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DETECTION OF PHENOLOIDS IN SOME HUNGARIAN *INULA* AND *CENTAUREA* SPECIES

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Phenoloid contents were detected, examined and compared with each other from seven composite species (*Inula ensifolia* L., *I. salicina* L., *I. spiraeifolia* L., *I. britannica* L., *I. conyza* DC., *Centaurea scabiosa* L., *C. micranthos* S. G. Gmel.). The six test solutions were the followings: apigenin, quercetin, hyperosid, chlorogenic acid, caffeic acid and rutin. There were significant differences between ray and disc florets from both quantitative and qualitative results, and the species with more significant insect visiting (*Inula ensifolia*, *I. salicina, I. spiraeifolia*) have got quantitative dominance.

Key words: Asteraceae, *Centaurea*, disc floret, *Inula*, nectar, phenoloid content, ray floret

INTRODUCTION

Flavonoids are widely occurring compounds which are the most varied structure and the most frequently occurring types (Szabó 1996). According to several explanations (Hegnauer 1964, Borhidi 1995) for flavonoids to make much use of them as chemotaxonomic markers in Asteraceae.

Too little is known up to now regarding the distribution of flavonoids in Inuleae tribe, but flavonoid structure has been found as a specific subtribal character yet (Harborne 1977, Emerenciano *et al.* 1987). With regard to flavonoid type, perhaps the most characteristic feature distinguishing members of the Inuleae from those of other composite tribes is the presence of flavonols lacking hydroxylation on ring B. The two common flavones, apigenin and luteolin, were found in several species in the tribe (Harborne 1977, Emerenciano *et al*. 1987).

The predominant types occurring in the Cynareae tribe are flavones and flavonols. Flavonons and flavanonols are restricted to the genera *Carthamus*, *Centaurea* and *Silybum* only. Normally they occur 3-O-glycosides in case of flavonols and 7-O-glycosides in case of flavanones. Flavone C-glycosides seem to occur sporadically. Sugar component of these glycosides is usually glucose, but glycuronic or galacturonic acid derivatives seem to be restricted to the *Centaurea* species. The most prominent aglycones are scutellarein, centaureidin, jaceidin, jaceosidin and hispidulin.

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The subtribes Carduinae and Centaureinae are characterised by the occurrence of cinnamic acids and their derivatives and chlorogenic acid and its isomers (Wagner 1977).

Nectar is a more or less concentrated, sweet liquid of different chemical composition (Nyárády 1958). The major components are water and sugars. Among the minor components organic and inorganic acids, starch, mineral salts, metallic elements, flavours and pigments occur (Örösi 1951, Nyárády 1958).

Our aim was to study the phytochemical pattern of phenoloids in some Hungarian *Inula* and *Centaurea* species. Among floral attractants phenoloids are predominant to flower colour and occur eventually in the nectar. Our examinations focused on:

- selection of a suitable, optimal development mixture;
- qualitative and quantitative analysis of phenoloids of ray and disc florets from extracts of the examined species;
- detection of phenoloids from their nectar.

MATERIAL AND METHODS

Study areas

The first sample area is located in South Hungary, in the southern part of the Mecsek Mts, on Tettye Hill. On its southern slope karstic shrub forest (*Inulo spiraeifoliae-Quercetum virgilianae*) is typical, with species of forest steppes and steppes, such as *Inula spiraeifolia*, *Orchis simia*. Characteristically southern species *Ligustrum vulgare*, *Berberis vulgaris*, *Lonicera caprifolium*, *Tamus communis*, *Ruscus aculeatus* and *Helleborus odorus* have been found as sub-Mediterranean elements. *Cleistogeni-Festucetum rupicolae* association has been found on the southeastern slope. The species *Festuca rupicola* and *Festuca valesiaca* are of high constancy. Rare species are *Pulsatilla grandis*, *Plantago argentea*, *Ophrys cornuta* (Borhidi 1996). We could find four *Inula* species (*I. ensifolia*, *I. salicina*, *I. spiraeifolia*, *I. conyza*).

The second study area is in the Aggtelek Karst (NE Hungary), northwest of Jósvafő, in the eastern side of the valley Tohonya-völgy. The vegetation of the area can be characterised by the associations *Polygalo-Brachypodietum* and *Caricetum humilis* in the Cirsio-Brachypodion association group. Dominant species in this area are for example: *Dorycnium germanicum*, *Coronilla varia*, *Teucrium chamaedrys*, *Teucrium montanum*, *Salvia verticillata*, *Stachys recta*, *Centaurea scabiosa*, *Inula salicina*, *I. ensifolia*, *Carex humulis*, etc. *Brachypodium pinnatum*, *Carex montana* and dicotyledons are in a patch pattern and covering as

much as 50% in the area. There are several important species, such as *Dracocephalum austriacum*, *Adonis vernalis*, *Centaurea triumfetti*, *Polygala major*, *Cirsium pannonicum*, *Cytisus procumbens* and so on. The original climax vegetation here must have been *Querco-Carpinetum* and *Corno-Quercetum* and larger or smaller patches of these plant association can be still found in many places in similar situations.

