leaf, did not show much variability, but the trichomes at the back side of the leaf were more branchy than those on the front side and their branches were narrower. On the basis of the chromatogram of the leaf ethanol extract and the photograph of the same in the UV spectrum, the presence of the flavonoids was estimated. In addition, a considerable resemblance among the populations in their qualitative structure was observed, particularly among the major components of the flavonoids. The importance of the micromorphological para-

metres and the qualitative composition of the flavonoid components as taxonomic markers in delimitation of *A. markgrafii* and related species from the *Odontarrhena* section was also discussed.

Key words: *Brassicaceae*, *Alyssum markgrafii*, trichomes, flavonoids, trichomes, flavonoids, SEM, HPLC, taxo-nomy.

Ključne reči: *Brassicaceae*, *Alyssum markgrafii* trihomi, flavonoidi, trihomi, flavonoidi, SEM, HPLC, ta-ksonomija.

## INTRODUCTION

*Alyssum* L. (*Brassicaceae*) genus includes about 170 species; its areas are confined to the territory of southwest Asia, eastern Mediterranean, south and southeastern Europe.

Species were classified in the following six sections: *Aurinia* (Desv.) Meyer, *Alyssum* (*Eualyssum*) Gris., *Psilonema* (Meyer) Hook, *Gamosepalum* (Hausskn.) Dudl., *Odontarrhena* (Meyer) Koch., and *Memiocus* (Desv.) Hook (Ančev, 1991).

There are 64 species in Europe, and 32 of them (47%) are European endemics (P. W. Ball et al., 1964). This high percentage of the continental endemics is closely connected to the flora of the eastern Mediterranean, or, more accurately, to the southeastern areas of the Balkan peninsula. On this area genus is represented with about 45 species, out of which 21 is endemic to the Balkans, with the areas limited to the territory of Crete, Greece, Albania, Yugoslavia and Bulgaria. There is 17 species of this genus in the Republic of Serbia (Diklić, 1972).

*A. markgrafii* is a perennial, herbaceous plant and belongs to the section of *Odontharrena* (Meyer) Koch. It populates areas in limestone, serpentine, most frequently in mountain region. It has been spread in Yugoslavia and Albania (Diklić, 1972).

In systematics revisions of different taxa, micromorphological characters of the leaf surface, fruits and seeds were often ignored or only seldom mentioned, regardless of their stability (Davis et Heywood, 1963). The use of the scanning electronic microscope (SEM) gave new possibilities in researching microcharacters in plants (Marin, 1989). Nowadays, a greater importance is given to the application of scanning electronic microscope for the analysis of micromorphological characters in taxonomy researches (Hardin, 1979 a and b; Hardin and Gensel, 1982; Husain et al., 1989, 1990; Ančev, 1991; Marin et al., 1994).

Taxonomical significance of the trichome micromorphology within the family of *Brassicaceae* has also been emphasized with *Bornmuellera dieckii* Degen (Marin et al., 1993), as well as with the genus of *Alyssum* L. (Ančev, 1991).

Diversity regarding morpho-anatomic aspect, as well as the way of life in a particular area is reflected in the chemical structure of the plants. Different groups of chemical compounds are from chemotaxonomical aspect more or less typical for certain taxonomic categories. 5-methylthiopentylglucosinolate was identified in this way as taxonomic character of the *Alyssum* L. genus (Hasapis et al., 1981). Then followed isothiocyanates, nitriles and epithiobutanes with *Alyssum minimum* Willd (Lockwood, Afsharipuor, 1986). These substances are the reason for application of the *Alyssum minimum* Willd in the traditional medicine. Flavonoid glucuronoides in *Alyssum minimum* were also researched from chemotaxonomic aspect (Afsharipuor et Lockwood, 1986). Considering chemotaxonomic aspect,

flavonoids possess a wide range of use on different levels within families (Harborne et Turner, 1984; Husain et al. 1989). The presence of alkanes on the leaves of *Borrmuelleria dieckii* Degen and *Alyssum markgrafii* Schulz (Marin et al. 1993) within family of *Brassicaceae* was researched.

An analysis of the variability of morphologic characters of the vegetative organs was done in this study, with an emphasis on the trichomes on the leaves in ten populations of the *Alyssum markgrafii* Schulz species on the following localities: Brdani, Smedraž, Stragari, (these localities are in the vicinity of Gornji Milanovac), Dobre strane near Kraljevo, Ozren near Sjenica, Sevce near Brezovica and Brezovica itself (Fig. 1). All the localities have serpentine basis. Also, a comparative chemical analysis of the leaf ethanol extract from the same populations was made in order to estimate interpopulation variabilities of the phenolic compound composition in leaves and its taxonomic importance.

