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Iridoids, flavonoids and terpenoids as taxonomic markers in *Lamiaceae, Scrophulariaceae,* and *Rubiaceae*

Abstract

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In a preliminary chemotaxonomic study, by thin-layer chromatography, samples of *Lamiaceae* (39 species of 25 genera), *Scrophulariaceae* (5 species of *Linaria* and 4 of *Veronica*) and *Rubiaceae* (15 species of *Galium*) were screened for iridoids, flavonids and triterpenoids. The usefulness of these substances as taxonomic markers is discussed in the light of congruence between the new results and the accepted classification.

Introduction

Iridoids and flavonoids are widely used as taxonomic markers, while ordinary terpenoids are ubiquitous secondary metabolites that appear to have little value for classification purposes (Waterman & Gray 1987). This preliminary communication lists the results of our analyses, by thin-layer chromatography (TLC), of iridoids, flavonoids and triterpenoids of various samples of *Lamiaceae* (39 species of 25 genera), *Scrophulariaceae* (21 samples of 5 *Linaria* species and 12 samples of 4 *Veronica* species) and *Rubiaceae* (17 samples of 15 *Galium* species), and discusses their taxonomic significance.

Materials and methods

Above-ground parts of plants in full anthesis were sampled, of which voucher specimens were deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM). Compounds were extracted with methanol, individual components were isolated by chromatographic procedures (Nikolova-Damyanova & al. 1994) and identified by spectroscopic methods. The relative concentrations of the main components were established by TLC densitometric analysis (Nikolova-Damyanova & al. 1994). Surface compounds were abluted with chloroform from the above-ground parts and analysed in the same way.

Table 1. Characteristic compounds found in the *Lamiaceae* studied. The classification follows Wunderlich (1967). – Compounds (details in the text): 1-4, flavonoids; 5, diterpenoid; 6, iridoid; 7-8, triterpenoids; tr = traces. (SOM): voucher Nos.

