UNIVERSITETI I PRISHTINËS
"HASAN PRISHTINA"
FAKULTETI I SHKENCAVE MATEMATIKE NATYRORE
Rr. Eqrem Çabej, 10000 Prishtinë, Republika e Kosovës
Tel: +381-38-249-873 • E-mail: fshmn@uni-pr.edu • www.uni-pr.edu

FSHMN Ref. nr. 708 Prishtinë, Dt. 11.03.26

KËRKESË

Për: Këshillin Mësimor të Departamentit të Biologjisë

Këshillin e Studimeve të Doktoratës të FSHMN-së

Këshillin e Fakultetit të shkencave Matematike-Natyrore

Lënda: Kërkes për formimin e komisionit për vlerësimin e doreshkrimit të temës së doktoratës

Duke u bazuar në Statusin e Universitetit të Prishtinës dhe Rregullorës ekzistuese për studime të doktoratës, i plotësoj kushtet për vlersimin e doreshkrimit, prandaj kërkoj nga organet e lartëpërmendura të FSHMN-së të më mundësoj formimin e komisionit për vlerësim të doreshkrimit të temës së doktoratës me titull: "**Analizë filogjenetike e disa llojeve të gjinisë *Centaurea* (sect. *Acrocentron*, *Asteraceae*) në Kosovë bazuar në sekuenca të ADN-së dhe në veçori fitokimike".**

Kërkesës ia bashkëngjisë:

1. Kopjen e doreshkrimit
2. Punimin shkencor nga lëmia e ngushtë
3. Pëlqimin e mentorit për dorzimin e doreshkrimit
4. Dëshmin për pjesëmarrje në konferenca
5. Formularin F6

Më: 11.03.26 Prishtinë

Kandidatja: MSc. Mimozë Buzhala

Tel: +38344687950

E-mail: buzhalamimoze@gmail.com

REZYMEJA

Familja *Asteracea* është familje e rëndësishme e bimëve, me lloje të përhapura në mbarë botën. Ndër gjinitë më të mëdha të kësaj familjeje është gjinia *Centaurea*, e pranishme në florën e Kosovës me lloje me rëndësi të veçantë biologjike dhe ekologjike. Në Kosovë, llojet e kësaj gjinie janë të pastudiuara në aspektin taksonomik, fitokimik dhe të aktivitetit antioksidativ. Vëmendje të veçantë kërkojnë llojet me status taksonomik të padefinuar qartë dhe studimi i tërësishëm i llojeve të kësaj gjinie është i domosdoshëm për njohjen, ruajtjen dhe konservimin e tyre.

Qëllimi i këtij studimi është vlerësimi i llojeve të kësaj gjinie në aspektin molekular, fitokimik dhe të aktivitetit antioksidativ. Llojet e përzgjedhura ishin: *C. melanocephala* Pančić L. (sin. *C. albertii* Rexhepi L., sin. *C. candelabrum*), *C. kosaninii* Hayek L., *C. atropurpurea* Waldst. & Kit. L., *C. kotschyana* Heuff. L., *C. salonitana* Vis L., *C. scabiosa* L. Komponimet e avullueshme u analizuan me *GC-FID-MS*. Fenolet, flavonoidet dhe aktiviteti antioksidativ u analizuan me metoda spektrofotometrike. Për analizat molekulare u përdorën markerët e ADN-së: *ITS, trnL, rbcL* dhe *psbA-trnH*. Amplifikimi i tyre u bë përmes Reaksionit Zinxhiror të Polimerazës (PCR), ndërsa sekuencimi me analizuesin kapilar të ADN-së. Kromatogramet u bashkërenduan me programin Chromas dhe iu nënshtruan kërkimeve BLAST. Trungjet filogjenetike janë konstruktuar me programin MrBayes.

Në vajrat esenciale gjithsej u përfituan 138 komponime organike, dhe në sasi më të lartë ishin: acidi heksadekanoik (10.5–31.1 %), heptakosani (4.3–20.04 %), n-trikosani (4.9–11.9 %), n-pentakosani (4.9–12.6 %), acidi linoleik (1.3–6.5 %), germakreni D (0.49–4.7 %), kariofillen oksidi (0.87–5.7 %), n-tetrakosani (1.2–2.3 %), kurse komponimet tjera kishin përqindje më të ulët.

Sasia e fenoleve totale rezultoi mjaft e lartë në gjethe (50.9–226.5 mg CAE/100 g), kurse në lulesa (22.2–35.5 mg CAE/100 g); flavonoidet gjithashtu rezultuan me vlera më të larta në gjethe (6.1–78.0 mg CE/100 g), kurse në lulesa (3.2–13.6 mg CE/100 g). Përqindja më e lartë e DPPH u regjistrua në gjethe (13.6–80.6 %), ndërsa në lulesa (7.25–19.6 %). Kapaciteti më i lartë antioksidativ, matur me radikalin FRAP, u gjet në gjethe (16.0–55.2 mg TE/g dm), ndërsa në lulesa (4.9–15.1 mg TE/g dm).

Sipas analizave fitokimike dhe molekulare, nuk rezultoi variabilitet gjenetik në mes llojeve *C. melanocephala, C. albertii* dhe *C. candelabrum*, çka nënkupton se këto lloje duhet të trajtohen si sinonime.

Fjalët kyçe: *Asteracea, Centaurea, fitokimi, taksonomi, aktiviteti antioksidativ, C. melanocephala, C. albertii, C. candelabrum.*

RESUME

The *Asteracea* family is an important family of plants, with species distributed throughout the world. Among the largest genera of this family, the genus *Centaurea* is present in the flora of Kosovo with species of particular biological and ecological importance. In Kosovo, the species of this genus are unstudied in terms of taxonomy, phytochemistry and antioxidant activity. Special attention is required for species with unclear taxonomic status and a comprehensive study of the species of this genus, which is necessary for their recognition, preservation and conservation.

The aim of this study is to evaluate the species of this genus in terms of molecular, phytochemical and antioxidant activity. The selected species were: *C. melanocephala* Pančić L. (syn. *C. albertii* Rexhepi L., syn. *C. candelabrum*), *C. kosaninii* Hayek L., *C. atropurpurea* Waldst. & Kit. L., *C. kotschyana* Heuff. L., *C. salonitana* Vis L., *C. scabiosa* L. Volatile compounds were analyzed by *GC-FID-MS*. Phenols, flavonoids and antioxidant activity were analyzed by spectrophotometric methods. For molecular analyses, DNA markers were used: *ITS, trnL, rbcL* and *psbA-trnH*. Their amplification was done by Polymerase Chain Reaction (PCR), while sequencing was performed using a capillary DNA analyzer. Chromatograms were aligned with the Chromas program and subjected to BLAST searches. Phylogenetic trees were constructed with the MrBayes program.

A total of 138 organic compounds were obtained in the essential oils and in higher quantities were: hexadecanoic acid (10.5–31.1 %), heptacosane (4.3–20.0 %), n-tricosane (4.9–11.9 %), n-pentacosane (4.9–12.6 %), linoleic acid (1.3–6.5 %), germacrene D (0.49–4.7 %), caryophyllene oxide (0.87–5.7 %), n-tetracosane (1.2–2.3 %), while other compounds had lower percentages.

Total phenols resulted quite high in leaves (50.9–226.5 mg CAE/100 g) and in flowers (22.2–35.5 mg CAE/100 g); flavonoids also resulted with higher values in leaves (6.1–78.0 mg CE/100 g) and in flowers (4.88–13.55 mg CE/100 g). The highest percentage of DPPH was recorded in leaves (13.6–80.6 %), while in inflorescences (7.25–19.6 %). The highest antioxidant capacity, measured with the FRAP radical, was found in leaves (16.0–55.2 mg TE/g dm), while in inflorescences (4.9–15.1 mg TE/g dm).

According to phytochemical and molecular analyses, no genetic variability was found between the species *C. melanocephala, C. albertii* and *C. candelabrum*, which means that these species should be treated as synonyms.