### MATERIALS AND METHODS

Herbarium materials gathered on the localities of Brdani, Smedraž, Stragari, Dobre strane, Ozren, Sevce and Brezovica (Fig. 1) were used for morphological, micromorphological and chemical investigation. Material used in this analysis was stored in a herbarium of the Institute of Botany and Botanical Garden „Jevremovac”, Faculty of Biology, University of Belgrade.

Height of plants, length and width of leaves were measured with a standard elastic ruler. Number of trichomes on the square millimeter on the both sides of the leaf along the main nerve where the leaf is the widest, was estimated using the stereo magnifying glass magnified for 56 x and the net of 1 mm<sup>2</sup> surface in the eyepiece.

The measured results were treated according to the following formulas:

$$X = \frac{\sum (X_i \times F_i)}{N}$$

$$D_i = X_i - X$$

$$\sigma = \frac{\sqrt{\sum (F_i \times D_i^2)}}{N}$$

$$CV = \frac{\sigma}{X} \times 100\%$$

X - average value,  $\Sigma$  - sum,  $X_i$  - value of the class,  $F_i$  - frequency of the class,  $D_i$  - average value deviation, N - number of specimens,  $\sigma$  - standard deviation, CV - variation coefficient.

During micromorphological analysis the samples of the leaves were attached to special metal cylinders and than evaporated with 30 nm thick layer of gold-paladium (80:15) in JEOL JEE 4B vacuum evaporator and analyzed on JEOL JSM T.35 scanning electronic microscope.

Ethanol extract from the leaves was acquired as follows: 0.05 of the leaves from each population was crushed in a ball mill and than 50 ml of 70% EtOH (ethanol) was added. Each sample was than reboiled for 2 min to inactivate the enzymes. Than the samples were left for 24 hours for extraction. Ethanol extract was strained through a filter paper and evaporated until dried in a vacuum evaporator (Harborne, 1984).