Plant	provenance	(SOM)	1	2	3	4	5	6	7	8
Ajugoideae										
Ajuga chamaepytis Schreb.	Sofija region	LE9210	–	–	–	–	–	+	+[2]	++[2]
A. reptans L.	Sofija region	LE9211	–	–	–	–	–	+	++[2]	+++[2]
Teucrium chamaedrys L.	Vitoša	LE9213	–	–	–	–	–	+	tr	tr
Stachyoideae, Marrubieae										
Sideritis syriaca L.	Strandža Mt.	LE9247	++[1]	+++[1]	++[1]	+[1]	tr[2]	+	+[1]	–
–	(cult.)	LE9248	+	–	++[1]	++[1]	++[2]	+	+++[1]	tr[1]
–	Albania	LE9249	tr[1]	+++[1]	++[1]	+[1]	tr[2]	+	+[1]	tr[1]
–	Crimea	LE9250	tr[1]	+[1]	++[1]	+[1]	+[2]	+	+++[1]	–
–	Greece	LE9251	–	+++[1]	+[1]	–	+[2]	+	++[1]	–
S. scardica Griseb.	Pirin	LE9252	+[1]	+++[1]	+[1]	++[1]	+[2]	+	++[1]	tr[1]
S. lanata L.	Pirin	LE9253	tr[1]	++[1]	+++[1]	+[1]	+[2]	+	+[1]	tr[1]
S. montana L.	Sandanski	LE9254	+[1]	tr[1]	+++[1]	++[1]	+[2]	+	+[1]	–
– Stachydeae										
Stachys germanica L.	Vitoša	LE9220	–	++[2]	+[2]	tr[2]	–	+	–	tr[1]
Betonica officinalis L.	Vitoša	LE9221	–	–	–	–	tr[1]	+	–	tr[1]
Lamium maculatum L.	Sofija region	LE9222	–	–	–	–	–	+	–	tr[1]
– (roots)	Sofija region	LE9223	–	–	–	–	–	+	+[1]	++[1]
L. amplexicaule L.	Sofija region	LE9224	–	–	–	–	–	+	+[1]	–
Lamiastrum galeobdolon (L.) Ehrend. & Polatschek	Vitoša	LE9225	–	–	–	tr[1]	–	+	+++[1]	tr[1]
Leonurus cardiaca L.	Sofija region	LE9227	–	–	–	–	–	+	tr[1]	–
Phlomis tuberosa L.	Sliven	LE9216	+[1]	–	++[1]	–	–	+	–	tr[2]
Ballota nigra L.	Vitoša	LE9217	–	–	+[1]	+[1]	tr[1]	–	–	tr[1]
Galeopsis ladanum L.	Sofija region	LE9218	–	–	–	–	–	+	+++[1]	–
Saturejoideae, Nepeteae										
Nepeta cataria L.	Vitoša	LE9228	–	–	–	–	–	+	+[1]	++
N. mussinii Henckel	Cauc. (cult.)	LE9229	–	–	–	–	–	+	++[1]	++[2]
N. nuda L.	Pirdop	LE9230	–	–	–	–	–	+[2]	+++[1]	+++[2]
N. grandiflora M. Bieb.	Cauc. (cult.)	LE9231	–	–	–	–	–	+	+++[1]	+++[1]
N. transcaucasica Grossh.	Cauc. (cult.)	LE9232	–	–	–	–	–	+	++[1]	++
– Glechomeae										
Glechoma hederacea L.	Vitoša	LE9233	–	–	–	–	–	–	tr[1]	+++[2]
G. hirsuta Waldst. & Kit.	Vitoša	LE9234	–	–	–	–	–	–	tr[1]	+++
– Saturejeae										
Satureja hortensis L.	(cult.)	LE9235	–	–	–	–	–	–	–	+++
Acinos arvensis Dandy	Vitoša	LE9226	–	–	–	–	–	–	+[1]	+++[2]
Melissa officinalis L.	Vitoša	LE9236	–	–	–	–	–	–	tr[1]	+++
Hyssopus officinalis L.	(cult.)	LE9237	–	–	–	–	–	–	tr[1]	+++
Thymus sp.	Vitoša	LE9238	–	–	–	–	–	–	tr	+++
Origanum vulgare L.	Vitoša	LE9239	–	–	–	–	–	–	–	+++[2]
O. majorana L.	(cult.)	LE9240	–	–	–	–	–	–	+	+++
Mentha piperita L.	(cult.)	LE9241	–	–	–	–	–		+++[1]	++
– Rosmarineae										
Rosmarinus officinalis L.	(cult.)	LE9242	–	–	–	–	–	–	++	+++
– Lavanduleae										
Lavandula angustifolia Mill.	(cult.)	LE9243	–	–	–	–	–	–	++[2]	+++[2]
– Salvieae										
Salvia officinalis L.	Albania	LE9244	tr[1]	tr[1]	–	–	–	–	+++[2]	+++
–	Jordan		–	–	–	–	–	–	+[2]	+++
S. tomentosa Mill.	Kardzali	LE9245	–	–	–	–	–	–	–	+++[2]
S. aethiopis L.	Rodopi	LE9256	–	–	–	–	–	–	–	tr[1]
– Ocimeae										
Ocimum basilicum L.	(cult.)	LE9246	–	–	–	–	–	–	+[1]	++
Orthosiphon stamineus Benth.	(cult.)	LE9255	–	–	–	–	–	–	tr	++[1]

[1] Compound found for the first time in the genus; [2] id. in the species.

Results and discussion

Lamiaceae. – The chemical composition of taxa representing 25 genera of *Lamiaceae* – 32 samples of Bulgarian and 7 samples of foreign origin – was studied by TLC. The results obtained are summarized in Table 1.

