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A Comparative Study of Morphological Features and Flavonoid Composition of *Galium* L. Genus Species

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ABSTRACT

The aim of the present study was to establish the correlation between the morphological features of 28 Bedstraw species (Galium L. genus, Rubiaceae Juss. family) and the content of flavonoids (cosmosiin, isorhoifolin, cynaroside, luteolin-7-O-arabino-glucoside, diosmetin-7-O-glucopyranoside, palustroside, diosmetin-7-O-xyloglucoside, nicotiflorine, hyperoside). Established was the correlation between the following: a glabrous stem, medium stem leaves borne in whorls of 6 to 8, corolla of 3 mm in diameter with lobes egg-shaped, and the presence of apigenin, luteolin and diosmetin derivatives. Conversely, these features do not correlate with the presence of quercetin and kaempferol derivatives. Only kaempferol glycoside (nikotiflorin) correlates with the such morphological features as creeping rhizomes, peduncle and pedicel hirsute. Only apigenin derivatives correlate with lobes oblong and fruiting pedicel glabrous. The data obtained can be used for a targeted search of biologically active compounds in the species of Galium L. genus.

Keywords: morphological features, flavonoids, correlation, *Galium* L., *Rubiaceae* Juss.

INTRODUCTION

The species of genus *Galium* L. have been the subject of research worldwide mostly from phytochemical and pharmacological points of view [**1-3**], with less attention paid to the chemotaxonomic research of the genus [**4-6**]. Normally, chemotaxonomic research makes use of compounds of primary and secondary biosynthesis as chemotaxonomic markers. However, due to the specificity of secondary metabolites prevailing markers in use are flavonoids and terpenoids [**7, 8**]. This can be explained by the universal distribution of these compounds in vascular plants and their relevance to the given genus or species, which enables the use of these compounds as criteria of family relationships at high levels of classification. Complexes of phenolic compounds and terpenoids are typical of the levels of the family, genus and species, thus enabling revision of phylogenetic systematics of plant species. Usually, within any species, a set of flavonoid aglycones is constant, whereas glycosides show a wide variability, and the glycoside variability tends to correlate with the morphological features of plants, yet depending on numerous factors, such as: the range of species, the intraspecies polymorphism and ecological differentiation, which supports the thesis about an adaptive role of flavonoids in plant physiology.

Our earlier research showed the presence of primary (sugars, amino acids, carboxylic acids) and secondary (hydroxycinnamic acids, coumarins, flavonoids, terpenoids) metabolites in *Galium* species. With the view to identifying prospect Bedstraw species, a morphological and chemotaxomonic study of the species was carried out and, on the basis of the results obtained and numeric taxonomy; a number of phytosubstances with marked biological activities were developed [**9, 10**].

As the knowledge of the relationship between morphological features of plants and their chemical composition significantly increases the likelihood of finding certain biologically active compounds (BAC) in naturally-occurring

species, we saw the aim of this work in establishing correlations between morphological features and chemical markers of *Galium* L. genus species.

MATERIALS AND METHODS

The object of the present research was 28 species of *Galium* L. genus, namely *G. aparine* L., *G. anisophyllum* Vill., *G. articulatum* Lam., *G. atropatanum* Grossh., *G. boreale* L., *G. brachyphyllum* Roem. et Schult, *G. calcareum* (Alb.) Pobed., *G. czerepanovii* Pobed., *G. erectum* Huds., *G. juzepczukii* Pobed., *G. hercynicum* Weig, *G. karakulense* Pobed., *G. krymense* Pobed., *G. mollugo* L., *G. palustre* L., *G. pamiro-alaicum* Pobed., *G. polonicum* Blocki, *G. ruthenicum* Willd., *G. rubioides* L., *G. ruprechtii* Pobed., *G. schultesii* Vest., *G. spurium* L., *G. tenuissimum* M.B., *G. turkestanicum* Pobed., *G. verum* L., *G. verticillatum* Danth., *G. uliginosum* L. and *G. ussuriense* Pobed.

