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# Chemotaxonomy in Bulgaria

**Abstract**

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This literature survey bears witness of a marked increase in number of Bulgarian contribution to the chemosystematic study of plants during the last decade. Interdisciplinary collaborative effort by biochemists and plant taxonomists is at the root of this positive development. the reviewed papers bear on several among the larger and more critical families of the Bulgarian flora *(Amaryllidaceae, Apiaceae, Asteraceae, Fabaceae, Geraniaceae, Guttiferae, Lamiaceae, Papaveraceae, Plantaginaceae, Ranunculaceae, Scrophulariaceae, Valerianaceae)* and on a variety of different classes of compounds (alkaloids, antraquinones, coumarins, essential oils, flavonoids, iridoids, proteins, sapogenins, valepotriates).

## Introduction

The advances of biochemistry in the last decade are impressive both as to the development of new, elegant methods and as to the important implications of their results. Taxonomists today must be familiar with basic concepts and techniques in a number of disciplines, including cytology, genetics, statistics, anatomy, and chemotaxonomy. With the accumulation of chemical data from various plant groups, some now ask for special categories for chemically defined taxa, such as chemovar, chemoforma and chemocultivar, to accommodate chemical races. Newly developed, rapid chromatographic techniques permit the quick detection of numerous chemical constituents which, as biochemical data, are useful for classification at the infrafamilial levels. Chromatographic data lend themselves easily to mathematical treatment. Potential advantages of chemical characters, for classification purposes, are:

- Chemical constituents are intimately linked with the origin and evolution of plant life.
- The compounds in a plant are markers for the progressive or retrogressive or adaptive evolution of a plant taxon.
- Biochemical data may help evaluate phylogenetic classifications schemes at the level of family and below.

The studies of Bulgarian chemotaxonomists during last decade have concentrated on secondary metabolites. The Bulgarian vascular flora comprises 178 families, 3600 species and 330 subspecies. Of these, flavonoids have been studied in the *Asteraceae, Geraniaceae,* and *Lamiaceae;* alkaloids, in the *Fabaceae, Ranunculaceae, Papaveraceae,* and *Amaryllidaceae;* valepotriates, in the *Valerianaceae;* iridoids, in the *Plantaginaceae;* coumarins, in the *Apiaceae;* proteins, in the *Scrophulariaceae* and *Asteraceae;* antraquinones, in the *Guttiferae;* essential oils, in the *Lamiaceae;* sapogenins, in the *Fabaceae.*

## Flavonoids

The tribe *Anthemideae (Asteraceae)* comprises a large number of species used as medicinal plants, particularly in folk medicine. Flavonoid patterns proved to be important in the study of intergeneric relationship. According to Wollenweber (1981), Ivančeva & Kuzmanov (1986, 1990), and Ivančeva & al. (1987), flavonoids in *Achillea* resemble those in other genera of the *Asteraceae* by the predominance of 6-methoxyflavonoids. Their external (epicuticular) accumulation, often observed, is of interest in the context of the frequent correlation between habitat preference (xeric, alpine) and flavonoid excretion. Epicuticular flavonoids are believed to act as an UV screen. The main components identified are listed in Table 1. Differences in flavonoid pattern between the investigated, mainly E Mediterranean *Achillea* species are presented in Table 2. Generally, the substitution pattern of the main flavones and flavonols showed a tendency to 6- and 4'-methylation.

The variation pattern of the molecular components of five enzymes, in the *Achillea millefolium* group, was subjected to multivariate statistical analysis (Kuzmanov & al. 1983b). Eight *Artemisia* species native to Bulgaria have been studied for flavonoid chemotaxonomic markers. Flavonol 3-O-methoxy derivatives and glycosides (Ivančeva & Kuzmanov 1989) were mainly reported. 6-methoxyflavonoid aglycones were found in *Tanacetum* (Ivančeva & Behar 1995).

In *Festuca (Poaceae),* 40 polyphenols were found whose distribution supports separation of a new subsection (Angelov & al. 1988). The flavonoid profiles in *Geraniaceae* (Ivančeva & Wollenweber 1989) includes methyl derivatives of kaempferol, quercetin and myricetin as chemotaxonomic markers. In *Lamiaceae* flavonoids are a common feature in *Nepeta* (Tomas-Berberan & al. 1992).