Four flavonoid glycosides, identified earlier in some *Stachys* species (Lenherr & Mabry 1987), were now found in *Stachys, Phlomis, Ballota,* and *Sideritis,* all belonging to the *Stachyoideae* – but not in the *Ajugoideae* and *Saturejoideae:*

- isoscutellarein-7-O-(6'''-O-acetyl-β-D-allopyranosyl-(1-2)-β-D-glucopyranoside (1);
- isoscutellarein-7-O-[6'''-O-acetyl-β-D-allopyranosyl-(1-2)-6''-O-acetyl-β-D-glucopyranoside (2);
- hypolaetin-4'-methylether-7-O-(6'''-O-acetyl-β-D-allopyranosyl-(1-2)-β-D-glucopyranoside (3);
- hypolaetin-4'-methylether-7-O-(6'''-O-acetyl-B-D-allopyranosyl-(1-2)-6''-O-acetyl-B-D-glucopyranoside (4).

The three subfamilies studied also differ in the composition of the leaf exudates. Detailed investigations of the complex mixtures that we isolated allowed us to identify a series of compounds. The ratio of the triterpenoids amyrin (7) to ursolic and oleanolic acid (8), which depends on the activity of the enzymes responsible for the oxidation of amyrin to triterpenoid acids, varied very much. While in the *Ajugoideae* the concentration of amyrin and ursolic acid were similar, in the *Stachyoideae* amyrin prevailed, and in the *Saturejoideae* the triterpenoid acids were present in higher concentrations.

The diterpenoid siderol (5) was found almost exclusively in *Sideritis* species, traces of it having also been detected in *Ballota nigra* L. and *Betonica officinalis* L.

The iridoid nepetalactone (6) was found only in *Nepeta.* This genus shares high iridoid concentrations with the *Ajugoideae* and *Stachyoideae,* whereas in the *Saturejoideae* iridoid glycosides occur only in traces. Moreover, the ratio amyrin to triterpenoid acids found in *Nepeta* species is closer to that of the *Ajugoideae* and *Stachyoideae* than of the *Saturejoideae.* These data indicate low chemical affinity of *Nepeta* with the *Saturejoideae* in which they are currently placed.

These preliminary results support the classifications of the *Lamiaceae* proposed by Wunderlich (1967) and El-Gazzar & Watson (1970).

Scrophulariaceae: Linaria. – The iridoid and flavonoid composition of 21 samples from native Bulgarian populations belonging to 5 *Linaria* species (Stojanov & al. 1967) were analysed (Table 2): *L. genistifolia* (L.) Mill. (with f. *genistifolia,* f. *linifolia* (Boiss.) P. H. Davis, var. *euxina* (Velen.) Stef. & Jordanov, subsp. *sofiana* (Velen.) Chater & D. A. Webb, and subsp. *dalmatica* (L.) Maire & Petitm.), *L. peloponnesiaca* Boiss. & Heldr., *L. pelisseriana* (L.) Mill., *L. vulgaris* Mill., and *L. simplex* (Willd.) DC. Two known flavonoid glycosides – pectolinarin (9) and acetylpectolinarin (10) – as well as 9 iridoid glycosides – antirrinoside (11), 5-O-glucosylantirrinoside (12), antirride (13), linarioside (14), E- and Z-p-coumaroylantirrinoside (15), 6β-hydroxyantirride (16), genistifolin (17), 5-O-allosylantirrinoside (18), and 7,8-epi-antirrinoside (19) – were isolated and identified. It appeared that their presence and amount differ in the different species and can thus be used for taxonomic purposes. Six of them proved to be new compounds.

Table 2. Flavonoid and iridoid glycosides found in 5 *Linaria* species on Bulgarian material. – Compounds (details in the text): 9-10, flavonoids; 11-19, iridoids; tr = traces. (SOM): voucher Nos.