Morphological features of the species under study were identified by matching with the herbarium specimens stored in the Herbarium of Pharmacognosy Department of National University of Pharmacy and in the Herbarium of V.N. Karazin Kharkiv National University. All in all, 348 markers were identified, of which 194 being of vegetative organs and 154 of generative ones, all used as taxonomic characteristics in further calculations.

Detection and identification of pharmacologically-valuable flavonoids were performed in ethanolic (70 %) extracts of the given species by bi-dimensional paper chromatography and thin layer chromatography. The mobility of flavonoids was determined on paper «Filtrac» (FN-4) or silica-gel 254 TLC plates in chromatographic systems, i.e. ethyl acetate-formic acid-water, 10 : 2 : 3 (first direction) and 15 % acetic acid (second direction) or *n*-butanol-acetic acid-water, 4 : I : 2 (first direction) and 15 % acetic acid (second direction). Upon drying, all plates were visualized directly with the help of UV at 254 nm and 366 nm in UV TLC viewer before and after spraying with a 3 % ethanolic solution of potassium hydroxide.

RESULTS AND DISSCUSION

Rutin was identified in all studied species and, therefore, can be assumed as a common compound. Flavonoids occurring in a number of species were as follows: *cosmosiin, isorhoifolin, cynaroside, luteolin-7-O-arabino-glucoside, diosmetin-7-O-glucopyranoside, palustroside (diosmetin-7-O-β-D-glucopyranosyl(6→1)-α-L-arabinoside), diosmetin-7-O-xyloglucoside, nicotiflorine (kaempferol-3-O-rutinoside), hyperoside* (Figure 1).

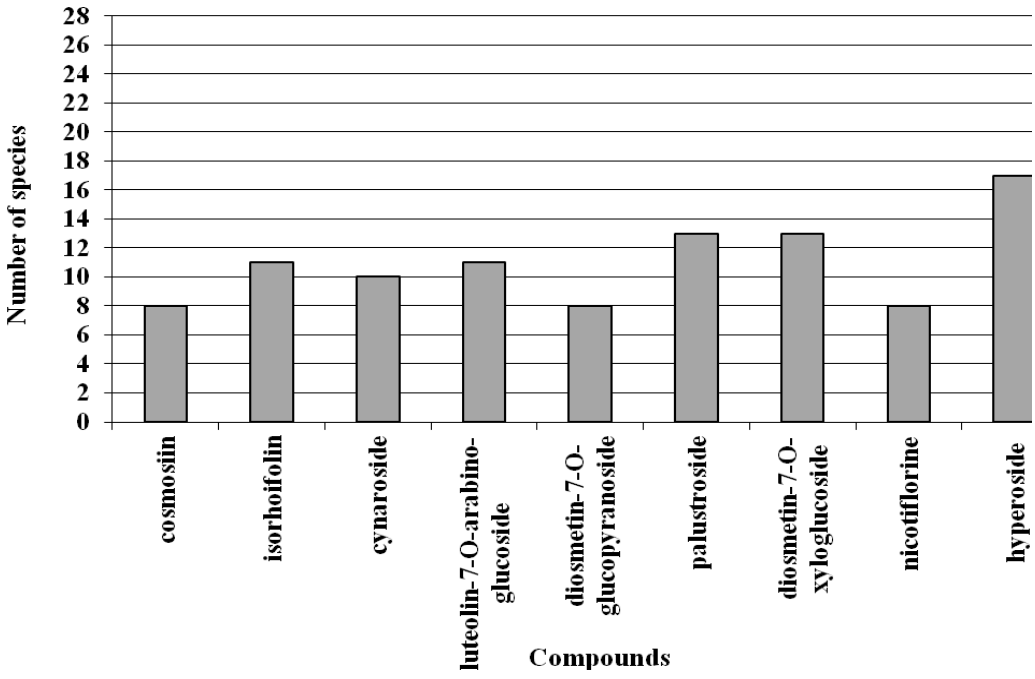

Figure 1: Identified Flavonoids

Cosmosiin was identified in 8 species, namely *G. palustre, G. karakulense, G. verum, G. ruthenicum, G. calcareum,* G. *juzepczukii*. *G. erectum, G. mollugo*.