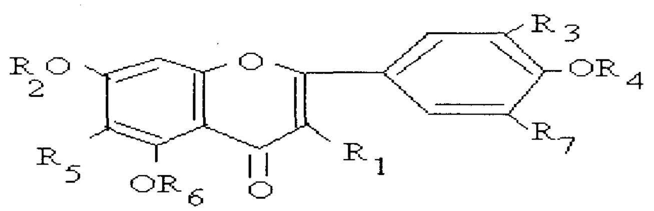


Fig. 1. Flavonoid skeleton. The $R_1$ to $R_7$ groups added in various aglycones found in *Achillea* are listed in Table 1

Table 1. Configuration of flavonoid aglycones found in *Achillea*. $R_1$ to $R_7$ are added to the skeleton illustrated in Fig. 1.

| N° Compound: | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ | $R_6$ | $R_7$ |
|---|---|---|---|---|---|---|---|
| 1. apigenin | H | H | H | H | H | H | H |
| 2. luteolin | H | H | OH | H | H | H | H |
| 3. 6-hydroxyluteolin | H | H | OH | H | OH | H | H |
| 4. 4'-methoxyluteolin | H | H | OH | $OCH_3$ | OH | H | H |
| 5. 5,7-dimethoxyluteolin | H | $OCH_3$ | OH | H | H | $OCH_3$ | H |
| 6. scutellarin (hispidulin) | H | H | H | H | $OCH_3$ | H | H |
| 7. 6-methoxyluteolin (nepetin) | H | H | OH | H | $OCH_3$ | H | H |
| 8. 3,6-dimethoxykaempferol | $OCH_3$ | H | OH | H | $OCH_3$ | H | H |
| 9. 3,6,3'-trimethoxyquercetagetin (jacidin) | $OCH_3$ | H | $OCH_3$ | H | $OCH_3$ | H | OH |
| 10. 3,6,7,4'-tetramethoxy-quercetagetin (casticin) | $OCH_3$ | H | $OCH_3$ | $CH_3$ | $OCH_3$ | H | OH |
| 11. 3-methoxykaempferol (isokaempferid) | $OCH_3$ | H | OH | H | H | H | H |
| 12. 3,7-dimethoxykaempferol (kumatakenin) | $OCH_3$ | $CH_3$ | OH | H | H | H | H |
| 13. 3-methoxyquercetin | $OCH_3$ | H | OH | H | H | H | OH |
| 14. 3,6,4'-trimethoxy-quercetagetin (centaureidin) | $OCH_3$ | H | OH | $CH_3$ | $OCH_3$ | H | OH |
| 15. 3,6,4'-trimethoxykaempferol (methylbetuletol) | $OCH_3$ | H | OH | $CH_3$ | H | H | H |
| 16. 3,6,7,3',4'-pentamethoxy-quercetagetin (artemetin) | $OCH_3$ | $CH_3$ | $OCH_3$ | $CH_3$ | $OCH_3$ | H | H |
| 17. 3,6,7,4'-tetramethoxy-kaempferol | $OCH_3$ | $CH_3$ | OH | $CH_3$ | H | H | H |
| 18. 3,4-dimethoxykaempferol (ermanin) | $OCH_3$ | H | OH | $CH_3$ | H | H | H |
| 19. 3,6-dimethoxyquercetagetin | $OCH_3$ | H | OH | H | $OCH_3$ | H | H |
| 20. 3,7-dimethoxyquercetin | $OCH_3$ | $CH_3$ | OH | H | H | H | OH |
| 21. 7-methoxyquercetin | OH | $CH_3$ | OH | H | OH | H | OH |
| 22. 3,6-dimethoxyquercetin (axillarin) | $OCH_3$ | H | OH | H | $OCH_3$ | H | OH |
| 23. 6,7-dimethoxyscutellarin (cirsimaritin) | H | $CH_3$ | H | H | $OCH_3$ | H | H |
| 24. 6,7,4'-trimethoxyscutellarian (salvigenin) | H | $CH_3$ | H | CH | $OCH_3$ | H | H |
| 25. 6,7,4'-trimethoxluteolin (eupatorin) | H | $CH_3$ | OH | $CH_3$ | $OCH_3$ | H | H |
| 26. 6,7,3'4'-tetramethoxyluteolin | H | $CH_3$ | $OCH_3$ | CH | $OCH_3$ | H | H |
| 27. 6,4'-dimethoxyscutellarin (pectolinaringenin) | H | H | H | $CH_3$ | $OCH_3$ | H | H |