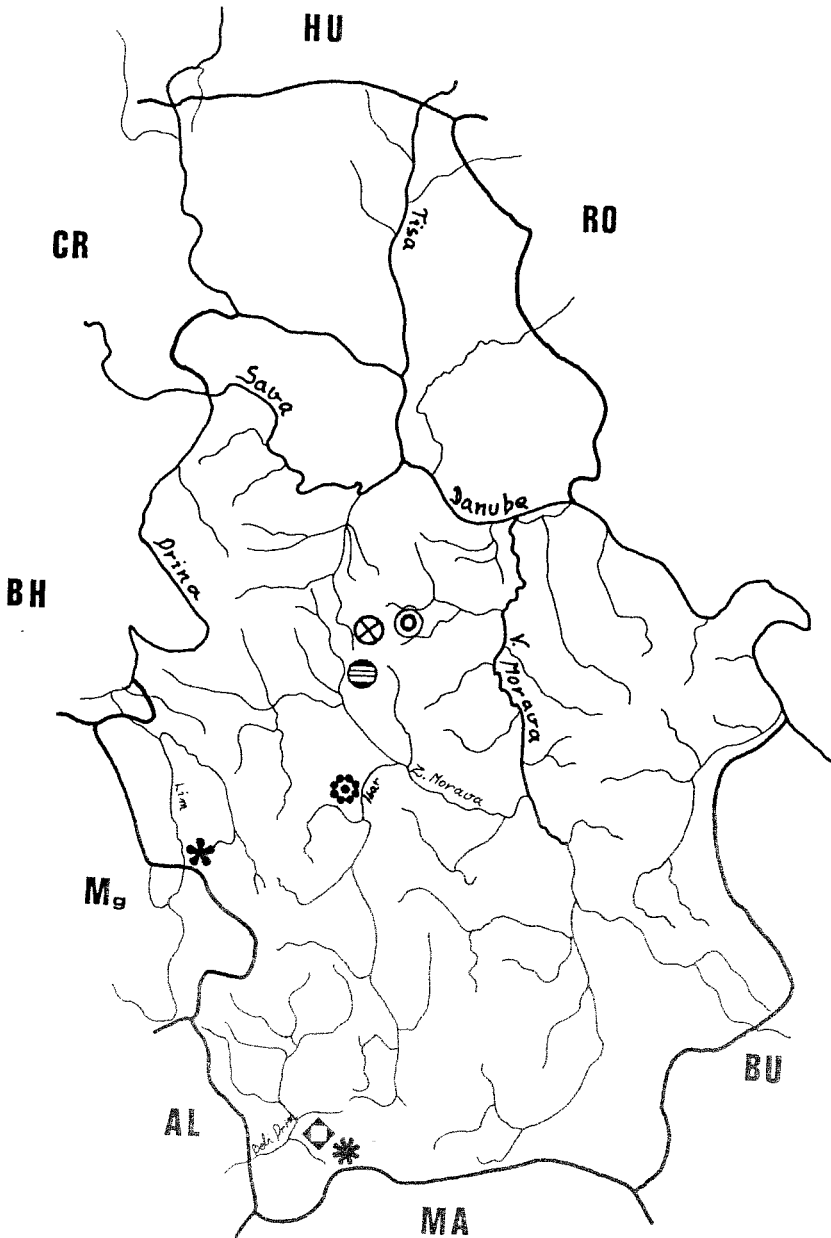


Fig. 1. – The localities of the analyzed populations of *Alyssum margrafii* Schulz

- ⊙ Stragari; ⊗ Brđani; ⊖ Semeđraž
- ⊛ Dobre stranc; \* Ozren; ◊ Sevice;
- \* Brezovica;

In order to remove physical and indissoluble admixtures, samples were refined with the membrane filter of the 3  $\mu\text{m}$  pore size, dissolved in 2 ml MeOH (methanol), filtered again and than diluted with another 2 ml MeOH.

Samples prepared in this way were chromatographed with a reverse-phase HPLC chromatography. OKTADECIL = Si 1003 YM column of the 125 x 4.6 mm dimension and 3  $\mu\text{m}$  particle size was used. Best separation was achieved with the gradient of MeOH and H<sub>2</sub>O eluents at the increase of MeOH concentration from 30% to 70% in a 30 min period. Detection was done with the UV Polychrom 9060 detector at the maximum absorption of 254 nm.

## RESULTS AND DISCUSSION

Morphological characteristics of the vegetative organs (such as length and width of leaves, leaf index - ratio between the length and width of leaves, height of the plant, number of trichomes on the square millimeter on both sides of the leaves), micromorphological characteristics of leaves, as well as the ethanol extract of leaves for ten populations of *A. markgrafii* on the above mentioned localities, were analyzed in this study.

Length of leaves differs from one populations to another although not considerably. Average value of length of leaves is the largest in A<sub>1</sub> population (21 mm) and the smallest in A<sub>9</sub> population (9.4 mm) (Fig. 2).

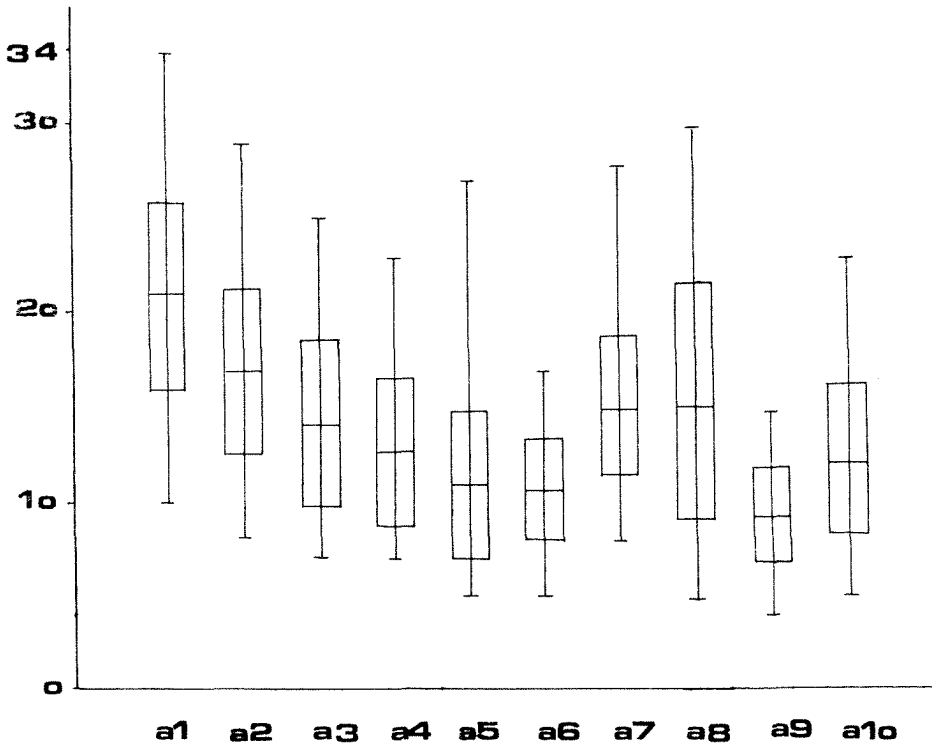


Fig. 2. - Variability of length of the leaves.