Within these 8 species it correlates with the following features: at the level of 62.5 % – roots reddish or brown, stem 25 cm long, square; medium stem leaves in whorls of 6-8, oblong, with revolute margins; cymes loose, peduncle twice trifid, glabrous, pedicel short or as long as flowers, fruiting pedicel yellow; at the level of 75 % – stem 30-35 cm or 70 cm long, branched, glabrous; leaves linear, pedicel glabrous, corolla 3 mm in diameter, lobes egg-shaped; at the level of 87.5 % – stem 40 - 60 cm long, leaves rough edged, upper bracts few, and fruit glabrous.

Isorhoifolin was identified in 11 species, namely *G. palustre*, *G. karakulense*, *G. verum*, *G. pamiro-alaicum*, *G. ruthenicum*, *G. calcareum*, G. *juzepczukii*, *G. erectum*, *G. mollugo*, *G. schultesii*, *G. polonicum*.

It correlates with the following morphological features: at the level of 54,5 % – roots reddish or brown; leaves oblong, lanceolate, with revolute margins, glabrous; pedicel glabrous, equal or longer than flowers, corolla white, 3.5 mm in diameter, lobes oblong, style bifid on the upper third; at the level of 64 % – stem 30 - 35 cm long, medium stem leaves in whorls of 6-7, short-pointed, hirsute; corolla 3 mm in diameter; at the level of 73 % – stem square, branched, medium stem leaves in whorls of 8, cymes loose, peduncle glabrous, fruiting pedicel glabrous; at the level of 82 % – stem 40 - 55 cm or 70 cm long, glabrous; leaves linear, rough edged, pedicel glabrous, lobes egg-shaped; at the level of 91 % – stem 60 cm long, and fruit glabrous.

Cynaroside was identified in 10 species, namely *G. hercynicum*, *G. verum*, *G. ruthenicum*, *G. mollugo*, *G. erectum*, *G. juzepczukii*, *G. calcareum*, *G. schultesii*, *G. polonicum*, *G. czerepanovii*.

It correlates with the following morphological features: at the level of 60 % – stem 35 - 55 cm or 70 cm long, medium stem leaves in whorls of 7, hirsute, with revolute margins, leaves 1.5 mm wide; pedicel longer than flowers, corolla white, 3 mm in diameter, fruiting pedicel yellow; at the level of 70 % – stem 25 cm or 60 cm long, medium stem leaves in whorls of 8, linear, short-pointed, glabrous; cymes loose, lobes egg-shaped; at the level of 80 % – stem 30 cm long, square, medium stem leaves in whorls of 6, upper bracts few, lower bracts paired; at the level of 90 % – stem glabrous, leaves rough edged, peduncle and pedicel glabrous, and fruit glabrous.

Luteolin-7-*O*-arabino-glucoside was identified in 11 species, namely *G. verticillatum*, *G. verum*, *G. ruthenicum*, *G. atropatanum*, *G. mollugo*, *G. erectum*, *G. juzepczukii*, *G. calcareum*, *G. schultesii*, *G. polonicum*, *G. czerepanovii*.

It correlates with the following features: at the level of 54.5 % – stem 55 cm or 70 cm long, leaves hirsute, pedicel short, corolla yellow, fruiting pedicel yellow; at the level of 64 % – stem 35 - 50 cm or 60 cm long, straight, branched, medium stem leaves in whorls of 7, lanceolate, glabrous; corolla 3 mm in diameter; at the level of 73 % – stem 30 cm long, square, glabrous, medium stem leaves in whorls of 6 or 8, short-pointed, upper bracts few, lobes egg-shaped; at the level of 82 % – leaves linear, rough edged, bracts paired, peduncle and pedicel glabrous, fruit glabrous; at the level of 91 % – cymes terminal.