Key words: *Asteracea, Centaurea, phytochemicals, taxonomy, antioxidant activity, C. melanocephala, C. albertii, C. candelabrum.*

Biochemical Systematics and Ecology 122 (2025) 105037

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Variation in VOCs, phenolics, flavonoids and antioxidant activity among natural populations of *Centaurea kosaninii* in Kosovo

Mimozë Buzhala [a], Behxhet Mustafa [a], Bledar Pulaj [a], Xhavit Mala [b], Kristian Peters [c], Christoph Rosche [d,e], Hazbije Sahiti [a], Avni Hajdari [a,*]

[a] Department of Biology, Faculty of Mathematical and Natural Science, University of Prishtina, Mother Theresa St, 10000 Prishtinë, Kosovo
[b] Sharri National Park, Prizren, Kosovo
[c] Bioinformatics and Systems Biology, Justus Liebig University Giessen, Gießen, Germany
[d] Institute of Biology/Geobotany and Botanical Garden, Martin Luther University Halle-Wittenberg, Am Kirchtor 1, D-06108 Halle (Saale), Germany
[e] German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig, Halle, Germany

ARTICLE INFO

Keywords:
Centaurea kosaninii
VOCs
Total phenolics content
Total flavonoids content
Antioxidant activity
Natural variability

ABSTRACT

Endemic plant species, characterised by a narrow geographic range, are increasingly threatened by global changes due to the complex ecological interactions with their environments. Ecometabolomic studies can reveal detailed insights into the molecular functioning of these interactions, providing fundamental implications for conservation efforts. We assessed the chemical variation in *Centaurea kosaninii*, a plant species endemic to the Balkan Peninsula. Plant materials were collected from five wild-growing populations in Kosovo to investigate the genetic variability, chemical diversity, composition of the volatile organic compounds (VOCs), total phenolics content (TPC), total flavonoids content (TFC) and antioxidant activity. The nuclear and chloroplast loci were amplified and sequenced, showing minimal genetic diversity among the examined populations. VOCs were extracted using hydro-distillation and analyzed using gas chromatography, revealing the separation of 107 VOC constituents. Principal component analysis, principal coordinates analysis, discriminant analysis, and Boruta, based on random forest variable selection, demonstrated distinct chemical compositions of VOCs across different populations. Additionally, spectrophotometric methods were utilised to determine TPC, TFC, and antioxidant activities, revealing significant differences between the leaves and inflorescences of *C. kosaninii*. We also found strong correlations between TPC and TFC with DPPH radical scavenging activity, while the correlations with FRAP activity were more moderate. This study provides the first insights into the genetic and chemical diversity of *C. kosaninii*, contributing to scientific understanding and laying the groundwork for future research initiatives, focusing on its ecological roles, adaptive traits, population dynamics, conservation strategies and potential biotechnological applications.

1. Introduction

Endemic plant species are restricted to narrow geographic regions and face an increasing threat of extinction due to global environmental changes (Médail, 2017; Rosche et al., 2022; Stein et al., 2014). As ecosystems undergo transformations driven by climate change, habitat loss, and human activity, several species may disappear before their ecological roles, taxonomic complexities, and biotic interactions are fully understood (Al-Gharaibeh et al., 2024; Lehnert et al., 2024). Eco-metabolomics can help to determine physiological stress at the molecular level, which is important for conservation assessments (Nagler et al., 2018). These techniques may also uncover the biotechnological potential of endangered species, offering insights into their applications in fields such as pharmacology, agriculture, and biochemistry (Patti et al., 2012). In an eco-evolutionary context, eco-metabolomics provides a valuable tool for exploring plant-environment interactions by analysing the biochemical profiles that underpin species adaptations and ecological strategies (Irimia et al., 2019; Peters et al., 2018; Uthe et al., 2021).

This study focuses on volatile organic compounds (VOCs) that play a crucial role in mediating plant-environment interactions by functioning as chemical defences against herbivores through their toxic effects or as

* Corresponding author.
E-mail address: avni.hajdari@uni-pr.edu (A. Hajdari).

https://doi.org/10.1016/j.bse.2025.105037
Received 2 March 2025; Received in revised form 2 May 2025; Accepted 3 May 2025
Available online 7 June 2025

signalling molecules that attract beneficial organisms, such as pollinators or natural enemies of herbivores (Kessler and Kalske, 2018; Petrén et al., 2024). Similarly, phenolic compounds, including flavonoids, contribute to plant defence against both biotic and abiotic stresses (Wen et al., 2020), while the antioxidant properties of these classes of compounds further enhance the adaptive capacity of plant species, supporting their survival in diverse environmental conditions.

The genus *Centaurea*, belonging to the Asteraceae family, is characterised by complex systematics. Its taxonomy has frequently been revised due to cryptic morphological variations and the occurrence of multiple cytotypes (Hilpold et al., 2014; Rosche et al., 2025). Understanding these complexities, particularly through metabolomics studies, is crucial for accurate species identification and conservation efforts. *Centaurea kosaninii* Hayek (Fig. 1) is a Balkan endemic species whose ecological interactions and potential biotechnological applications remain largely unexplored.

This relict species is commonly found on serpentine substrates (Stevanović et al., 2003), but rarely observed on calcareous substrates (Siljak-Yakovlev, 2022) (Fig. 1). Its habitats are distributed across Albania (Kuzmanović et al., 2016; v. Hayek, 1914), Kosovo (Blečić et al., 1969; Kuzmanović et al., 2016; Siljak-Yakovlev, 2022; Tomović et al., 2014), North Macedonia (Micevski and Matevski, 1987), and South Serbia (Kuzmanović et al., 2016). In our study region, Kosovo, it primarily inhabits rocky grassland habitats on serpentine and calcareous substrates in the southern part of Pashtrik Mountain. In serpentine substrates, *C. kosaninii* is associated with *Euphorbia glabriflorae*, building the characteristic plant association *Centaureo kosaninii-Euphorbietum glabriflorae* (Jovanović et al., 2017). *C. kosaninii* has been included in the Kosovo List of Protected and Strictly Protected Species due to its restricted distribution in Kosovo (Administrative Instruction No. December 2020 of the Ministry of Environment and Spatial Planning (MESP, 2020)). Nevertheless, with over 1000 individuals in most of its habitats, this species is classified as a "Least Concern" in terms of the IUCN criteria (Administrative Instruction No. December 2020 (MESP, 2020)).

Although species of the genus *Centaurea*, such as *C. cyanus*, have been used in traditional Kosovan medicine to treat respiratory disorders and eye infections (Mustafa et al., 2012) and as a blue colouring agent for textiles (Mustafa et al., 2012), there is no evidence of traditional ecological knowledge on the uses of *C. kosaninii*. Besides this, the current information about its taxonomic status, population genetics, chemical diversity, and biological activities is insufficient. To the best of our knowledge, there is only one publication that mentions the presence of sesquiterpene lactones (Tešević et al., 1998), and another recent short communication paper of preliminary data on total phenolics content (TPC), total flavonoids content (TFC), and antioxidant activities of this species from a single population in Shterpcë, Kosovo (Buzhala et al., 2022). However, no previous research has reported the chemical composition of VOCs and any metabolomic variability among natural populations of *C. kosaninii*.

Even though data on the VOCs of *C. kosaninii* are lacking, the VOC profiles of related species (*Acrocentron* section of the *Centaurea* genus) have previously been documented, including *C. scabiosa* (Carev et al., 2022), *C. rupestris* and *C. salonitana* from Croatia (Carev et al., 2023); *C. orientalis*, and *C. atropurpurea* (Novaković et al., 2016).*C. murbeckii* and *C. chrysolepis* (Novaković et al., 2019), *C. melanocephala*, *C. kotchyana*, *C. zlatiborensis*, *C. orientalis*, *C. calocephala*, *C. atropurpurea* and *C. grbavacensis*) (Novaković et al., 2019), *C. orientalis*, and *C. atropurpurea* (Novaković et al., 2016) from Serbia; *C. immanuelis-loewii* from Bulgaria (Bancheva et al., 2022); *C. finazzeri* and *C. rupestris* from North Macedonia (Novaković et al., 2022).

To address the apparent knowledge gaps, our study aims to assess variation among *C. kosaninii* populations in Kosovo in terms of the genetic variability based on DNA markers, the chemical diversity of VOCs, and to confirm and expand the initial findings on TPC, TFC, and antioxidant activity.