Flavonoid patterns of several provenances of *Veronica triloba, V. sublobata* and *V. hederifolia* were investigated (Peev 1982). 7-0-glucoronides of apigenin and luteolin as well as of 6-substituted (6-hydroxyluteolin and hispidulin) and 3'-methylated (chrysoeriol) derivatives show a characteristic tendency to accumulate in the *V. hederifolia* group. The three species differ slightly in their flavonoid profiles. The pattern of *V. hederifolia* is cumulative of those of *V. triloba* and *V. sublobata*, thus supporting the hypothesis of an alloploid origin of the former.

Six Bulgarian species of *Peucedanum*, representing two sections, were studied for their flavonoids and coumarins (Kuzmanov & al. 1981a). 23 coumarins and 20 flavonoid compounds were found. A chemotaxonomic analysis of the species was considered in relation with the systematic position of the Balkan endemic *Peucedanum* species.

**Alkaloids**

The genus *Glaucium (Papavereae, Papaveraceae)* with its 21 species is widespread in the Mediterranean area. In *Glaucium,* Filipov & al. (1991) and Kuzmanov & al. (1992) isolated and characterized isoquinoline alkaloids. The main biogenetic pathway builds on the aporphine skeleton. The correlation between chemical, morphological and cytological characteristics, in the six genera of this tribe, suggest recognition of two subtribes: *Glauciinae* (monogeneric) and *Papaverinae* (including the remaining genera).

The Bulgarian species of *Chamaecytisus* and *Genista* in the tribe *Genisteae (Fabaceae)* have been investigated for quinolizidine alkaloids, characteristic for that family (Dučevska & al. 1986, Hristov & al. 1991). The oxidation level of quinolizidine alkaloids in different species was used for assessing phylogenetic progression in *Chamaecytisus,* where *C. supinus* with its capitulate inflorescence should be considered evolutionary more advanced as it has alkaloids of a higher oxidation level. New data on the quinolizidinine alkaloid content of *Lupinus graecus* and *L. albus* (Hristov & Evstatieva 1985) confirmed the established taxonomic scheme.

L-sparteine is the main alkaloid in *Chamaecytisus austriacus, C. absinthoides, C. ciliatus,* and *C. polytrichus.* The correlation between morphological and chemical data supports the separation of *Chamaecytisus* from *Cytisus* (Dučevska & al. 1988). In 10 Bulgarian *Genista* species cytisine and N-methylcytisine were found as main alkaloids (Hristov & al. 1991).

More than one-hundred native Bulgarian populations of *Corydalis* taxa *(Papaveraceae)* were studied for their alkaloid content (Kuzmanov & al. 1984a) and enzyme systems (Kuzmanov & al. 1983c). Two different alkaloid patterns were found to correlate with *Corydalis solida-C. slivenensis* and *C. bulbosa-C. marschalliana,* respectively. On the evidence of chemical data, *Corydalis* in Bulgaria is best treated as consisting of two species, *C. solida* and *C. bulbosa,* each subdivided into infraspecific taxa (Kuzmanov & al. 1984c).

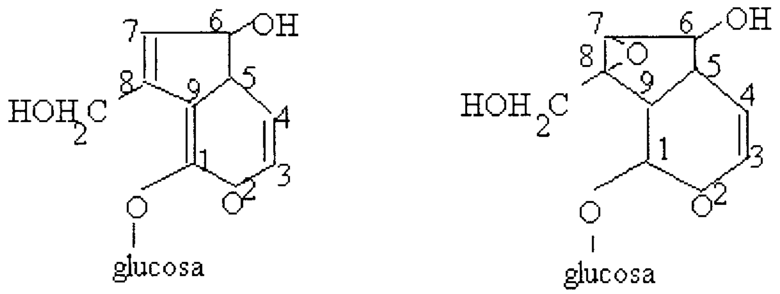


Fig. 2. $C_9$-Iridoids found in *Plantago* subg. *Plantago.* Left: aucubin; right: catalpol (From Kuzmanov & al. 1984b).

