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## The determination of the content of the polyphenols in the areal parts of the species *Centaurea rupestris* L. and *C. fritschii* Hayek (Asteraceae)

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The content of flavonoids, total polyphenols and tannins in the areal parts of the endemic plant species *Centaurea rupestris* L. and *C. fritschii* Hayek are determined. These compounds were investigated during the three vegetation seasons. Flavonoids are present in the most quantity in the leaves of *C. fritschii*, where they make on an average 0.90 – 1.35% and in flowers of *C. rupestris* which contains on an average 0.73 – 0.95% flavonoids. In the other investigated samples this content is somewhat lower. The content of the total polyphenols is the highest in the leaves of investigated plants. They make on an average 5.90 – 7.80% of *C. rupestris* and 7.60 – 11.20% of *C. fritschii* leaves. The richest in tannins are the leaves of *C. fritschii*, where they make on an average 1.60 – 3.80%. In *C. rupestris* tannins are present in the greatest amount in the stems, where they do on an average 0.80 – 2.30%. It is visible from quoted results that the areal parts of *C. fritschii* are markedly richer in the polyphenols in relation to *C. rupestris*.

**Key words:** *Centaurea rupestris* L. (Asteraceae); *Centaurea fritschii* Hayek (Asteraceae); polyphenols

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The chemistry of the members of the genus *Centaurea* attracts attention of researchers in connection with introduction of different compounds in plants and their possible application in therapy (1 – 5), as well as because of taxonomic character of some separated compounds (6). However, endemic species of the genus *Centaurea* in the Balkan region are with regard to chemical aspects almost completely uninvestigated (7). Therefore, for chemical investigations the Illyrian-Adriatic endemic plant *Centaurea rupestris* L. and the Balkan endemic species *C. fritschii* Hayek were chosen.

*C. rupestris* is described (8, 9) as perennial herb of 15 – 70 cm height. The stem is erect, slender, fluted, unramified or poorly ramified from the middle, covered with cobwebby hairs. Branches terminate with single capitula. Florets are golden- to lemon-

\* Correspondence

-yellow. According to the same literature data *C. fritschii* is perennial herb up to 2 m high. The stem is erect, fluted, from the middle ramified and branches terminate with single capitula of purple-red colour.

The chemistry of the species *C. rupestris* and *C. fritschii* was completely uninvestigated. This work encircles the determination of content of the flavonoids, total polyphenols and tannins in the leaf, flower, stem and shoot in whole of the quoted species during the three vegetation seasons.

## MATERIALS AND METHODS

### Materials

*C. rupestris* was collected in the first half of July during the three years (1988 – 1990) in the adriatic coastal region Uvala Scott by Kraljevica. *C. fritschii* was collected at the beginning of July in the same three-year interval, in the mountains region Samoborsko gorje. Both investigated species were identified by Pavletić and Trinajstić (10).

### Methods

Content of flavonoids, total polyphenols and tannins was determined three times in all the samples and from this results an average value was calculated.

*The determination of the content of the flavonoids according to Römisch (11).* – Preparation of the extract: An amount of 0.5 g air-dried and powdered plant material was extracted on water bath with 25 mL methanol during 30 min. The extract was filtered through cotton wool which containing the rest of the drug was extracted once again with 20 mL methanol. The fused extracts were diluted with methanol to 50 mL. An amount of 5 mL methanolic extract was mixed with 2 mL tetrachlorocarbon and 3 mL water and after that the mixture was low centrifuged for purpose of separation of the layers. Water-methanolic layer was separated and diluted with methanol to 10 mL.

Measure of the absorption of the extract: To the specimen of 2 mL of extract 0.6 mL concentrated acetic acid, 10 mL reagent (made by mixing 20 mL pyridine and 80 mL water) and 2.5 mL 12% methanolic solution of aluminium chloride hexahydrate were added. Such extract was diluted with water to 25 mL and filtered. The first 5 mL of filtrate were discarded. The absorption of the rest of the filtrate was measured against water at 420 nm.

Determination of the content of the flavonoids: Based on the value of the absorption, the content of the flavonoids was read off gauged straight line.

*Determination of the content of total polyphenols and tannins according to Schneider (12).* – Preparation of the extract: An amount of 0.25 g air-dried and powdered plant material was extracted on water bath with 80 mL methanol during 15 minutes. Extract was filtered and filtrate was diluted with 30% methanol to 100 mL. A volume of 2 mL filtrate was mixed with 8 mL water and 10 mL sodium acetate solution (1.92 g sodium acetate and 0.34 mL concentrated acetic acid diluted to 100 mL).

The mixture prepared in such a way represents the solution A. A volume of 10 mL of the solution A was shaken with 50 mg casein during 60 minutes and after that it was filtered. This filtrate represents the solution B.

Measure of the absorption of the extract: A quantity of 1 mL of each, solution A and solution B, was mixed separately with 0.5 mL reagent of Folin (13) and then both solutions were diluted to 10 mL with 33% sodium carbonate solution. The absorptions of such prepared solutions were measured against water at 720 nm.

Determination of the content of total polyphenols and tannins: Content of the total polyphenols was read off gauged straight line based on the value of the absorption of the solution A. Content of the tannins linked to casein was read off gauged straight line based on the difference of the absorption between solution A and solution B.

## RESULTS AND DISCUSSION

Contents of the flavonoids, total polyphenols and tannins in leaf, flower, stem and shoot in whole of *C. rupestris* and *C. fritschii* during three vegetation seasons are represented in Tables I and II.