2. Materials and methods

2.1. Plant materials

The plant materials were gathered from five natural populations of *C. kosaninni* in Kosovo between June and August of 2022 in Shtërpcë, Pashtrik, Kishnicë, Qafë Prush, and Devë (Table 1). Fresh leaves were collected from all locations and preserved in silica gel for DNA analysis. Leaves and inflorescences were collected separately from five individuals per locality in four populations (Table 1) for TPC, TFC, and antioxidant activities analyses. The materials were dried in a cabinet at

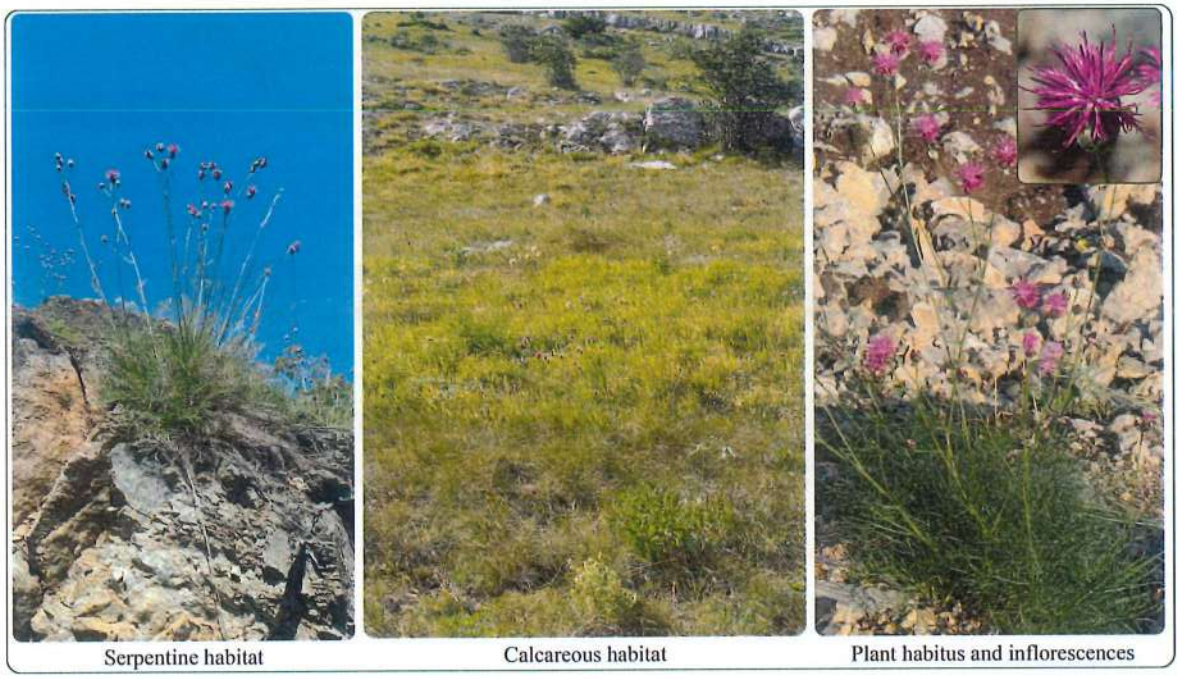

Fig. 1. *Centaurea kosaninii* present in the serpentine substrate, calcareous substrate and plant habitus (from left to right).

Table 1
Basic characteristics of the collection sites, voucher information, and GenBank accession numbers of the *Centaurea kosaninii* samples used for this study.

Collection locality	Longitude	Latitude	Altitude	Substrate	Sampled for	ITS	trnL-trnF	rbcl	psba-trnH	Herb. Acces. no
Kishnicë	42°37′5.8"	21°13′30"	681 m	Serpentine	1, 2,	OR906344	PP035464	PP297742	PP297799	00002031
Shterpcë	42°13′31"	21°0′33.4"	894 m	Serpentine	1, 2, 3	OR906345	PP035465	PP297743	PP297800	00002032
Devë	42°19′51"	20°20′55"	599 m	Serpentine	1, 3	OR906346	PP035466	PP297744	PP297801	00002033
Qafë Prush	42°18′30"	20°22′37"	738 m	Serpentine	1, 3	OR906347	PP035467	PP297745	PP297802	00002030
Pashtrik	42°12′12"	20°32′2.3"	1465 m	Calcareous	1, 2, 3	N/A	PP035468	PP297746	PP297803	00002029

Samples for: 1: DNA analysis, 2: essential oil composition, 3: TPC, TFC, and antioxidant.

35 °C for five days. Only inflorescences were collected from three locations to assess the VOCs (Table 1). As a larger amount of plant material (30 g) was needed for extraction, inflorescences from at least ten individuals were collected and combined to create a bulk sample for distillation. Three samples were collected per population, and the materials were stored in a freezer at −18 °C until further analysis. Voucher herbarium specimens are deposited at the Herbarium of the Department of Biology, University of Prishtina, with the herbarium accession numbers 00002029, 00002030, 00002031, 00002032, 00002033, 00002034 (Table 1).

2.2. Molecular analyses

2.2.1. DNA extraction, chain reaction (PCR), and sequencing

Genomic DNA was extracted from silica gel-dried material using the DNeasy Plant Mini Kit (Qiagen Hilden, Germany) according to the manufacturer's instructions. The DNA quality was checked using agarose gel electrophoresis. The nuclear internal transcribed spacer region (*ITS (ITS1, complete 5.8S rDNA gene, ITS2 and a part of 26S rDNA*) and the chloroplast *trnL-trnF, psbA-trnH, rbcL* markers were amplified and sequenced (Table 1). PCR amplifications were performed with a Mic qPCR Cycler, and then the amplified PCR fragments (2 μL of PCR products) were checked using electrophoresis in 1 % agarose gels. Sequencing was performed by Microsynth Austria (Vienna, Austria) using Applied Biosystems 3730 × l 96 capillary DNA analyser (Thermo Fisher Scientific). Sequences of this species (19 in total) were manually edited using CHROMAS ver. 2.6.6 (Technelysium, South Brisbane, Australia) and were aligned with MEGA X software (Kumar et al., 2018). Comprehensive information on DNA extraction, amplification, and sequencing can be found in the protocol used by Hajdari et al. (2021). DNA sequences were deposited to GenBank (accession numbers in Table 1), while the edited sequences were subjected to BLAST searches for preliminary analysis (Altschul et al., 1990).

2.3. Chemical composition of the VOCs

2.3.1. Distillation of plant materials

The VOCs were obtained using the hydro-distillation method. Here, 30 g of chopped plant material (inflorescences) and 0.5 l of distilled water were placed in a 1-L flask and then distilled at a 3 ml/min rate in a Clevenger apparatus for 2 h. The volatiles were collected with n-hexane and stored in the dark at −18 °C in a freezer until further analysis.

2.3.2. GC-FID and GC-MS analyses

GC-FID analyses were performed using a gas chromatography (GC) system coupled with a flame ionisation detector (FID) (Agilent 7890A). The separation was conducted on an HP-5MS column. Helium was used as carrier gas with a 0.6 ml/min flow. The front inlet was operated at 250 °C. The split ratio was set as 50:1, while the GC oven temperature was increased from 60 °C to 280 °C at a rate of 5 °C/min. FID operated at 250 °C, with 350 ml/min airflow and a 35 ml/min hydrogen flow.

GC-MS analyses were performed using a GC system coupled to a 5975C mass selective detector (MSD) (Agilent 7890A). The GC condition for separating the constituents was the same as for GC-FID analyses, while the mass spectra (MS) ionisation energy was 70 eV with a mass range of 40–400 m/z. The injection volume was 1.0 μl.

2.3.3. Identification of the chemical constituents

The identification of the VOCs was performed by comparing their arithmetic retention indices (ARI) with those in the literature (Adams, 2017), by comparing the mass spectra of each constituent with those stored in the NIST 08. L and WILEY MS 9th databases, and with mass spectra from the literature (Adams, 2017). The percentage of constituents from the GC peak areas was calculated using the normalization method without correction factors.

2.4. Total phenolic and total flavonoid contents and antioxidant activity assays

2.4.1. Methanolic extraction of plant materials

The leaves and inflorescences were extracted separately. For extraction, 150 mg of the grounded sample was mixed with 25 ml of 50 % MeOH and shaken in an ultrasonic bath for 30 min. The samples were filtered and stored in a dark freezer at −18 °C until further analysis. Photometric measurements of the samples in the following analyses were carried out using a microplate UV–Vis spectrophotometer (Sunrise™, Tecan Group Ltd., Switzerland). Each sample was measured in triplicate.

2.4.2. Determination of total phenolics and total flavonoids

The total phenolic content in the methanolic extracts 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 (Chizzola et al., 2008).

The total flavonoid was also determined using a photometric method. Here, catechin (0–10 mg/ml) was used to construct the calibration curve, and absorbance was measured at 510 nm. The total content of flavonoids was expressed as mg catechin equivalent/g plant dry weight (Leontowicz et al., 2003).

2.4.3. Evaluation of antioxidant activity

To assess the 2.2-diphenyl-*l*-picrylhydrazyl (DPPH) radical scavenging activity, Trolox (2.5 mM in methanol) were used as a reference substance to construct a 0–3.7 mg/ml calibration curve. The absorbance was measured at 515 nm against the blank. The results are expressed as a % inhibition of DPPH free radicals (Chizzola et al., 2008).