Linaria	provenance	(SOM)	9	10	11	12	13	14	15	16	17	18	19
genistifolia f. *genistifolia*	N.E. Bulgaria	151010	–	–	++++	+	–	tr	–	+	+	++	–
–	N.E. Bulgaria	151011	–	–	++++	+	–	tr	–	+	+	++	–
–	Black Sea coast	151012	–	–	++++	+	–	++	–	+	+	+++	–
f. *linifolia*	N.E. Bulgaria	151016	–	–	++++	+	–	tr	–	+	+	++	–
–	N.E. Bulgaria	151017	–	–	++++	+	–	tr	–	+	+	++	–
–	Stara planina	151013	–	–	++++	+	–	+	–	++	+	++	–
–	Black Sea coast	151014	–	–	++++	+	–	tr	–	+	+	++	–
–	Mt Osogovska	151015	–	–	++++	++	–	++	–	+	+	+	–
var. *euxina*	Black Sea coast	151018	–	–	++	+	–	–	–	++++	+	++	–
subsp. *sofiana*	Sofija region	151019	–	–	++++	+	–	–	–	+	+	++	–
subsp. *dalmatica*	Mt Vitoša	150736	++	+++	++++	+	–	+	–	+	–	tr	+
–	Sredna gora	151009	++	+++	++++	+	–	+	–	+	–	tr	+
peloponnesiaca	Znepole reg.	151020	++	+++	++	tr	–	–	–	+	–	+++	–
pelisseriana	Mt Kožuh	150733	–	–	++	+	–	tr	–	–	–	–	–
–	Mt Osogovska	151004	–	–	++	+	–	tr	–	–	–	–	–
vulgaris	Herba		++	+++	++	++	–	++	+	+	–	+	–
–	Stara planina	150735	++	+++	++	++	–	++	+	+	–	+	–
–	N.E. Bulgaria	151005	++	+++	++	++	–	++	+	+	–	+	–
–	Stara planina	151006	++	+++	++	++	–	++	+	+	–	+	–
–	Sofija region	151008	+	++	++	tr	–	–	+	++	–	+	–
simplex	Kožuh Mt	150732	+	++	+	+	+	tr	–	–	–	–	–

Linaria genistifolia, except subsp. *dalmatica,* differs from all other taxa studied in the absence of flavonoids and concomitant presence of a characteristic iridoid, genistifolin. This indicates an isolated position for *L. genistifolia* subsp. *dalmatica,* treated by many authors as a separate species (*L. dalmatica* (L.) Mill.). Conversely, *L. genistifolia* subsp. *sofiana,* treated as a separate species (*L. concolor* Griseb.) by Stojanov & al. (1967), according to our results does belong to *L. genistifolia,* in agreement with Chater & al. (1972).

The two studied varieties of *L. genistifolia* subsp. *genistifolia,* var. *genistifolia* and var. *euxina,* can be easily distinguished chemically, the latter lacking linarioside, having a higher amount of 6β-hydroxyantirride and a lower one of antirrinoside. The two formae recognized in var. *genistifolia,* f. *genistifolia* and f. *linifolia,* however, are not chemically distinct.

Instead of pectolinarin and acetylpectolinarin, characteristic for the genus, *Linaria pelisseriana* contained other, unidentified flavonoid glycosides.

Some iridoids appear to be characteristic for individual species. Thus, E- and Z-p-coumaroylantirrinosides were detected only in *Linaria vulgaris*, 7, 8-epi-antirrinoside in *L. dalmatica,* and genistifolin in *L. genistifolia.*

Scrophulariaceae: Veronica. – The iridoid composition of 12 samples of four *Veronica* species (*V. chamaedrys* L., *V. officinalis* L., *V. serpyllifolia* L., and *V. urticifolia* Jacq.) was investigated. The iridoids in the latter species were studied for the first time. In Table 3, the data on the presence of aucubin (20), catalpol (21), mussaenoside (22), ver-

Table 3. Iridoid compounds found in 4 *Veronica* species on Bulgarian material. – Compounds: 20, aucubin; 21, catalpol; 22, mussaenoside; 23, verproside; 24, verminoside; tr = traces. (SOM): voucher Nos.