Diosmetin-7-*O*-glucopyranoside was identified in 8 species, namely *G. verum*, *G. pamiro-alaicum*, *G. ruthenicum*, *G. mollugo*, *G. erectum*, *G. juzepczukii*, *G. calcareum*, *G. czerepanovii*.

It correlates with the following morphological features: at the level of 62.5 % – roots reddish or brown, stem 35 cm or 70 cm long, straight; narrow loose elongated cymes, peduncle twice trifid, corolla yellow, 3 mm in diameter, lobes egg-shaped, fruiting pedicel yellow, one of two mericarps is obsolescent; at the level of 75 % – stem 25-30 cm or 40-60 cm long, square, medium stem leaves in whorls of 7-8, linear, short-pointed, with revolute margins, glabrous, rough edged; at the level of 87.5 % – stem glabrous, medium stem leaves in whorls of 6, peduncle and pedicel glabrous, and fruit glabrous.

Palustroside (diosmetin-7-*O*-*β*-D-glucopyranosyl(6→1)-*α*-L-arabinoside) and diosmetin-7-*O*-xyloglucoside were identified in 13 species, namely *G. palustre*, *G. karakulense*, *G. ruprechtii*, *G. verum*, *G. pamiro-alaicum*, *G. ruthenicum*, *G. atropatanum*, *G. mollugo*, *G. erectum*, *G. juzepczukii*, *G. calcareum*, *G. polonicum*, *G. czerepanovii*. These compounds correlate with the following morphological features: at the level of 54 % – roots reddish or brown, stem 25 cm, 35 cm long, medium stem leaves in whorls of 7, oblong, short-pointed, with revolute margins, 1.5 mm wide, hirsute; pedicel longer than flowers, corolla white, 3 mm in diameter, style bifid on the upper third; at the level of 61.5 % – stem 55 cm or 70 cm long, branched, medium stem leaves in whorls of 6 or 8, peduncle glabrous, fruiting pedicel yellow; at the level of 69 % – stem 30 cm or 40-50 cm, or 60 cm long, square, glabrous, leaves linear, rough edged, cymes loose, pedicel glabrous, lobes egg-shaped; at the level of 84.5 % – fruit glabrous.

Nicotiflorine (kaempferol-3-*O*-rutinoside) was identified in 8 species, namely *G. anisophyllum*, *G. uliginosum*, *G. articulatum*, *G. rubioides*, *G. turkestanicum*, *G. verum*, *G. ruthenicum*, *G. brachyphyllum*.

Nicotiflorine correlates with the following morphological features: at the level of 62 % – roots reddish or brown, stem 35 cm, 60-70 cm long, straight, leaves lanceolate, hirsute on the lower surface along the vein and on the edges; corolla 3.5 mm in diameter. fruiting pedicel yellow; at the level of 86 % – creeping rhizome, stem 30 cm or 40-55 cm long, leaves linear, 1 vein, peduncle and pedicel hirsute, and fruit glabrous.

Hyperoside was identified in 17 species, namely *G. aparine*, *G. spurium*, *G. tenuissimum*, *G. verticillatum*, *G. krymense*, *G. uliginosum*, *G. articulatum*, *G. rubioides*, *G. ussuriense*, *G. boreale*, *G. turkestanicum*, *G. verum*, *G. pamiro-alaicum*, *G. ruthenicum*, *G. mollugo*, *G. czerepanovii*, *G. brachyphyllum*.

Hyperoside correlates with the following morphological features: at the level of 53 % – stem 70 cm long, square, leaves glabrous on the upper surface, peduncle glabrous, fruiting pedicel yellow; at the level of 59 % – stem 35 cm long, leaves lanceolate, rough edged, pedicel glabrous, corolla white; at the level of 65 % – stem 60 cm long, straight, 1 vein, hirsute on the edges; at the level of 70.5 % – stem 30 cm, 40-45 cm, 55 cm long, fruit glabrous; at the level of 76.5 % – leaves linear, cymes terminal.