Average value of width of leaves in all population is within the range of 1.8 - 3.1 mm (Fig. 3).

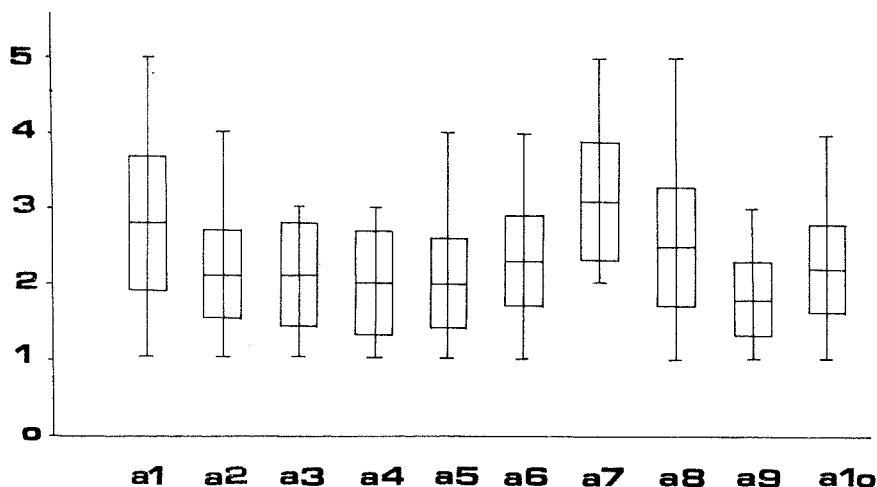


Fig. 3. – Variability of width of the leaves.

Leaf index presents the ratio between length and width of leaves and unites this two morphological characteristics. Population A<sub>2</sub> (8) has the largest average value of leaf index, while the smallest one belongs to A<sub>6</sub> population (4.5). Leaf index shows a greater importance in interpretation of variability of these populations compared to length and width of leaves in separate (Fig. 4).

Height of plants is very variable. A<sub>1</sub> population has the largest average height value (47.9 cm) and A<sub>9</sub> population has the smallest one (17.9 cm) (Fig. 5).

A<sub>6</sub> population has the largest number of trichomes on the front side of the leaf (7.4/mm<sup>2</sup>) and A<sub>8</sub> and A<sub>9</sub> populations have the smallest number of them (3.8/mm<sup>2</sup>) (Fig. 6).

A<sub>8</sub> population has the largest number of trichomes on back side of the leaf (29.9/mm<sup>2</sup>) and A<sub>2</sub> population have the smallest number (21.6/mm<sup>2</sup>) (Fig. 7).

Larger number of trichomes on the back side of the leaf than on the front side is connected to self protection of the plant. Trichomes protect the plant from excessed transpirations as well as from phytophagous insects. Parts of the plant with a small number of trichomes or those parts not having them at all, perianth for example, are often attacked and damaged by phytophagous insects. It should be mentioned that some phenolic compounds in leavers are, besides trichomes, probably the reason why phytophagous insects rarely attack the leavers of these plants (Marin et al., 1993). Small number of trichomes as well as their poor branching on the front side of the leaf is connected to more intensive photosynthesis of the leaf (Ančev, 1991).

Analyzing micromorphological characteristics of the leaf surface in *A. markgrafii*, the presence of dual and tripartite branching trichomes was noticed (Fig. 8, a-d). Relief wartlike bulgers are visible at the trichome surface both on the front and back side of the leaf.