The results of the determination of flavonoids in the investigated plants showed that these compounds are present in the largest quantity in the leaves of *C. fritschii*, where they make on an average 0.90 – 1.35%. In flowers of *C. rupestris* this content is considerable too, and ranges from 0.73 – 0.95%, whereas the quantity of these compounds in remaining samples is somewhat lower. The content of the total polyphenols is the highest in the leaves of both investigated species. In the leaves of *C. rupestris* this content ranges on an average from 5.90 – 7.80%, and in the leaves of *C. fritschii*

Table I. Dried basis content of flavonoids, total polyphenols and tannins in the areal parts of plant species *Centaurea rupestris* L. during the three vegetation seasons

| Year of collecting | Samples | Method of determination        |                       |             |
|--------------------|---------|--------------------------------|-----------------------|-------------|
|                    |         | After Römisch                  | By means of casein    |             |
|                    |         | Flavonoids calcd. as rutin (%) | Total polyphenols (%) | Tannins (%) |
| 1988               | Leaf    | 0.30                           | 5.9                   | 0.6         |
|                    | Flower  | 0.73                           | 5.4                   | 0.4         |
|                    | Shoot   | 0.50                           | 5.8                   | 2.0         |
|                    | Stem    | 0.20                           | 4.6                   | 1.2         |
| 1989               | Leaf    | 0.55                           | 7.8                   | 1.8         |
|                    | Flower  | 0.95                           | 6.0                   | 0.8         |
|                    | Shoot   | 0.63                           | 6.2                   | 1.8         |
|                    | Stem    | 0.50                           | 6.0                   | 2.3         |
| 1990               | Leaf    | 0.35                           | 7.4                   | 1.8         |
|                    | Flower  | 0.90                           | 4.8                   | 0.6         |
|                    | Shoot   | 0.55                           | 6.5                   | 1.6         |
|                    | Stem    | 0.20                           | 3.6                   | 0.8         |

Table II. Dried basis content of flavonoids, total polyphenols and tannins in the areal parts of plant species *Centaurea fritschii* Hayek during the three vegetation seasons

| Year of collecting | Samples | Method of determination        |                       |             |
|--------------------|---------|--------------------------------|-----------------------|-------------|
|                    |         | After Römisch                  | By means of casein    |             |
|                    |         | Flavonoids calcd. as rutin (%) | Total polyphenols (%) | Tannins (%) |
| 1988               | Leaf    | 0.90                           | 10.4                  | 2.8         |
|                    | Flower  | 0.45                           | 6.4                   | 1.0         |
|                    | Shoot   | 0.80                           | 6.2                   | 1.4         |
|                    | Stem    | 0.55                           | 4.0                   | 1.2         |
| 1989               | Leaf    | 1.35                           | 11.2                  | 3.8         |
|                    | Flower  | 0.50                           | 5.6                   | 0.4         |
|                    | Shoot   | 0.65                           | 7.6                   | 2.4         |
|                    | Stem    | 0.50                           | 4.8                   | 0.8         |
| 1990               | Leaf    | 1.00                           | 7.6                   | 1.6         |
|                    | Flower  | 0.45                           | 6.0                   | 0.8         |
|                    | Shoot   | 0.70                           | 7.0                   | 2.0         |
|                    | Stem    | 0.35                           | 3.8                   | 1.0         |

from 7.60 – 11.20%. In *C. fritschii* the content of tannins is the highest in the leaves too, and ranges from 1.60 – 3.80%. Contrary to expectation, the content of tannins in species *C. rupestris* is the highest in the stems in general, where the tannins are present within 0.80 – 2.30%. The difference in the content of the total polyphenols between the leaves and stems of *C. rupestris* is not so significant in favour of leaves as in *C. fritschii*. This fact can explain the higher content of tannins in the stems of *C. rupestris* in relation to leaves.

From the mentioned results it is visible that the areal parts of *C. fritschii* are considerably richer in polyphenols in relation to *C. rupestris* (Tables I and II).

The polyphenols of genus *Centaurea*, especially flavonoids, are in qualitative sense comprehensively investigated (4, 5, 14). On the contrary, the number of the papers about the quantitative analysis of polyphenols in genus *Centaurea* is very limited (15). Therefore it is not possible to compare the results of this work with the results of another *Centaurea* species. The quoted authors (15) have determined the content of total anthocyanins in blue *Centaurea* flowers by means of spectrometric methods.

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## S A Ž E T A K

### Određivanje sadržaja polifenola u nadzemnim dijelovima vrsta *Centaurea rupestris* L. i *C. fritschii* Hayek (Asteraceae)

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Određen je sadržaj flavonoida, ukupnih polifenola i tanina u nadzemnim dijelovima vrsta *Centaurea rupestris* L. i *C. fritschii* Hayek u toku tri vegetacijske sezone. Flavonoidi su u najvećoj količini prisutni u listovima vrste *C. fritschii* gdje čine prosječno 0,90 – 1,35% i u cvjetovima vrste *C. rupestris* koji sadrže 0,73 – 0,95% flavonoida. U ostalim istraživanim uzorcima sadržaj flavonoida je nešto niži. Sadržaj ukupnih polifenola je najviši u listovima istraživanih vrsta. Ovi su spojevi u listovima vrste *C. rupestris* zastupljeni s prosječno 5,90 – 7,80%, a u listovima vrste *C. fritschii* s 7,60 – 11,20%. Najbogatiji taninima su listovi vrste *C. fritschii*, gdje se njihov sadržaj prosječno kreće od 1,60 – 3,80%. U vrste *C. rupestris* tanini su prisutni u najvećoj mjeri u stabljikama, gdje čine prosječno 0,80 – 2,30%. Iz iznesenih rezultata je vidljivo da su nadzemni dijelovi vrste *C. fritschii* značajno bogatiji polifenolnim spojevima u odnosu na vrstu *C. rupestris*.

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