Table 2. Flavonoids found in some Bulgarian *Achillea* species. × = large amount; o = small amount. Numbers refer to the compounds listed in Table 1.

| *Achillea* sect. | species | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Millefolium* | *urumoffii* | | o | | | | × | × | × | o | o | o | o | o | | | | | | | | | | | | | | |
| | *kotschyi* | | | | o | o | | | × | × | | | | | | | | | | | | | | | | | | |
| *Ptarmica* | *grandifolia* | | | | | | | × | | | | | | | | | | | | | | | | | | | | |
| | *umbellata* | o | o | | | | | × | × | | | | | | o | × | | o | | | | | o | | | | | |
| *Santolinoideae* | *spinulifolia* | | | | | | o | o | | | | | | | | | | × | o | | | | | × | × | × | o | o |
| *Filipendulinae* | *clypeolata* | | | | | | | | | | | | o | | × | × | o | o | o | | | | | | | | | |
| | *coarctata* | | | | | | | | | | | | | | × | × | o | | | | | | | | | | | |
| | *thracica* | | | | | | | | | | | | o | × | | | | | | × | o | o | | | | | | |

*Thalictrum* is one of the larger genera in the *Ranunculaceae*. So far, more than half of its c. 70 species have been cytologically studied, of which 13 were found to be diploid and the remainder, polyploid. Kuzmanov & Dučevska (1982) distinguished two groups of polyploids, these groups being further supported by phytochemical data on isoquinoline alkaloids. A clear correlation between the polyploidy level and the type of the alkaloids produced could be shown: in diploids the alkaloids are bisbenzylisoquinolines; in decaploids only aporphine-benzylisoquinolines were found. Protoberberine alkaloids, mainly berberine, are characteristic of both cytotypes.

By morphological, cytological, embryological and chemosystematic analyses, Stefanov (1990) established three races of *Leucojum aestivum (Amaryllidaceae),* with galanthaminic, likoreinic and likorinic alkaloids, respectively.

## Iridoids

A postulated evolutionary pattern in *Plantago* is supported by phytochemical studies of iridoids (Kuzmanov & al. 1984b), which in *P.* subg. *Plantago* were found to be of the $C_9$ group: aucubin and catalpol (Fig. 2), whereas taxa of *P.* subg. *Psyllium* contain two $C_{10}$-iridoids. The phytochemical differences corroborate the distinction of two subgenera.

## Valepotriates

Valepotriates were used in a chemosystematic study of *Valerianaceae* (Evstatieva & al. 1993). Native Bulgarian populations of *Valeriana officinalis* showed considerable variability, with strong regional and local differences. The species was divided into two morphologically and phytochemically characterized subspecies with definite areas of distribution: *V. officinalis* subsp. *officinalis,* with cytotypes *"officinalis"* ($2n$ = 14) and *"nitida"* ($2n$ = 28), and *V. officinalis* subsp. *collina,* with cytotypes *"collina"* ($2n$ = 28) and *"illyrica"* ($2n$ = 14). Boundaries between the subspecies and cytotypes were obscured due to cross pollination.

## Proteins

The molecular components of esterase and the soluble acid and basic proteins of ripe seeds were investigated for 20 populations of 20 native Bulgarian *Anthemis* species representing 2 subgenera and 5 sections (Kuzmanov & al. 1981b). The difference of Species of *A.* sect. *Anthemis* and *A.* subg. *cota* more advanced phylogenetically than are those of *A.* sect. *Hiorthia,* the highest degree of specialization being shown by sect. *Anthemis (A. arvensis, A. auriculata,* and *A. ruthenica).* Bulgarian endemics have evolved in both subgenera of *Anthemis,* and in different sections, both as diploids and tetraploids. By cluster analysis, the taxonomic status and affinities of the 20 *Anthemis* species were estimated on the basis of their soluble proteins (Kuzmanov & al. 1983a).

The water soluble protein and dehydrogenase isoenzyme contents were studied on extracts from fresh leaves of 5 *Veronica* taxa (Peev & Babalăkova 1978). Inter- and intraspecific comparisons were made, using PA (paired affinity index) and Rf. Results are consistent with the assumption that the proposed taxonomic scheme reflects natural relationships among the taxa studied.

## Sesquiterpene lactones and terpenoids

Sesquiterpene lactones are characteristic secondary metabolites of the *Asteraceae.* In *Anthemideae,* the lactones of *Tanacetum* species, in particular, were investigated (Todorova & Ognjanov 1985; Ognjanov & Todorova 1983; Ognjanov & al. 1991). Santamarin, tatridin-A, tatridin-B, 11,13-dehydrodesacetylmatricarin, and desacetylpyretrosin were found. These secondary metabolites show strong qualitative variation and a marked tendency to form discrete chemotypes. The investigated population was determined as a "thujon chemotype".