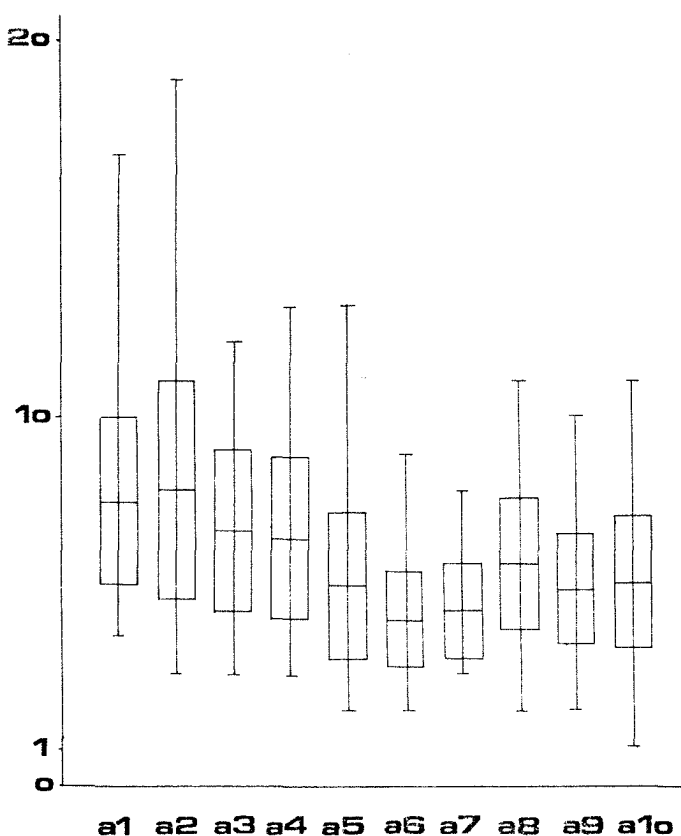


Fig. 4. - Leaf index.

Species from *Odontarrhena* section that were analyzed before, have relatively similar morphological characteristics of trichomes (Ančev, 1991). The trichomes of species from *Odontarrhena* section have a narrow central part of the trichome, more or less furrowed and scattered or evenly covered with warts, unevenly conical as in *A. bertolonii* Desv., or hemispheric in *A. borzaeanum* Niar. The warts gradually become smaller to the top of the branch. Only in *A. murale* Wik ssp. *pichlerii* (Vel.) Stoj. et Stef. trichomes have poorly furrowed central part. Starlike trichomes are most frequently covered with conical or semispheric warts of different size, as in *A. bertolonii* Desv., *A. obtusifolium* Steud. ex DC. and *A. murale* Wik ssp. *pichlerii* (Vel.) Stoj. et Stef. As a rule, more massive warts take the central part of the trichome.

*A. markgrafii*, which had not been analyzed from this aspect by now, has somewhat different trichomes than other members of this section. *A. markgrafii* trichomes have both fewer and thinner branches and less prominent wartlike bulgers than in other related species from this section.

It could be said that trichomes are, with all their micromorphological characteristics, a stable morphological character of taxonomic importance.

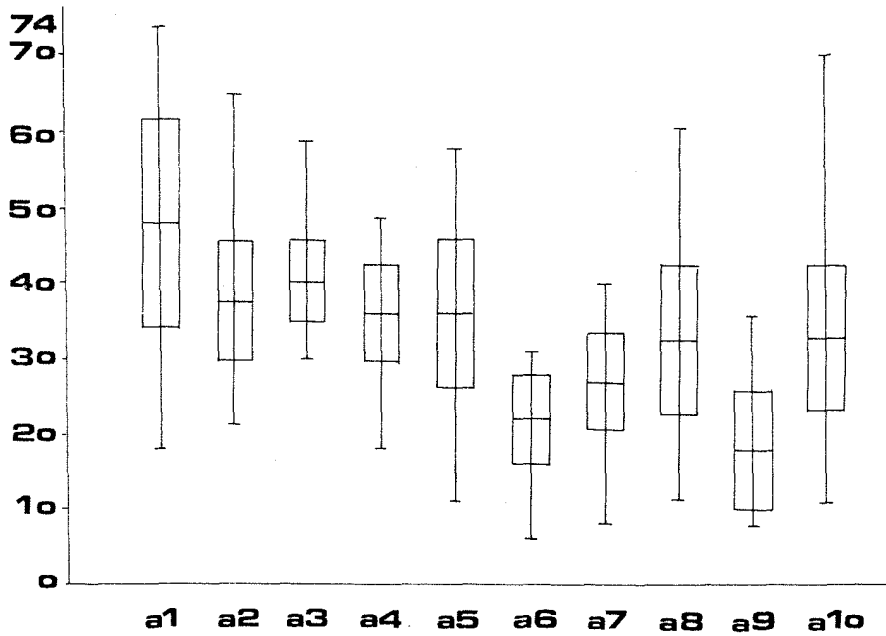


Fig. 5. - Variability of height of the plants.