Trolox (0–2.40 mg/ml) was also used to construct the calibration curve to evaluate the ferric-reducing antioxidant power (FRAP). The absorbance was measured at a wavelength of 593 nm (Chizzola et al., 2008). The results were expressed as % inhibition.

2.5. Statistical analysis

In prior statistical analyses, the matrix containing the abundances of VOCs determined by GC-MS was normalised using a log-transform and the missing values were imputed with zero. Principal coordinate analysis (PCoA) was accomplished with the function PCoA of the ape R package (Legendre and Legendre, 2012). A Euclidean distance measure was selected as the matrix only contained a few zero values and had a

near-normal distribution. The descriptive statistical analyses, discriminant analysis (DA) and principal component analysis (PCA) were performed using the statistical software XLSTAT Version 2023.1.2 (Addinsoft, New York, USA) to determine if the VOCs could reflect the natural variability among populations. Only the VOCs with concentrations higher than 1 % (bold in Table 2) were selected for PCA and DA for statistical analysis.

The selection of variables that were significantly correlated to the location was accomplished by applying the Boruta method using the R packages Boruta, random forest and rpart (Breiman, 2001; Kursa and Rudnicki, 2010). Boruta eliminates irrelevant variables selected by random forest (RF) by performing permutation tests and comparing the importance of variables with a background dataset of random variables (Kursa and Rudnicki, 2010). To validate the Boruta model, a regression tree using the function rtree was constructed, and prediction accuracy metrics were calculated using the R packages pROC (Robin et al., 2011), PRROC (Grau et al., 2015) and multiROC (Wei et al., 2018). To visualise relationships, a heatmap was implemented using the function heatmap.2 in the R package iESTIMATE (https://github.com/ipb-halle/iESTIMATE) (Peters et al., 2024), selecting Euclidean dissimilarity measures and the Ward.D method to agglomerate values in columns and rows, respectively.

An analysis of variance (ANOVA with Tukey's test) was utilised to determine the variations in TPC, TFC and antioxidant activities between different populations. Pearson correlation coefficients were calculated to assess the relationship between TPC, TFC and two antioxidant test systems. Pearson correlation and ANOVA were conducted using PAST software for Windows, version 4.13 (Hammer and Harper, 2001).

3. Results and discussion

3.1. Molecular analysis

The ITS sequences of the four individuals (Kishnicë, Shtërpc, Devë and Qafë Prush) presented identical sequence composition and comprised 690 base pairs. No sequences of *C. kosaninii* for the *ITS* region were available in the GenBank database, but sequences of *C. salonitana* from Spain (FJ459690) (Font et al., 2009) and *C. salonitana* from Ukraine (MW383491) were identified as the closest relatives of our ITS sequences with a 99.69 % similarity. Additionally, the *Rbcl* sequences comprising 585 base pairs and the *trnL-trnF* sequences comprising 920 base pairs were identical across all analyzed individuals. The *psbA-trnH* sequences indicated that individuals from Kishnicë, Shtërpc, and Devë were similar (comprising 460 base pairs), but they differed by fifteen bases from individuals in Qafë Prush and Pashtrik. However, individuals from Qafë Prush and Pashtrik were similar (for 475 base pairs). No sequences were available for the *Rbcl, trnL-trnF,* and *psbA-trnH* genes in the GenBank database for this species, and our sequences did not show any similarity with other species found in the database. In summary, despite the pronounced isolation of our study populations, we found little genetic differentiation with our markers, contrasting with several studies on fragmented species (Rosche et al., 2018). However, it is important to note that we used conservative markers primarily used for phylogenic assessments, and classical population genetic markers could provide a more detailed picture of the genetic differentiation among the *C. kosaninii* populations in Kosovo.

3.2. Metabolite profiling of the VOCs

From the inflorescences, a total of 107 VOC constituents were separated, of which 97 were identified (Table 2). The main VOCs were heptacosane (11.4–20.0 %), followed by hexadecanoic acid (10.5–15.0 %), n-tricosane (7.7–10.8 %), n-pentacosane (6.4–9.7 %), [Z,Z,Z]-9,12,15-octadecatrienoic acid methyl ester (4.2–6.4 %), linoleic acid (3.6–5.8 %), germacrene D (2.4–4.7 %), (E)-isovalencenol (1.5–2.5 %), n-tetracosane (1.3–2.1 %), isopimara-9(11),15-diene (1.1–2.0 %), and α-acorenol (1.1–3.0 %) which has higher concertation that 1 % in all analyzed population (Table 2).

Due to the lack of available data on the chemical composition of the VOCs of *C. kosaninii*, we compared our findings with closely related species from sect. *Acrocentron* originates from Kosovo's neighbouring countries. Heptacosane was one of the main constituents of the VOCs, and had a concentration similar to that reported for *C. scabiosa* (Carev et al., 2022), *C. rupestris* and *C. salonitana* from Croatia (Carev et al., 2023), *C. murbeckii* and *C. chrysolepis* from Serbia (Novaković et al., 2019), as well as for *C. immanuelis-loewii* from Bulgaria (Bancheva et al., 2022), and for *C. finazzeri* from North Macedonia (Novaković et al., 2022). Heptacosane is an important female sex pheromone found in both bees and beetles, which acts as a contact pheromone, stimulating male precopulatory behaviour (Francke and Schulz, 2010). Such interactions may play a vital ecological role in the relationships between plants and insects.

Along with heptacosane, hexadecanoic acid was one of the main VOCs whose concentration in our samples was similar to that of *C. rupestris* from Croatia (Carev et al., 2023). Previous research has shown that hexadecanoic acid and *its derivatives* may play an important role in how plants adapt to their environments, significantly suppressing the growth of soil-borne pathogens while simultaneously encouraging seedling development (Abdel-Naime et al., 2019; Davis et al., 1997; Ding et al., 2019). In our sample, the concentration of n-tricosane is higher than that found in *Centaurea* species (sect. *Acrocentron*) (Novaković et al., 2019), *C. orientalis* and *C. atropurpurea from Serbia* (Novaković et al., 2016), *C. rupestris* and *C. salonitana* from Croatia (Carev et al., 2023), and *C. finazzeri* and *C. rupestris* from North Macedonia (Novaković et al., 2022). Tricosane and its derivatives are crucial components of the female sex pheromone in various insects, including bees, beetles, moths, borers, and navel orangeworms (Francke and Schulz, 2010). Other VOCs with high concentrations in our samples include n-pentacosane, which, together with its derivatives play a significant role in the female sex pheromones of bees, beetles, wasps, navel orangeworms, and pyralid moths (Francke and Schulz, 2010); linoleic acid and its derivatives which, acts as an egg dispersion pheromone for moths, a brood pheromone in bees and also functions as an aggregation pheromone and antiaphrodisiac (Francke and Schulz, 2010); and germacrene-D, which may act as a deterrent against herbivores, exhibit insecticidal properties against mosquitoes (Ravi Kiran and Sita Devi, 2007), and shows repellent effects against aphids (Bruce et al., 2005) and ticks (Birkett et al., 2008).

Considering the main classes of VOCs of *C. kosaninii*, the predominant chemical constituents were other hydrocarbons (57.9–71.3 %), followed by oxygenated sesquiterpenes (13.2–17.6 %), unknown compounds (5.8–6.4 %), sesquiterpenes (4.2–8.4 %), diterpenes (4.7–6.8 %), oxygenated diterpenes (2.3–3.8 %), and monoterpenes (tr–0.36 %) (Table 2). While the concentration of individual compounds plays an important role in plant-environment interactions, a comprehensive understanding of these dynamics requires examining the diverse classes of phytochemicals or the plant's overall phytochemical profile (Petrén et al., 2024). Synergistic and antagonistic interactions among these compounds can significantly influence plant responses and shape ecological relationships within their environments.

3.3. Chemical composition and diversity among wild populations of C. kosaninii

Various prediction models (PCA, PCoA, DA, and RF) were used as statistical tools to constitute relationships among the VOCs between plant populations. The localities were used as grouping variables to classify or discriminate between different populations, while the chemical constituents were used as independent variables.

The PCA biplot indicates a clear grouping of the populations into two distinct clusters, with the Pashtrik population exhibiting the most pronounced separation from the Shtëpcë and Kishnicë populations (Fig. 2a).

Table 2
Composition (%) of the VOCs of *C. kosaninii* from different locations.