Sampling method

The sampling of inflorescences and nectar was made in July and August 1998 (Tettye: *I. ensifolia*, *I. salicina*, *I. spiraeifolia*, *I. conyza*; Jósvafő: *I. britannica*, *Centaurea scabiosa*, *C. micranthos*). The inflorescences were dried on airy shadow place after collection from the study areas. Then the inflorescences were taken apart for ray and disc florets.

Nectar samples were taken from isolated inflorescence, with Whatman no. 1 paper wicks (McKenna and Thomson 1988).

Phenoloid analysis

Six compounds were identified from sample solution by test solutions: apigenin (1 mg/ml), quercetin (1 mg/ml), caffeic acid (1 mg/ml), hyperosid (1 mg/ml), chlorogenic acid (1 mg/ml) and rutin (1 mg/ml).

The procedure of phenoloid analyses followed Botz *et al*. (1995).

The sample solution: 2.0 g powdered drug were boiled into 10–15 ml methanol for 15 s at 65 °C. The test solutions and sample solutions were dropped to Silica gel 60 F_{254} (20×10) aluminium sheets (Merck) by Minicaps. Development was carried out once, in the saturated chamber (CAMAG).

Different development mixtures were used:

1. ethyl acetate:formic acid:acetic acid:water (100:11:11:27);
2. ethyl acetate:formic acid:acetic acid:ethyl methyl keton:water (50:7:3:30:10);
3. chloroform:aceton:formic acid (75:16.6:8.5);
4. chloroform:ethyl acetate (60:40);
5. n-butanol:acetic acid:water (upper phase) (40:10:50);
6. chloroform.

The plates were dipped twice into a solution consisting of A and B solution (= Naturstoff reagent) (A solution: 1 g diphenyl-boric acid- -ethyl-amino-esther into 100 ml methanol; B solution: 5 g poliethylen-glycol 4000 into 100 ml ethanol). Afterwards, they were heated in the thermostat at 105 °C for 5 s. We have chosen the most suitable plate which was developmented by development mixture 1.

Nectar analysis

The obtained nectar was unfastened from Whatman paper. The nectar analysis was carried out by thin layer chromatography (TLC), where the stationary phase was Silica gel 60 F_{524} TLC foils (Merck), with the sample application we used 1 µl Minicaps (Grösz and Braunsteiner 1989). The unfastened nectar was dripped up in different concentration to the Silica gel. Further methodical steps identical with phenoloid analysis.

RESULTS

Qualitative analysis

The samples do not contain rutin in detectable quantity which is not characterised in Asteraceae. Caffeic acid and chlorogenic acid, which are general phenoloic constituents, occur in each examined species. Presence of apigenin, quercetin and hyperosid has varied depending on species and floret type. Quercetin has been detected only from *Inula conyza* which might occur with some frequency in Inuleae tribe. Apigenin and hyperosid were not found in ray florets of *I. spiraeifolia* and inflorescences of *I. conyza*, although it occurs in several species in the tribe (Table 1).

Table 1
Pattern of flavonoids in the examined species

	Apigenin	Quercetin	Caffeic acid	Hyperosid	Chlorogenic acid	Rutin
Inula ensifolia (r)	+	−	+	+	+	−
I. ensifolia (d)	+	−	+	+	+	−
I. salicina (r)	+	−	+	+	+	−
I. salicina (d)	+	−	+	+	+	−
I. spiraeifolia (r)	−	−	+	−	+	−
I. spiraeifolia (d)	+	−	+	+	+	−
I. britannica (r)	+	−	+	+	+	−
I. britannica (d)	+	−	+	+	+	−
I. conyza	−	+	+	−	+	−
Centaurea micranthos	+	−	+	+	+	−
C. scabiosa	+	−	+	+	+	−

(r = ray floret, d = disc floret)

Quantitative analysis

Quantitative analysis was carried out in case of three phenoloids, chlorogenic acid, hyperosid and caffeic acid (Figs 1–2). Considering the *Inula* species, the three measured phenoloid components usually had higher values in ray florets, except for *I. salicina*, *I. spiraeifolia* and *I. conyza*. Ray florets of *I. salicina* contained more caffeic acid and chlorogenic acid. Inflorescences of *I. spiraeifolia* and *I. conyza* did not contain hyperosid in the samples (Fig. 1).

The maximum value of chlorogenic acid is found in *I. salicina*, and caffeic acid had maximum value in *I. ensifolia*. In every examined *Inula* species hyperosid was the lowest compared to the other compounds (Fig. 1).

There is difference between Cynareae and Inuleae tribes. Caffeic acid and chlorogenic acid are in low quantity to *Centaurea* species which are, according to the literature, relatively rare in Cynareae tribe. Hyperosid as opposed to caffeic acid and chlorogenic acid has got the highest value to two *Centaurea* species (especially *C. micranthos*) (Fig. 1).