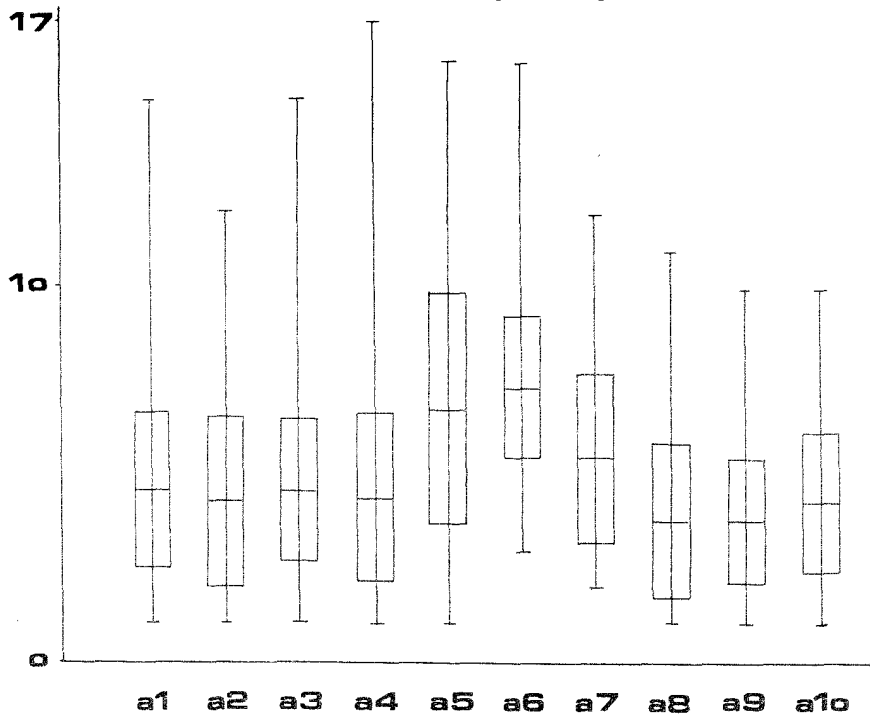


Fig. 6. - Number of trichoms/mm<sup>2</sup> on the front side of the leaf.



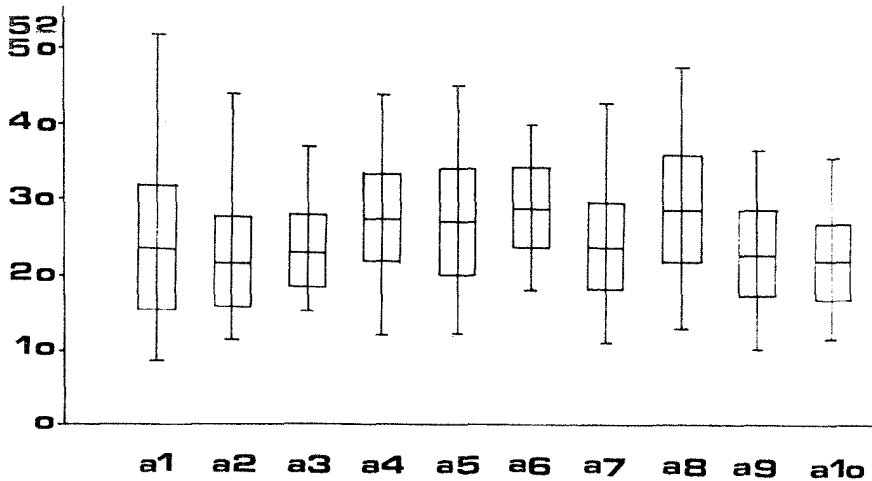


Fig. 7. – Number of trichomes/mm<sup>2</sup> on the back side of the leaf.

On the chromatogram of the leaf ethanol extract of analyzed populations (Fig. 9), peaks from I - XIII, represented more or less in all populations, were noticed. Quantitative differences can be seen in the presence or absence of particular peaks in all populations. Namely, only A<sub>2</sub> population has all the peaks, while A<sub>1</sub> population does not have peaks IX, XI and XII; A<sub>3</sub> does not have peaks VII and XI; A<sub>4</sub> does not have peaks II, XI, XI and XII; A<sub>5</sub> does not have peaks III, VI, VII, XI and XII; A<sub>6</sub> does not have peaks VIII and XII; A<sub>7</sub> does not have peak XII; A<sub>8</sub> does not have peaks VIII and XII; A<sub>9</sub> does not have peaks IV, VII, VIII, IX, X and XIII and A<sub>10</sub> does not have peaks III, VI and XI. According to chromatograms, all populations have peaks I and V.

Calculating the percentage of the surfaces with treated peaks (I-XIII) on and with corresponding retention times, certain qualitative differences between treated populations were established (Chart 1).