ARI literature	ARI calculated	Constituents	Pashtrik	Shtërpcë	Kishnicë
846	850	(2E)-Hexenal	0.10	0.47	0.58
932	934	α-Pinene	tr	0.22	0.21
987	992	Furfuryl acetate	–	0.10	0.19
998	999	Furfuryl methyl sulfide	tr	0.30	0.20
1024	1028	Limonene	–	0.14	0.11
1036	1040	Benzene acetaldehyde	tr	0.24	tr
1095	1098	6-Camphenone	tr	0.10	0.11
1100	1105	n-Nonanal	tr	0.33	0.33
1137	1142	cis-Verbenol	tr	tr	tr
1144	1146	trans-Verbenol	tr	0.17	0.21
1166	1168	ρ-Mentha-1,5-dien-8-ol	tr	0.30	0.12
1201	1206	n-Decanal	tr	tr	0.17
1271	1271	Citronellyl formate	0.10	0.16	0.24
1315	1317	(2E,4E)-Decadienal	0.27	0.38	0.39
1335	1340	δ-Elemene	0.12	0.21	0.34
1339	1346	trans-Carvyl acetate	tr	tr	tr
1417	1421	(E)-Caryophyllene	0.84	1.53	1.64
1448	1450	cis-Muurola-3,5-diene	tr	tr	0.12
1452	1457	α-Humulene	0.10	0.26	0.40
1454	1459	(E)-β-Farnesene	tr	0.24	–
1464	1465	(2E)-Dodecenal	0.13	0.25	0.19
1480	**1484**	**Germacrene D**	**2.41**	**3.63**	**4.68**
1488	1489	β-Selinene	tr	0.18	–
1492	1492	iso-Menthyl lactate	tr	0.14	0.15
1502	1503	*trans*-β-Guaiene	–	tr	–
1508	1508	Germacrene A	tr	0.12	tr
1522	1526	δ-Cadinene	tr	0.21	0.13
1527	1530	(E)-γ-Macrocarpene	0.17	0.34	0.19
1543	1542	cis-Sesquisabinene hydrate	tr	0.13	0.26
1548	1549	Pentanoic acid, nonyl ester	tr	0.13	0.26
1553	1553	Isobutyl isovalerate	0.18	0.53	0.53
1559	1561	Germacrene B	0.31	0.69	0.76
1565	1565	Dodecanoic acid	tr	tr	tr
1575	1579	Spathulenol	0.26	0.31	0.16
1582	1587	Caryophyllene oxide	0.87	1.64	1.45
1595	1595	ar-dihydro-Turmerone	0.14	0.12	0.26
1594	1600	Salvial-4(14)-en-1-one	0.15	0.10	0.16
1608	1611	β-Atlantol	0.20	0.18	0.12
1608	1613	Humulene epoxide II	tr	tr	tr
1612	1615	β-Biotol	0.28	0.11	0.45
1622	1622	10-epi-γ-Eudesmol	0.39	0.38	0.36
1630	1630	γ-Eudesmol	0.29	0.14	0.31
1632	**1633**	**α-Acorenol**	**1.09**	**1.27**	**3.04**
1634	1637	Camphoric acid	0.16	0.15	0.16
1639	1641	Caryophylla-4(12),8(13)-dien-5α-ol	0.47	0.64	0.96
1644	1646	α-Muurolol	0.24	0.61	0.52
1645	1650	Cubenol	0.10	0.22	0.38
1652	1654	α-Eudesmol	–	1.05	0.32
1652	1658	α-Cadinol	0.58	0.90	0.65
1666	1667	14-hydroxy-(Z)-Caryophyllene	0.23	0.15	0.34
1668	1670	14-hydroxy-9-epi-(E)-Caryophyllene	0.16	0.12	0.12
1674	1675	(Z)-α-Santalol	0.38	0.35	0.35
1679	1684	Khusinol	0.23	tr	0.22
1685	1690	Germacra-4(15),5,10(14)-trien-1-α-ol	0.91	1.06	1.97
1700	1700	n-Heptadecane	0.14	0.24	0.14
1711	1715	Pentadecanal	tr	0.60	0.16
1725	1725	Guaiol acetate	0.16	0.22	0.43
1764	1765	Tetradecanoic acid	0.39	1.16	0.94
1768	1768	Unknown 1	0.58	0.49	2.20
1773	1775	α-Costol	0.12	0.18	0.55
1793	**1800**	**(E)-Isovalencenol**	**1.50**	**2.07**	**2.53**
1803	1807	14-hydroxy-δ-Cadinene	0.27	0.17	0.98
1823	1824	Khusinol acetate	0.65	0.83	0.93
1847	1846	6,10,14-trimethyl-2-Pentadecanone	0.59	0.58	0.55
1865	1868	Pentadecanoic acid	0.21	0.60	1.06
1876	1876	Unknown 2	0.32	0.98	0.44
1874	1881	n-Hexadecanol	0.42	0.94	0.92
1881	1883	Laurenene	tr	0.16	tr
1889	1888	(5Z,9E)-Farnesyl acetone	0.15	0.13	0.13
1890	1896	β-Chenopodiol-6-acetate	0.12	0.31	0.22
1900	1900	n-Nonadecane	0.17	0.56	0.24
1905	**1912**	**Isopimara-9(11),15-diene**	**1.14**	**1.58**	**2.04**
1922	1923	Totarene	0.33	0.64	0.55
1927	1927	Unknown 3	0.13	0.27	0.28

(*continued on next page*)

Table 2 (*continued*)

ARI literature	ARI calculated	Constituents	Pashtrik	Shtërpcë	Kishnicë
1937	1935	Cembrene	0.23	0.35	0.39
1933	1934	Cyclohexadecanolide	0.60	0.94	0.73
1959	**1965**	**Hexadecanoic acid**	**10.49**	**14.98**	**13.80**
1871	1871	Unknown 4	0.27	0.39	0.29
2000	2000	n-Eicosane	0.23	0.40	0.19
2016	2014	Phyllocladene	0.34	0.47	0.66
2022	2022	Abieta-8,12-diene	0.25	0.35	0.23
2056	2054	Manool	0.37	0.60	0.20
2059	2060	13-epi-Manool	0.29	0.40	0.21
2077	2081	n-Octadecanol	0.44	1.07	0.42
2095	2096	Methyl linoleate	0.32	0.46	0.52
2100	2100	n-Heneicosane	0.67	0.69	0.73
2115	2114	Laurenan-2-one	0.25	0.39	0.40
2123	2123	Unknown5	0.24	0.49	0.36
2132	**2140**	**Linoleic acid**	**5.76**	**3.56**	**4.28**
2143	**2147**	**[Z,Z,Z]-9,12,15-Octadecatrienoic acid, methyl ester**	**6.44**	**4.98**	**4.16**
2165	2165	Unknown 6	0.63	0.61	0.31
2184	2190	Sandaracopimarinal	0.30	0.28	0.19
2200	2200	n-Docosane	0.56	0.60	0.45
2220	2228	Methyl eperuate	1.96	0.23	1.02
2231	2236	(Z)-Methyl communate	0.35	tr	0.17
2257	2265	(E)-Methyl communate	0.23	tr	0.10
2274	2274	Unknown 7	1.41	0.56	0.57
2300	2300	**n-Tricosane**	**10.80**	**8.43**	**7.65**
2328	2328	Unknown 8	1.06	0.79	0.37
2400	**2400**	**n-Tetracosane**	**2.14**	**1.30**	**1.52**
2430	2430	Labd-(13E)-8,15-diol	0.39	0.25	0.12
2474	2474	Unknown 10	1.28	0.86	0.50
2500	**2500**	**n-Pentacosane**	**9.73**	**6.96**	**6.41**
2600	2600	Hexacosane	1.20	0.80	0.88
2700	**2700**	**Heptacosane**	**20.04**	**13.08**	**11.39**
2800	2800	Octacosane	0.25	0.22	0.16
2900	2900	Nonacosane	0.13	tr	0.11
		Monoterpenes	tr	0.36	0.32
		Oxygenated monoterpenes	0.62	1.2	1.23
		Sesquiterpenes	4.21	7.48	8.24
		Oxygenated sesquiterpenes	9.19	13.12	17.67
		Diterpenes	2.31	3.57	3.86
		Oxygenated diterpenes	1.13	1.23	0.91
		Other hydrocarbons	76.09	66.6	61.89
		Unidentified	6.4	6.44	5.88

ARI - Arithmetic Retention Index calculated against a C9-C22 n-alkanes mixture on the HP5 MS column. Compounds are listed in order of elution from a HP-5MS column, and their percentages were obtained by FID peak-area normalization. The percentage for each population represents the mean values a of tree calculated samples. Boldface marked compounds (with concentrations higher than 1 %) were chosen for DA and PCA statistical analyses. - = missing; tr = trace <0.1 %.