Considering the amount of measured phenoloid components *I. ensifolia* and *I. salicina* have got the most highest values (Fig. 2). They are followed by *I. spiraeifolia* and *I. britannica* with more difference and less values. *I. conyza* and the two *Centaurea* species supplied insignificant data in accordance with qualitative composition (Figs 1–2). Difference of *C. micranthos* and *C. scabiosa* from *Inula* species is insignificant according to the examined phenoloids. By con-

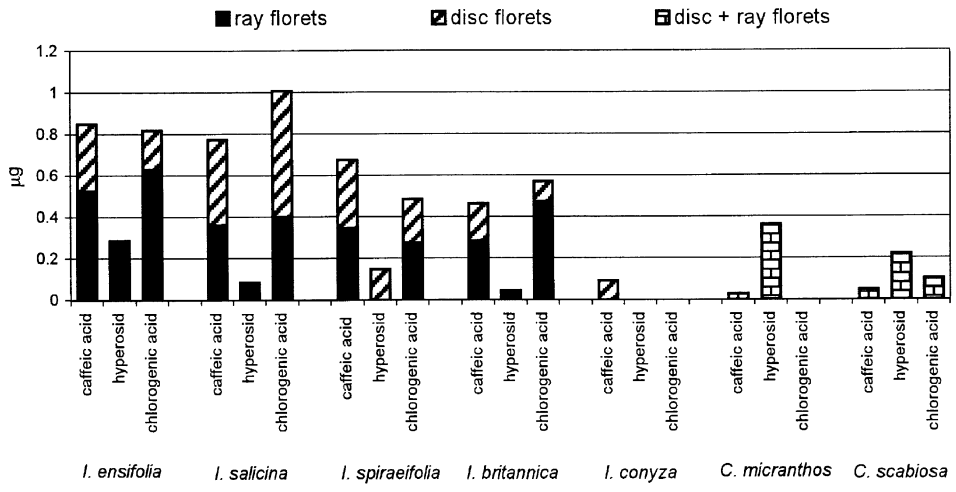

Fig. 1. Quantities of three measured phenoloid components to examined species in their inflorescences

trast, *Centaurea* species are richer in phenoloids than *Inula* species, but their other phenoloids were not analysed.

Nectar analysis

Based on the preliminary nectar analysis, samples of the biggest sugar content were chosen for analysis. The same samples were used for the measuring of phenoloid content of the nectar. After unfasten of 24 hourly we dripped up the sugary-mix in different (1 µl, 20 µl, 50 µl) concentrations to the Silica gel. We have not found any patch on the Silica gel after nectar analysis, so nectars of examined species did not contain phenoloids.

DISCUSSION

Among floral attractants, phytochemical pattern of phenoloids (apigenin, quercetin, hyperosid, chlorogenic acid, coffeic acid, rutin) of seven flowering plant species (5 *Inula* and 2 *Centaurea* species) were studied. These compounds are predominant to flower colour and usually occur in the nectar. Differences in the phenoloid composition between the species were revealed in case of apigenin, quercetin and hyperosid in the qualitative analysis.

By contrast, we have examined results to caffeic acid by quantitative analysis. Differences of phenoloid contents between ray and disc florets emphasised by several authors (Hörhammer *et al.* 1963, Pekic *et al.* 1999) were

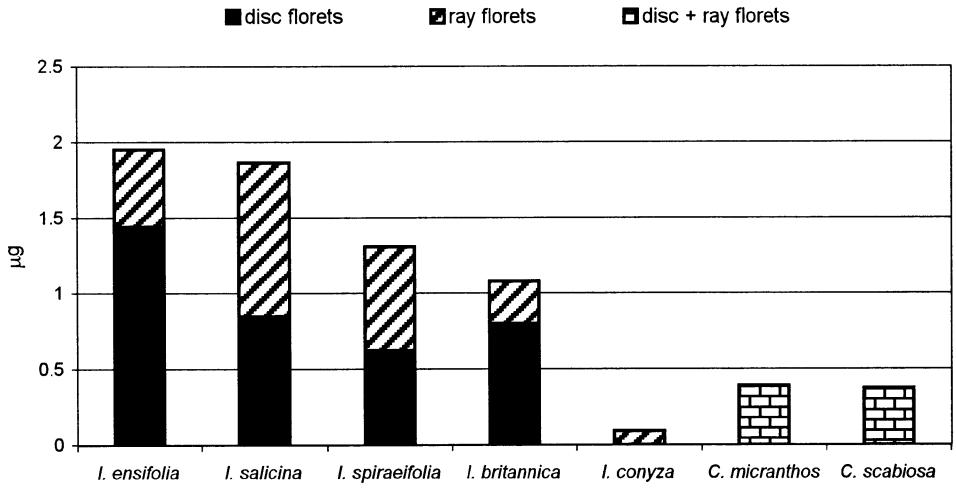

Fig. 2. Difference between ray florets in considering of three measured phenoloid components

also detected in our study. *I. ensifolia*, *I. salicina* and *I. spiraeifolia*, living in steppes and forest-steppes with significant insect visiting, contained the most phenoloids examined. *I. conyza* and *I. britannica*, having insignificant pollination by insects, contained less phenoloids.

No phenoloids could be detected in the nectar of the examined species.

These results on the pattern of flavonoids can be used in the family as taxonomic markers.

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