Chart 1. – Qualitative composition of the phenolic compounds

Rf	2.7	4.7	9.0	15.8	17.1	18.8	21.5	23.4	25.1	28.3	30.5	32.6	34.3
P	2.7	4.7	9.0	15.8	17.1	18.8	21.5	23.4	25.1	28.3	30.5	32.6	34.3
A1	1.3	0.3	0.5	1.3	3.3	9.2	3.4	74.1	-	3.5	-	-	3.4
A2	9.9	2.9	1.5	12.9	13.6	30	6.9	2	1.9	0.2	0.2	1.9	16.1
A3	5	0.4	0.4	4	12.4	45.7	-	3.6	2.3	1.6	-	4.4	19.9
A4	4.4	-	2.2	2.6	10.6	-	41.3	4.8	5.4	2.2	-	-	26.1
A5	4.6	5.7	-	6.1	58.6	-	-	-	2.1	-	-	-	22.5
A6	5.9	0.4	4.7	1.4	7.3	0.6	27	-	5.6	4.1	2.2	-	40.3
A8	5.2	1.3	17.6	6.7	9.2	1.3	24.2	-	7	3.7	2.6	-	27.8
A9	2.9	10	0.7	-	12.9	11.6	-	-	-	-	-	61.8	-
A10	6.6	7.9	-	0.8	0.7	-	45.2	2.3	9.8	2.4	-	7.1	17
PEAK	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII

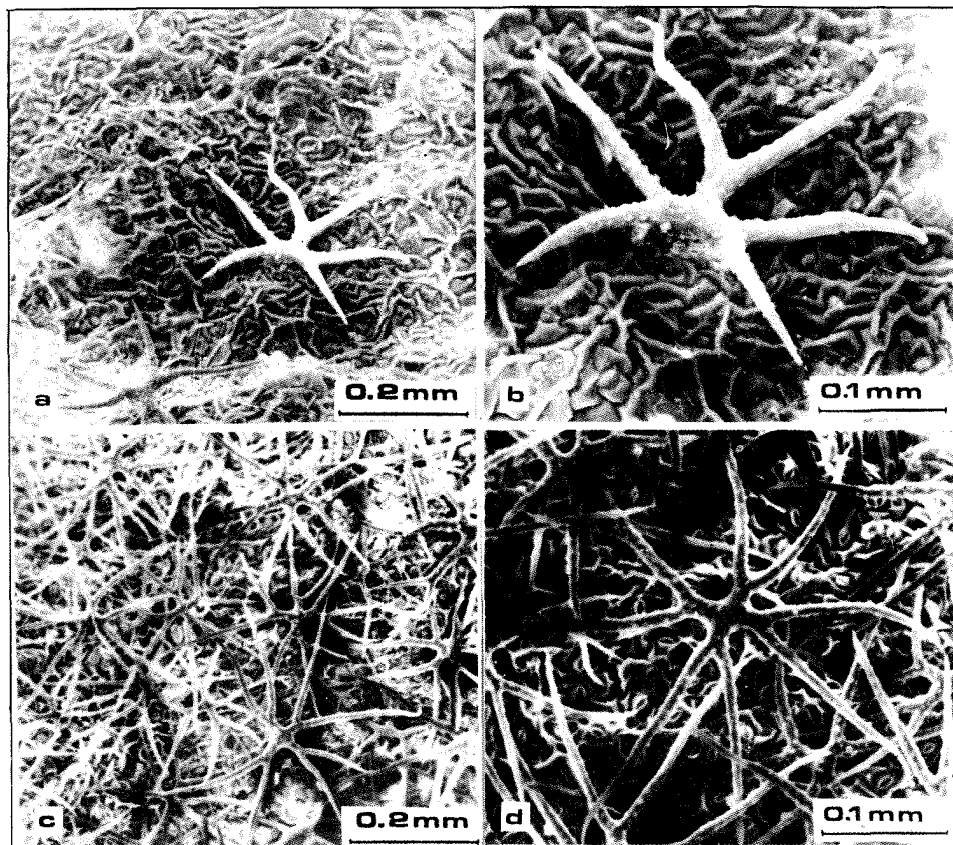


Fig. 8. – SEM of the leaves  
a-b. Trichomes on the front side of the leaf  
c-d. Trichomes on the back side of the leaf

On the basis of UV specters analysis of the treated peaks with the help of HPLC, the presence of phenolic compounds in leaves, probably flavonoids was established.

Regardless of the acquired quantitative differences in composition of flavonoids in different populations, the qualitative composition of major components in particular, is stable and could be used as a taxonomic character compared to other species of the *Alyssum* genus.