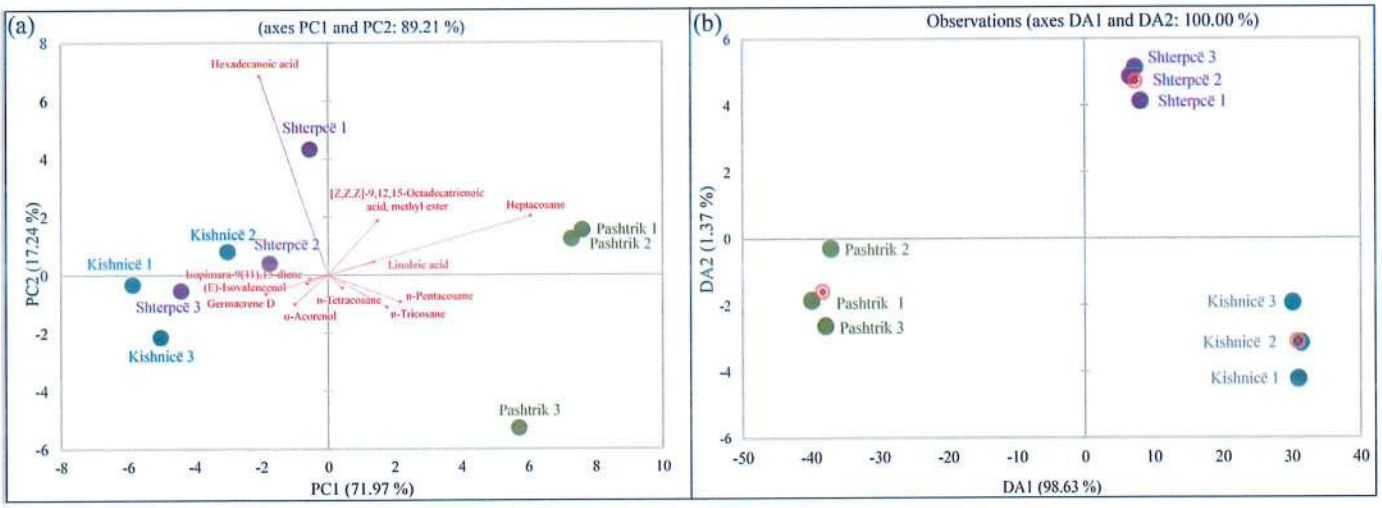

Fig. 2. (a) PCA biplot, and (b) scatter plot of discriminant analysis of the soil constituents obtained from the inflorescence of *C. kosaninii*. Only the VOCs with concentrations higher than 1 % were selected for analysis. Green dots represent the samples from Pashtrik; blue dots represent the samples from Kishnicë; violet dots represent the samples from Shterpcë. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

While samples from Shtëpcë and Kishnicë also show differences, they are relatively closer in proximity to each other. The PCoA analysis demonstrated a sample distribution consistent with that observed in the PCA. The data from the DA scatter plot (Fig. 2b) mostly corroborated the findings obtained from PCA and PCoA. The samples from the Pashtrik population demonstrated the most significant differentiation from the Shtëpcë and Kishnicë populations. Contrary to the PCA and PCoA biplots, the samples from Shtëpcë and Kishnicë are delineated through DA. The heatmap of the selected VOCs using Boruta (Fig. 3) shows that the samples from Kishnicë display the most significant differences compared to those collected from Pashtik and Shterpcë. The samples from Pashtrik and Shterpcë, therefore, show more similarities, in contrast to the abovementioned findings.

However, the heatmap (Fig. 3) presents a clear clustering of samples based on their geographic origins, thus aligning with the PCA, PCoA and DA. The heatmap (Fig. 4) showed that fatty acid esters, methyl esters, seven specific classes of mono-, di-, and sesquiterpenoids and heteroaromatic compounds had higher abundance in the Kishnicë population, whereas the Shterpcë population was additionally characterized by low abundance of tertiary alcohols and fatty alcohol esters and high abundance of bicyclic monoterpenoids. The Pashtrik population presented lower abundance in almost all of the selected classes except diterpenoids.

The differences in the chemical composition of the VOCs of *C. kosaninni* from the Pashtrik, Shtërpcë, and Kishnicë populations may be attributed to various factors. The population from Pashtrik grows in a calcareous substrate, while the populations from Shtërpcë and Kishnicë typically grow in a serpentine substrate. The effect of calcareous versus serpentine substrate on the VOC profile has also been documented in other taxa, such as *Satureja montana* (Hajdari et al., 2016) and *Teucrium montanum* (Zlatić et al., 2022). In addition, the overall lower abundances of selected compound classes in Pashtrik (Fig. 4) may be attributed to its significantly higher altitude than the Kishnicë and Shtërpcë locations, potentially causing higher abiotic stress and lower abundances of consumers (Moreira et al., 2018; Robinson et al., 2023). Furthermore, the Pashtrik location has a unique continental climate influenced by the Alpine climate due to high altitude and Mediterranean climate, which came from the Adriatic Sea through the Drini River. Increasing altitude alters environmental factors and shortens the growing season, which may stimulate the synthesis of various phytochemicals that facilitate essential biological processes within a limited timeframe to optimise reproductive success. In this context, *C. kosaninni* appears to produce different chemicals, including heptacosane, n-tricosane, n-pentacosane, and linoleic acid, which are important pheromones (Francke and Schulz, 2010), and whose concentration is higher in Pashtrik compared to Shtëpcë and Kishnicë. The altitude and specific climatic conditions have been reported to affect both the content and chemical composition of VOCs significantly in various species, including *Satureja montana* (Hajdari et al., 2016), *Juniperus communis* (Hajdari et al., 2015b), *J. oxycedrus* (Hajdari et al., 2014), *Pinus peuce* (Hajdari et al., 2014), and *P. mugo* (Hajdari et al., 2015a).

Conversely, the predominant compound classes identified at the Kishnicë and Shtërpcë sites may play a role in alleviating oxidative stress, which could be a consequence of the elevated levels of various chemical elements found in the serpentine substrate. This suggests that plants may enhance their resilience to abiotic stress by facilitating direct interactions between terpenoids and reactive oxidants (Boncan et al., 2020). Because interactions remain unclear, our result warrants further investigation and subsequent research, focused on the biochemical pathways involved, the potential trade-offs in metabolic investment, and

