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Short communication

Total phenolic, total flavonoid and anti-oxidant activity of methanolic extracts of some *Centaurea* species from Kosovo

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Introduction

The genus *Centaurea* (Asteraceae) has several native species in Kosovo, growing in different habitats. Some of them, i.e. *C. melanocephala* and *C. kosaninii* are Balkan endemic species.

The species of this genus are rich in various bioactive compounds, including phenolic acids, flavonoids, lactones, terpenes, lignans (Khammar, and Djeddi 2012), alkaloids and steroids (Sharonova et al., 2021). Due to the presence of diverse chemical compounds, species of this genus have shown different biological activities, such as anti-inflammatory, analgesic, anti-oxidant, anti-bacterial, anti-fungal activity (Khammar & Djeddi 2012, Sharonova et al. 2021), wound-healing, anti-ulcer, hepatoprotective (Khammar & Djeddi, 2012), anti-tumor, anti-diabetic, anti-depressant, anti-rheumatic (Sharonova et al., 2021).

Although numerous *Centaurea* species are present in Kosovo, information about their chemical composition and biological activities is missing. Thus, this work aims to assess the content of total phenolic, total flavonoid, and the anti-oxidative activity (DPPH and FRAP test systems) on the methanolic extract of the C. *melanocephala*, *C. kosaninii*, *C. scabiosa*, *C. saloniatana*, *C. kotschyana* and *C. atropurpurea*.

Materials and methods

Plant Materials

Plant materials (leaves and inflorescences of five indviduals) was collected from Jun to August 2021 in four different localities (Badovc, Bajgorë, Pashtrik, and Shtërpcë) in Kosovo. Voucher specimens were deposited at the Herbarium of the Department of Biology, University of Prishtina. Plant material was dried in the drying cabinets at 35°C for five days.

Extraction of Plant Materials

Before extraction, leaves and inflorescences were separated and then grounded. Grounded samples (150 mg) were extracted with 25 ml of 50% MeOH for 30 min in an ultrasonic bath. The samples were filtered and stored in the dark at -18 °C in until further analysis.

Determination of Total Phenolic and Total Flavonoid.

The total phenolic content was determined using the Folin-Ciocalteu method. Caffeic acid (0-8 mg/ml) was used to construct the calibration curve, and absorbance was measured at 725 nm against the blank. The results were expressed as mg Caffeic acid equivalent/g plant dry weight (mg CAE/g dw).

The total flavonoid was also determined using the spectrophotometric method. Catechin (0-10 mg/ml) was used to construct the calibration curve. Absorbance was measured at a wavelength of 510 nm. The total content of flavonoid was expressed as mg Catechin equivalent/g plant dry weight (mg CE/g dw).

Evaluation of Anti-oxidant Activity.

To assess the DPPH (2.2-diphenyl-l-picrylhydrazyl) radical scavenging activity, the Trolox (0-3 to7 mg/ml) were used as reference substances. The absorbance was

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measured at 515 nm against the blank. The results are expressed as a % inhibition of DPPH free radicals.

The Trolox (0-2.4 mg/ml) was used to construct the calibration curve for evaluation of the ferric reducing anti-oxidant power (FRAP). Absorbance was measured at 593 nm and the result were expressed as mg Trolox equivalent/g plant dry weight (mg TE/g dw).

All spectrophotometric measurements were performed using a UV-Vis microplate-reader (Sunrise™ Tecan).

Results and discussion

The results (mean value and standard deviation) of total phenolic, total flavonoid, and anti-oxidant activity (DPPH and FRAP) are presented for all analysed species.

The highest concentration of the total phenolic was recorded in the leaves of *C. atropurpurea* (218.5±50.2), followed by *C. melanocephala* (162.8±24.1), *C. kosaninii* (141.2±83.2), *C. scabiosa* (125.1±14.5), *C. kotschyana* (50.9±2.3) and *C. saloniatana* (34.4±5.0 mgCAE/g dw). The total phenolic content was lower in the inflorescences compared to its content in the leaves. The highest total phenolics in inflorescences was as follows: *C. kosaninii* (49.1±43.0), *C. kotschyana* (26.3±1.2), *C. melanocephala* (23.6±2.6), *C. scabiosa* (22.4±1.0), *C. saloniatana* (21.9±1.6) and *C. atropurpurea* (21.4±6.2 mg CAE/g dw).

The content of total flavonoids differed among the species and plant organs too. The highest content was recorded in leaves of *C. atropurpurea* (56.1±14.1), followed by *C. melanocephala* (53.6±3.9), *C. kosaninii* (47.4±21.0), *C. scabiosa* (44.8±4.4), C. *saloniatana* (7.3±0.9) and *C. kotschyana* (6.1±0.2 mg CE/g dw). In inflorescence, the highest content was in extracts of *C. kosaninii* (18.2±17.4), followed by *C. scabiosa* (7.1±0.4), *C. melanocephala* (6.6±1.6), *C. atropurpurea* (5.7±0.9), *C. kotschyana* (5.4±0.3) and *C. saloniatana* (4.7±0.4 mg CE/g dw).

Leaves of the *Centaurea* species showed the higher DPPH radical scavenging capacity compared with inflorescences. The highest % inhibition in leaves was recorded in *C. atropurpurea* (80.6±1.0) extracts, followed by *C. melanocephala* (73.3±4.5), *C. scabiosa* (60.8±4.7), *C. kosaninii* (56.6±25.6) *C. kotschyana* (43.4±2.2) and *C. saloniatana* (18.3±2.0 % inhibition). In inflorescences, the highest % inhibition was recorded in extracts of *C. kosaninii* (27.9±7.3) followed by *C. saloniatana* (12.0±2.8), *C. kotschyana* (11.2±0.8), *C. atropurpurea* (10.1±3.8), *C. scabiosa* (9.8±0.5) and *C. melanocephala* (7.0±2.3%).

The highest FRAP anti-oxidant capacity was found in the leaves of the *Centaurea* sp., whereas the lowest was found in inflorescences. In the leaves, the highest anti-oxidant capacity was recorded in *C. atropurpurea* (55.3±7.0), followed by *C. scabiosa* (35.8±0.7), *C. melanocephala* (33.9±4.9), *C. kosaninii* (31.4±7.3), *C. kotschyana* (28.7±2.9), *C. saloniatana* (18.4±0.6 mg TE/g dw). In the inflorescences, the highest anti-oxidant capacity was recorded in the *C. kosaninii* (13.3±5.9), followed by *C. saloniatana* (6.1±0.8), *C. melanocephala* (5.5±1.9), *C. atropurpurea* (5.5±0.4), *C. kotschyana* (5.0±0.3) and *C. scabiosa* (4.8±0.3 mg TE/g dm dw).

Conclusion

The results of this study outline that the most significant difference in total phenolic and total flavonoid content, as well as the anti-oxidant activity (DPPH and FRAP), were amonge the plant organs. This can be attributed to differences in the gene expression profiles, as different plant organs have entirely different gene expression adapted to the function of the respective organ.

Furthermore, an interspecies variation (smaller than between organs) among the analysed species (for the respective tested parameters) was recorded too. This seems to reflect the genetic background and the environmental impact, influenced by differences in habitat composition, altitude, and microclimatic conditions.

Further research is needed to screen the chemical profile of the analysed species, as well as to evaluate the correlation between specific chemical constituents and anti-oxidant activity.

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