Veronica	provenance	(SOM)	20	21	22	23	24
chamaedrys subsp. *chamaedrys*	Rila, Samokov (1000 m)	DP2	+	+			tr
–	Rila, Borovec (1100 m)	DP3	+	+			tr
–	Rila, Rido (1200 m)	DP4	+	+			tr
–	Rila, Zeleni grad (750 m)	DP6	+	+			tr
–	Rila, Banja (700 m)	DP7	+	+			tr
–	Rila, Malojovica (2400 m	DP13	+	+			tr
–	Rila, Borovec (1000 m)	DP14	+	+			tr
subsp. *orbelica*	Rodopi, Rožen (900 m)	DP15	+	+			tr
officinalis	Rila, Maljovica (1800 m)	DP9	tr	+	+	+	+
–	Rila, Jastrebec (2300 m)	DP10	+	+	++	+++	–
serpyllifolia subsp. *serpyllifolia*	Rila, Maljovica (2000 m)	DP11	tr	tr	–	tr	+
urticifolia	Rodopi, Beglica (1500 m)	DP12	++	+	++	–	–

Table 4. Iridoid glycosides found in 15 *Galium* species on Bulgarian material. – 25, asperuloside; 26, secogalioside; A: iridoid acids (desacetylasperulosidic acid, monotropein and scandoside); B, Me esters of these iridoid acids; tr = traces. (SOM): voucher Nos.

Galium	provenance	(SOM)	25	26	A	B
album subsp. *album*	Znepole region	MA-1	+	+	+	+
subsp. *pycnotrichum*	Znepole region	A9286	+	+++	+	+
lovcense	Mt Konjavska	A9214	+	+++	+	+
macedonicum	Struma valley	A9275	++	–	++	++
verum	Struma valley	A9249	++++	–	++	+
palustre	Dragomansko blato	A9239	+++	–	++	+
humifusum	Danube plain	A9283	+	–	+	–
odoratum	Mt Osogovska	A9218	++	–	+	+
–	Mt Vitoša	A9282	+	–	+	+
mirum	Besaparski ridove	A9234	+	–	+++	+
octonarium	Besaparski ridove	A9223	–	–	+	–
pseudoaristatum	Mt Osogovska	A9289	tr	–	++	tr
rivale	Struma valley	A9297	–	–	+	tr
schultesii	Mt Osogovska	A9290	+	–	+	tr
tricornutum	Besaparski ridove	A9227	++	–	tr	–
divaricatum	Mt Pirin	A9250	++	–	tr	–
rhodopeum	Besaparski ridove	A9232	tr	–	+	–

proside (23) and verminoside (24) are summarized. *V. officinalis* yielded the most complex iridoid pattern, with high concentrations of verminoside, verproside, and mussaenoside; *V. serpyllifolia* and *V. urticifolia,* the simplest. All samples of *V. chamaedrys* (both of subsp. *chamaedrys* and subsp. *orbelica* Peev) showed the same iridoid spectrum, independently of collecting season and habitat.

Rubiaceae: Galium. – The iridoid composition of 17 samples from native Bulgarian populations, belonging to 15 species, was investigated (Table 4). So far, detailed studies on the iridoids had been carried out only on *G. odoratum* (L.) Scop. (*Asperula odorata* L.), *G. album* Mill., *G. mollugo* L. and *G. verum* L. All samples studied by us contained neutral and acid iridoids, most of them biogenetically related to asperuloside (25).

Galium album (subsp. *album* and subsp. *pycnotrichum* (Heinr. Braun) Krendl) and *G. lovcense* Urum., of the *G. mollugo* group, all contain a compound found exclusively in this group, secogalioside (26). Further studies may well confirm this as a taxonomic marker for the entire group.

In 6 species (*Galium macedonicum* Krendl, *G. verum, G. humifusum* M. Bieb., *G. odoratum, G. schultesii* Vest, and *G. palustre* L.), asperuloside and non-acetylated iridoid acids (desacetylasperulosidic acid, monotropein and scandoside; A) were found in about equal amounts. In *G. mirum* Rech f., *G. rhodopeum* Velen., *G. rivale* (Sm.) Griseb., *G. octonarium* (Klokov) Soó, and *G. pseudoaristatum* Schur, the latter dominate over the former, while in *G. tricornutum* Dandy and *G. divaricatum* Lam., asperuloside prevails.

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