A comparison of results revealed that a number of morphological features correlate with the presence of the majority of the chemical compounds identified in the species understudy at approximately the same level. These features include: stem 30 - 40 or 60 cm - 70 cm long, square, leaves linear, rough-edged, peduncle and pedicel glabrous, fruiting pedicel yellow, and fruit glabrous (Table 1).

Table 1: Common Morphological Features that Correlate with Flavonoids of the Species of *Galium*

Morphological features	Correlation Coefficient, %							
	Cosmosiin	Isorhoifolin	Cynaroside	Luteolin-7-*O*-arabino-glucoside	Diosmetin-7-*O*-glucopyranoside	Palustroside, diosmetin-7-*O*-xyloglucoside	Nicotiflorine	Hyperoside
Stem 30 cm	75	64	80	73	75	69	86	70,5
Stem 35 cm	75	64	60	64	62,5	54	62	59
Stem 40 cm	87,5	82	60	64	75	69	86	70,5
Stem 60 cm	87,5	91	70	64	75	69	62	65
Stem 70 cm	75	82	60	54,5	62,5	61,5	62	53
Stem square	62,5	73	80	73	75	69	-	53
Leaves linear	75	82	70	82	75	69	86	76,5
Leaves rough edged	87,5	82	90	82	75	69	62	59
Peduncle pedicel glabrous	62,5	73	90	82	87,5	61,5	-	53
Pedicel glabrous	75	82	90	82	87,5	69	-	59
Fruiting pedicel yellow	62,5	-	60	54,5	62,5	61,5	62	53
Fruit glabrous	87,5	91	90	82	87,5	84,5	86	70,5

There is a correlation between the following features: stem glabrous, medium stem leaves in whorls of 6-8, corolla 3 mm in diameter, lobes egg-shaped, and the presence of apigenin, luteolin and diosmetin derivatives. Conversely, these features do not correlate with the presence of quercetin and kaempferol derivatives (Table 2).

Table 2: Correlation between Morphological Features and Glycosides of Apigenin, Luteolin and Diosmetyn of the Species of Galium

Morphological features	Correlation Coefficient, %							
	Cosmosiin	Isorhoifolin	Cynaroside	Luteolin-7-*O*-arabino-glucoside	Diosmetin-7-*O*-glucopyranoside	Palustroside, diosmetin-7-*O*-xyloglucoside	Nicotiflorine	Hyperoside
Stem glabrous	75	82	90	73	87,5	69	-	-
Medium stem leaves in whorls of 6	62,5	64	80	73	87,5	61,5	-	-
Medium stem leaves in whorls of 7	62,5	64	60	64	75	54	-	-
Medium stem leaves in whorls of 8	62,5	73	70	73	75	61,5	-	-
Corolla 3 mm in diameter	75	64	60	64	62,5	54	-	-
Lobes egg-shaped	75	82	70	73	62,5	69	-	-

Only kaempferol glycoside (nikotiflorin) correlates with the following morphological features: creeping rhizomes, peduncle and pedicel hirsute. Only apigenin derivatives correlate with lobes oblong and fruiting pedicel glabrous (Table 3).

Table 3: Correlation between Specific Morphological Features and Specific Flavonoids of the Species of *Galium*

Morphological features	Correlation Coefficient, %	
	Isorhoifolin	Nicotiflorine
Lobes oblong, fruiting pedicel glabrous	54,5/73	-
Creeping rhizomes, peduncle and pedicel hirsute	-	86

CONCLUSION

Within the scope of this research in 28 species of *Galium* L., 348 morphological features were analyzed and established the most common features correlating with the presence of the following flavonoids, i.e., *cosmosiin, isorhoifolin, cynaroside, luteolin-7-O-arabino-glucoside, diosmetin-7-O-glucopyranoside, palustroside, diosmetin-7-O-xyloglucoside, nicotiflorine*, and *hyperoside*.

The data obtained can be used for a targeted search of BAC in the species of *Galium* L. genus.

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