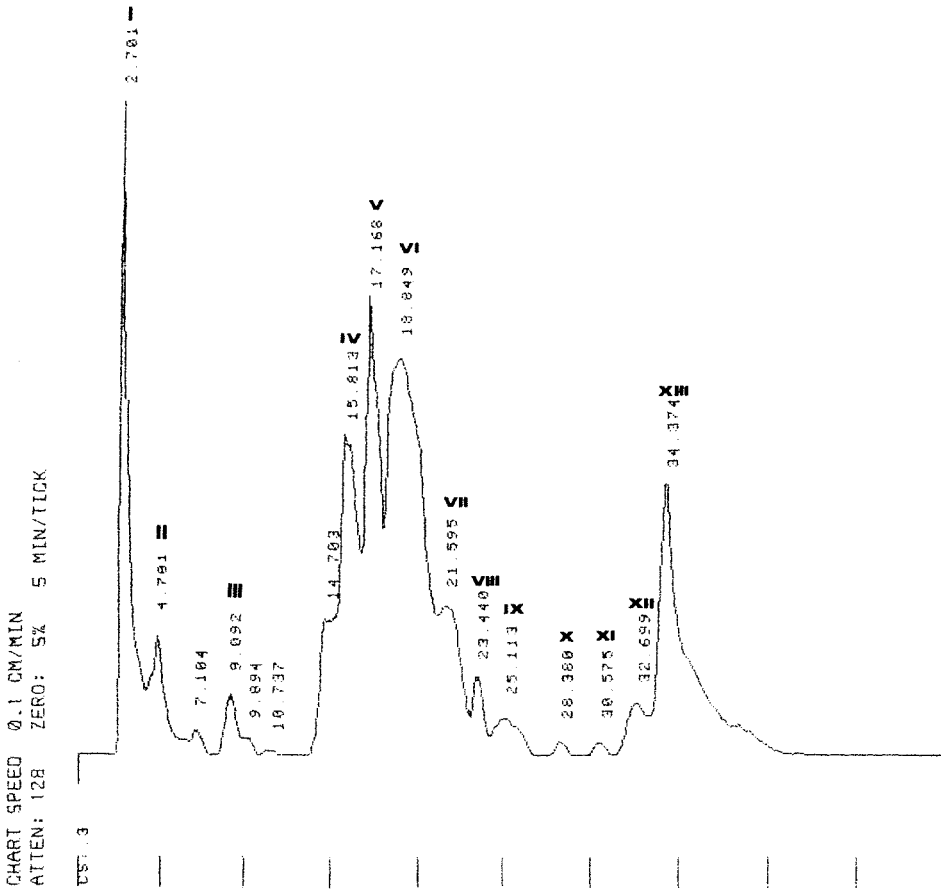


Fig. 9. - The chromatogram of the leaf ethanol extract

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## Rezime

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### MORFOLOŠKA I HEMIJSKA VARIJABILNOST POPULACIJA VRSTE *ALYSSUM MARKGRAFII* SCHULZ (*BRASSICACEAE*)

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U ovom radu izvršena je analiza varijabilnosti morfoloških karaktera vegetativnih organa kod deset populacija vrsta *Alyssum markgrafii* Schulz (*Brassicaceae*) na području Republike Srbije, sa posebnim akcentom na trihome na listovima. Sem toga, izvršena je i analiza listova pomoću skening elektronskog mikroskopa (SEM), kao i uporedna hemijska analiza etanolnog ekstrakta iz listova pomoću tečne hromatografije (HPLC). Dobijeni rezultati su pokazali da: dužina i širina listova ne pokazuju veliku varijabilnost između istraženih populacija; varijabilnost visine biljaka ispitanih populacija kreće se u granicama od 17.9 - 47.9 cm, što se poklapa sa literaturnim podacima; broj trihoma po mm<sup>2</sup> na licu, a naročito na naličju lista ne pokazuje veliku varijabilnost, a trihomi na naličju lista su razgranatiji od istih na licu i imaju uže grane. Na osnovu mikromorfološke analize listova može se zaključiti da su trihomi stabilan karakter i da se mogu koristiti kao dodatni parametar u delimitaciji *A. markgrafii* i srodnih vrsta u okviru sekcije *Odontarrhena*. Na osnovu hromatograma etanolnog ekstrakta iz listova uočena je velika sličnost između populacija. Ovi preliminarni hemijski parametri upućuju na mogućnost primene flavonoida, kao taksonomskih karaktera, s obzirom na malu varijabilnost između populacija.