On the basis of essential oils, two chemotypes can be recognized in *Satureja pilosa,* with thymol and carvacrol (Genova 1984), as well as in *Mentha spicata,* with carvon and linalol, respectively (Stoeva 1989).

## Saponins

In Bulgarian species of the genus *Astragalus (Fabaceae)* saponins and sapogenines occur as soyasapogenol and sapogenin II, respectively (Nikolov & al. 1985). The first of these compounds is characteristic of Bulgarian *Astragalus.*

## Antraquinons

A chemosystematic study of 12 sections of *Hypericum (Guttiferae)* showed that hypericin and pseudohypericin may be present. Primitive sections *(H.* sect. *Ascyreia, Androsaemum,* sect. *Roscyna,* and sect. *Spachium)* lack hypericin, whereas more advanced sections contain this compound and its derivatives (Kitanov 1988).

## Conclusions

Over the past decade there has been an increase in number of publications of a chemosystematics nature, in Bulgaria. This overview bears witness of many joint efforts of systematists and biochemists. New developments in chemosystematics must depend on such collaborative interdisciplinary research.

## References


Angelov, G., Edreva, A. & Kožuharov, S. 1988: Biosistematično proučvane na vidove ot rod *Festuca* L. I. Hromatografski analiz na polifenolnite säedienenija. – Fitologija **35:** 3-12

Dučevska, H., Hristov, V. & Kuzmanov, B. 1986: Chemosystematics of Bulgarian *Genisteae* and their use in the pharmaceutical practice. – Acta Hort. **188:** 48-52.

– , – & – 1988: Chemosystematics on Bulgarian species of *Chamaecytisus (Fabaceae).* – Izv. Him. (Sofija) **21:** 267-272.

Evstatieva, L., Handjieva, N., Popov, S. & Pašinska, P. 1993: A biosystematic study of *Valeriana officinalis (Valerianaceae)* distributed in Bulgaria. – Pl. Syst. Evol. **185:** 167-179.

Filipov, S. & Dučevska, H. & Kuzmanov, B. 1991: Chemotaxonomy of genus *Glaucium* Mill. – Izv. Him. (Sofija) **24:** 291-296.

Genova, E. 1984. Proučvane na *Satureja montana* L.: *S. pilosa* Vel. v Bălgarija. – Thesis, Sofija.

Hristov, V. & Evstatieva, L. 1985: Alkaloid content of *Lupinus graecus* Boiss. *(Fabaceae).* – Dokl. Bulg. Akad. Nauk **39:** 55-56

– , Dučevska, H. & Kuzmanov, B. 1991: Alkaloides quinolizidiniques des espèces bulgares de la tribu *Genisteae.* – Pl. Méd. Phytothérap. **25(2/2):** 127-133

Ivančeva, S. & Behar, M. 1995: Flavonoids in *Tanacetum vulgare.* – Fitoterapia **66:** 373.

– & Kuzmanov, B. 1986: Flavonoids in genus *Achillea – A. nobilis* group. – Bull. Liais. Groupe Polyphénols **13:** 576-579.

– & – 1989: Flavonoidi v bălgarski vidove ot rod *Artemisia* L. – Fitologija **36:** 53-56.

– & – 1990: Epicuticular flavonoids in *Achillea* sect. *Filipendulinae;* a comparative study. – Farmacija (Sofija) **40(11):** 20-24.

– & Wollenweber, E. 1989: Leaf exudate flavonoids in *Geraniaceae.* – Indian Drugs **27:** 167-168.

– , – & Kuzmanov, B. 1987: Flavonoiden săstav na *Achillea coarctata* Poir. – Pp. 50-52 in: Kuzmanov, B. & al. (ed.), Trudove na četvărtata nacionalna konferencija po botanika, **2.** – Sofija.

Kitanov, G. 1988: Fitohimičeskoe isučenie i analizi vidov *Hypericum* L. proizrastajučtih v Bălgarii. – Rastitel'n. Resursy **24:** 114-123.

Kuzmanov, B., Andreev, N. & Kozovska, V. 1981a: Chemotaxonomic study on Bulgarian species of *Peucedanum* L. I. – Anales Jard. Bot. Madrid **37:** 779-788.