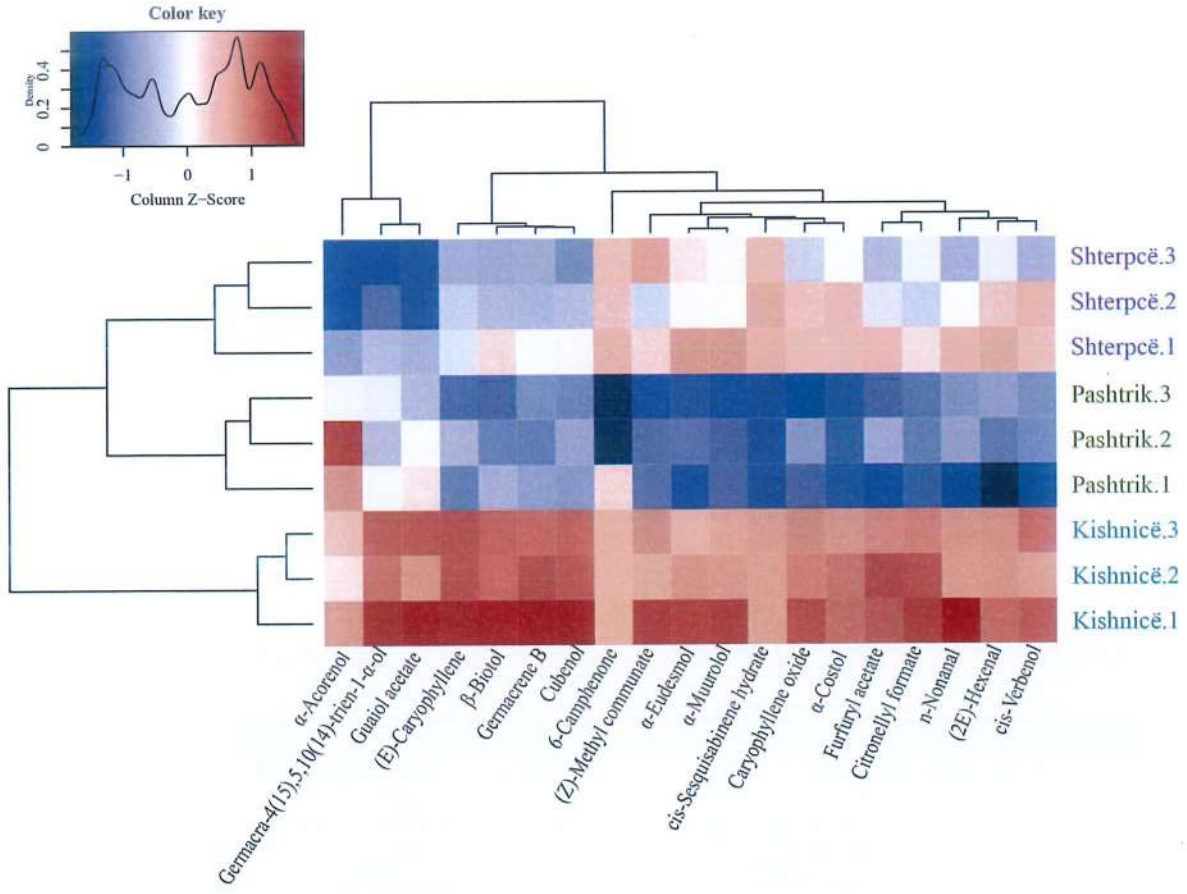

Fig. 3. Heatmap illustrating the selected classes of VOCs across three wild populations of *C. kosaninii.* Red indicates an upregulation and blue a downregulation in terms of mean variable variance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

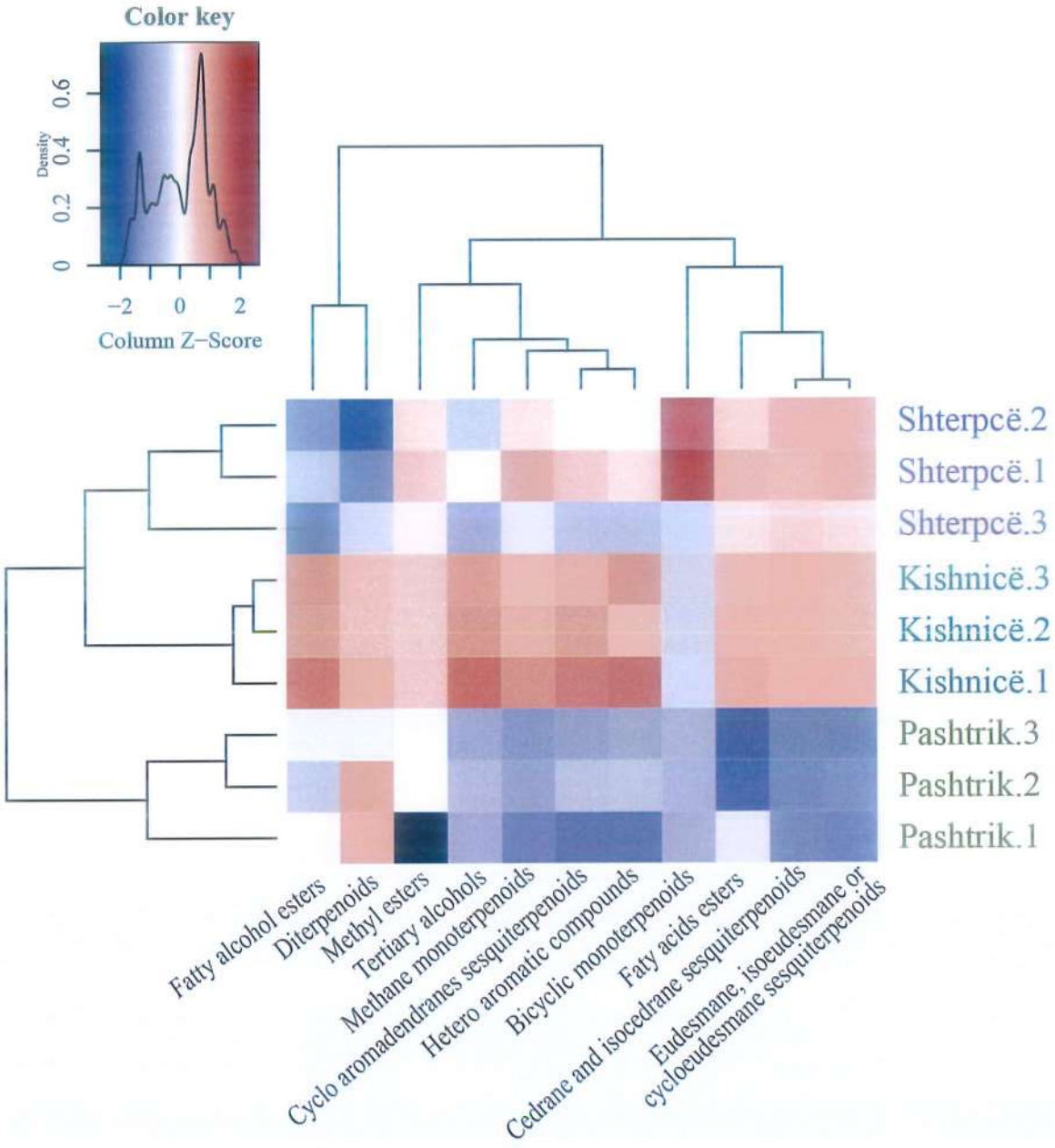

Fig. 4. Heatmap illustrating the selected VOCs across three wild populations of *C. kosaninii.* Red indicates an upregulation and blue a downregulation in terms of mean variable variance. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the ecological significance of these compounds.

3.4. Total phenolic content (TPC) and total flavonoid content (TFC)

The leaves of *C. kosaninii* showed the highest TPC concentration, ranging from 129.9 ± 29.0 to 192.8 ± 26.4 mg CAE/100 g (Fig. 5a). In contrast, the TPC concentration in the inflorescences was significantly lower than in the leaves, ranging from 23.6 ± 1.7 to 35.5 ± 7.4 mg CAE/100 g (Fig. 5a). There were no significant differences in TPC in the leaves of *C. kosaninii* among the analyzed populations ($P \leq 0.05$) (Fig. 5a). However, the TPC content in the leaves was greater in populations living on serpentine than on calcareous substrate. The TPC concentration was significantly lower in Devë than in the Pashtrik population in terms of inflorescences. The high TPC in the inflorescence of Pashtrik populations may be linked to the considerably shorter vegetative period at high altitude. The TPC concentration in the inflorescences of *C. kosaninii* was comparable to that in *C. kosaninii, C. kotschyana, C. melanocephala, C. scabiosa, C. saloniatana,* and *C. atropurpurea* previously reported (Buzhala et al., 2022). Furthermore, the TPC concentration in the leaves of *C. melanocephala, C. kosaninii and C. scabiosa* was consistent with our results and slightly exceeded that of *C. atropurpurea*; however, it was notably lower in *C. kotschyana* and *C. saloniatana* than in our samples (Buzhala et al., 2022).

The TFC concentration was higher in the leaves than in inflorescences, ranging from 59.3 ± 1.5 to 129.9 ± 29.0 mg CA/g dm, and from 7.2 ± 1.4 to 13.6 ± 3.2 mg CA/g dm, respectively (Fig. 5b). The TFC levels in leaf samples showed no significant differences between the localities. Like TPC, the TFC in the leaves was highest in populations living on the serpentine substrate. In terms of inflorescence, the TFC content in samples from Devë was significantly lower compared to