– & Dučevska, H. 1982: Evolutionary pattern and alkaloid biosynthesis in *Thalictrum.* – J. Nat. Prod. **45:** 766-771.

– , Evstatieva, L., Kirijakov, C. & Iskrenova, E. 1984a: Hemotaksonomično izsledvane na bălgarskite vidove ot rod *Corydalis* Vent. I. Alkaloiden săstav. – Fitologija **27:** 24-34.

– , –, Marekov, N. & Popov, S. 1984b: Chemosystematic study on genus *Plantago* L. – Fitologija **24:** 29-34.

– , –, Vitkova, A., Aladžemov, M. & Nejkov, N. 1984c: Hemotaksonomično izsledvane na bălgarskite vidove ot rod *Corydalis* Vent. 3. Klăstăr analiz. – Fitologija **25:** 3-12

– , Filipov, S. & Deligjozova-Gegova, I. 1992: Comparative phytochemical and chemosystematic research of populations of *Glaucium flavum* Crantz in Bulgaria. – Fitologija **43:** 52-57.

– , Vitkova, A., Nejkov, N. 1983a: Hemotaksonomično izsledvane na bălgarski vidove ot rod *Anthemis* L. 2. Klăstăr analiz. – Pp. 285-292 in: Velčev, V. & al. (ed.), Treta nacionalna konferencija po botanika. – Sofija.

– , – & – 1983b: Enzyme polymorphism in natural populations of the species of *Achillea millefolium* group in Bulgaria. – Bios (Thessaloniki) **[1]:** 121-127.

– , –, – & Aladžemov, M. 1983c: Hemotaksonomično izsledvane na bălgarskite vidove ot rod *Corydalis* Vent. 2. Elektroforeza na raztvorimi beltăci (kiseli i alkalni), malatdehidrogenaza i esteraza. – Pp. 293-300 in: Velčev, V. & al. (ed.), Treta nacionalna konferencija po botanika. – Sofija.

– , –, Thin, N., Aleksiev, A. & Nejkov, N. 1981b: Hemotaksonomično proučvane na bălgarskite vidove ot rod *Anthemis* L. Izsledvane na molekulno-heterogennija harakter na esterazata, raztvorimite kiseli i alkalni beltăci. – God. Sofijsk. Univ. Biol. Fak., 2 Bot. **74:** 46-62.

Nikolov, S. & Jonokova, I. & Panova, D. 1985: Soyasapogenol B from *Astragalus angustifolius* Lam. – Dokl. Bulg. Akad. Nauk **38:** 875-877.

Ognjanov, I. & Todorova, M. 1983: Sesquiterpene lactones in *Tanacetum*. – J. Med. Pl. Res. **48:** 181-183.

– , Min, F. & Todorova, M 1991: Chemotypes in some Bulgarian populations of *Chrysanthemum vulgare* (L.) Bernh. – Dokl. Bulg. Akad. Nauk **45:** 29-31.

Peev, D. 1982: Different substitution tendencies of leaf flavones in the *Veronica hederifolia* group *(Scrophulariaceae)*. – Pl. Syst. Evol. **140:** 235-242.

– & Babalăkova, N. 1978: Opit za proučvane na izoenzimnija săstav na njakoi dehidrogenazi i sravnenie na elektroforetičnite beltăčni spektri pri vidove ot rod *Veronica* L. – Fitologija **9:** 34-40.

Stefanov, J. 1990: Ekologo-biologični i fitohimični proučvanija vărhu populacii na *Leucojum aestivum* L. – Thesis, Sofija.

Stoeva, T. 1989: Izmenčivost v polusibni potomstva na *Mentha spicata* (L.) Hudson. – Thesis, Sofija.

Todorova, M. & Ognjanov, I. 1985: Sesquiterpen lactones in *Tanacetum macrophyllum*. – Pl. Med. **2:** 174-175.

Tomas-Berberan, Gil, M. & Ivančeva, S. 1992: External and vacuolar flavonoids from *Nepeta transcaucasica*. – Biochem. Syst. Ecol. **20:** 589-590.

Wollenweber, E., Valant-Vetschera, K., Ivančeva, S. & Kuzmanov, B. 1987: Flavonoid aglycones from the leaf surfaces of some *Achillea* species. – Phytochemistry **26:** 181-182.

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