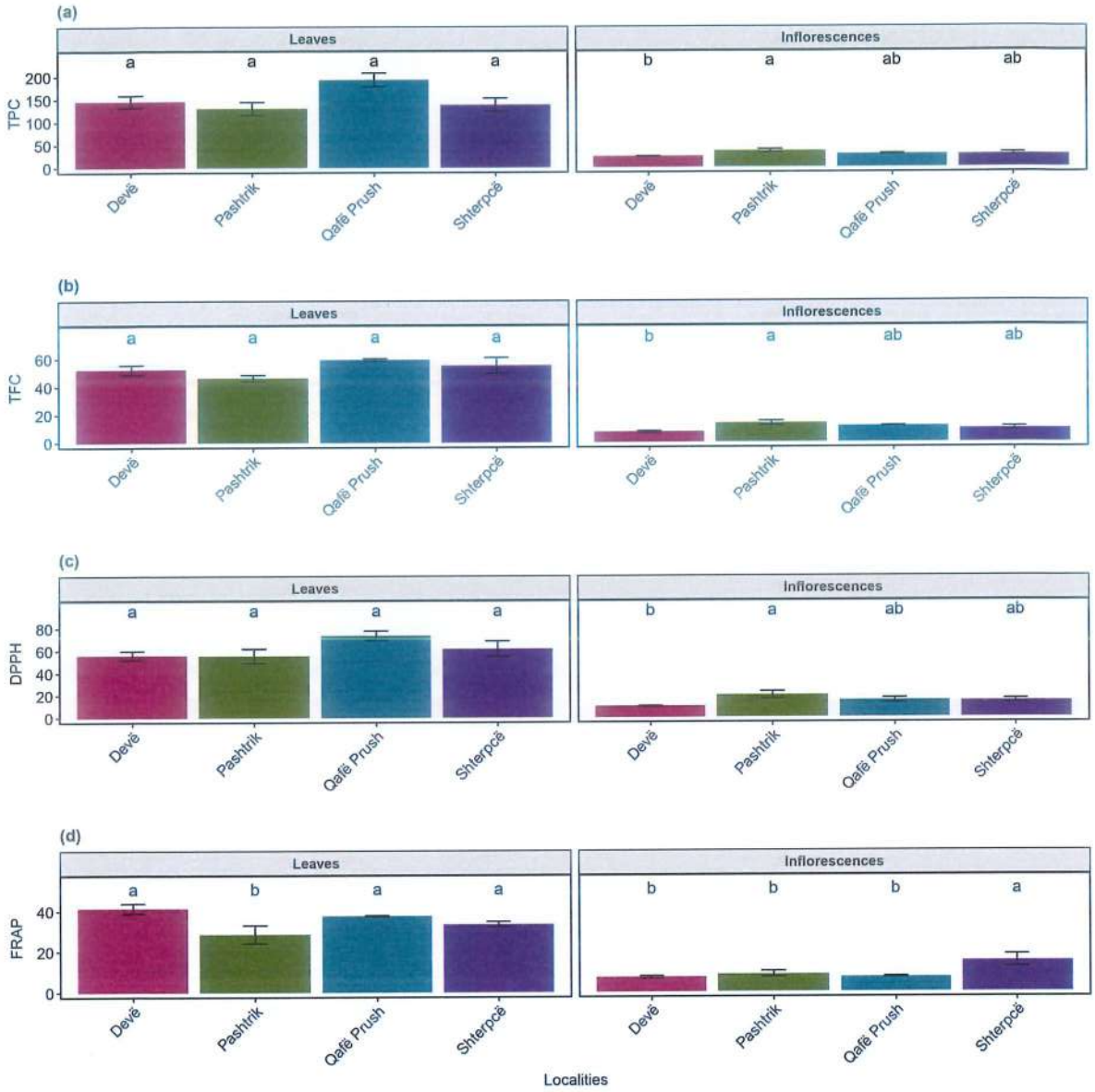

Fig. 5. Mean values and standard deviations of total flavonoids, total phenolics, DPPH and FRAP antioxidant activity in leaves and inflorescence of *C. kosaninii*. Subscript letters denote significant differences ($P < 0.05$) among populations (localities), as determined by Tukey's test within leaves or inflorescences.

Pashtrik, while Qafë Prush and Shterpcë presented intermediate values that were not significantly different from the other locations, except for Pashtrik (Fig. 5b), where the high TFC in the inflorescence may again be linked to the high altitude. Comparing TFC in our samples to previous studies, its concentrations were similar to *C. atropurpurea* and *C. melanocephala*, and slightly higher than *C. kosaninii* and *C. scabiosa*, while significantly lower than in leaves samples of *C. salonitana* and *C. kotschyana* (Buzhala et al., 2022). Additionally, the TFC content in the inflorescences of our samples was comparable to that of *C. scabiosa*, slightly lower than *C. kosaninii*, and higher than in the inflorescences of *C. melanocephala, C. atropurpurea, C. kotschyana,* and *C. salonitana* (Buzhala et al., 2022).

The high content of TPC and TFC in plants growing in serpentine substrates is likely influenced by the substrate's chemical composition, heavy metal concentrations and low nutrient availability. These harsh environmental conditions place significant stress on plants, prompting them to produce secondary metabolites, such as phenolic and flavonoid compounds, which serve as a defence mechanism and help the plant adapt to such an environment. Other studies have reported high TPC (Keleş et al., 2021; Maleva et al., 2022) and TFC (Krasteva et al., 2013) in plants growing in serpentine environments, suggesting that these phytochemicals are related to the protective mechanisms that plants employ to cope with such extreme conditions (Zhiponova et al., 2020).

The high TPC and TFC found in the inflorescences of plants from the Pashtrik population, which is situated at a higher altitude than the other two locations, may be influenced by microclimatic conditions associated with high-altitude environments. Specifically, factors such as increased UV radiation (Dong et al., 2020) and a shorter vegetative season necessitates rapid development and reproductive cycles (Rawat et al., 2018).

3.5. Antioxidant activity (DPPH and FRAP)

Antioxidant activity is a crucial defence mechanism that protects plants from oxidative stress induced by environmental factors such as UV radiation, heavy metals, drought, temperature extremes, and pathogens (REF). These stressors trigger the production of reactive oxygen species (ROS), which can cause cellular damage. In response, plants synthesise antioxidant compounds, including phenolics, flavonoids, and enzymatic antioxidants, to scavenge ROS, prevent oxidative damage,

and maintain redox homeostasis. The antioxidant defence system is essential for plant survival, and thus, assessing antioxidant levels serves as an important indicator of the degree of environmental stress a species experiences (Hasanuzzaman et al., 2020).

The DPPH radical scavenging capacities were significantly higher in the leaves than in the inflorescences (Fig. 5c). The lowest DPPH radical scavenging capacity of the leaves was 55.4 ± 12.8 %, while the highest was 73.5 ± 7.4 %. Meanwhile, the DPPH radical scavenging capacity in inflorescence samples ranged from 9.8 ± 0.7 to 19.6 ± 6.6 %. The leaf samples did not show significant DPPH radical scavenging capacity among populations, while the inflorescences of the Devë population presented significantly lower DPPH than Pashtrik. Compared to previous research, leaf extracts of *C. kosaninii* demonstrated a higher DPPH radical scavenging capacity than *C. kotschyana* and *C. saloniatana*. However, this activity was like that of *C. melanocephala* and *C. scabiosa* and slightly lower than that in the leaf extracts of *C. atropurpurea* (Buzhala et al., 2022). Furthermore, the % DPPH inhibition of our inflorescence samples was similar to *C. saloniatana, C. kotschyana, C. atropurpurea,* and *C. scabiosa*, while slightly higher than in *C. melanocephala* and somewhat lower than in *C. kosaninii* (Buzhala et al., 2022).

Similarly to the DPPH analyses, the leaves also demonstrated higher FRAP antioxidant activity (range: 28.6 ± 8.9 to 41.4 ± 4.9 mg TE/100 g) than the inflorescences (range: 7.2 ± 1.2 to 15.1 ± 5.4 mg TE/100 g, (Fig. 5d). The leaf samples from the Pashtrik localities exhibited lower FRAP antioxidant activity than other localities, while inflorescence samples from Shterpcë displayed significantly higher FRAP antioxidant activity (Fig. 5d). The FRAP antioxidant activity in our leaf samples was determined to be slightly lower than in *C. atropurpurea* and somewhat higher than in *C. saloniatana*, while it exhibited comparable antioxidant activity to the leaf extracts of *C. scabiosa, C. melanocephala, C. kosaninii,* and *C. kotschyana* (Buzhala et al., 2022). In our inflorescence samples, the FRAP antioxidant activity was slightly higher than in *C. kosaninii*, but somewhat lower than in the samples of *C. saloniatana, C. melanocephala, C. atropurpurea, C. kotschyana* and *C. scabiosa* (Buzhala et al., 2022).

The DPPH and FRAP antioxidant activities in the leaves were higher in populations living on the serpentine substrate (see Fig. 5c and d), which may be linked to oxidative stress induced by heavy metals and other environmental stressors (Sharma et al., 2019). In the inflorescence, DPPH antioxidant activity was significantly higher in the Pashtrik population, which seems to be linked to the higher altitude, where plants exposed to increased UV radiation may also exhibit elevated antioxidant activity as a protective response (Zhao et al., 2023).

The most significant variations in TPC, TFC, DPPH, and FRAP antioxidant activity were observed between leaves and inflorescences. These variations may be attributed to unique gene expression profiles in different plant organs, each adapted to specific functions (Hajdari et al., 2015a).

3.5.1. Correlation between TPC, TFC and antioxidant activity test systems (DPPH and FRAP)

Pearson correlation analysis was conducted to evaluate the antioxidant properties of TPC and TFP by using DPPH and FRAP assays in the leaves and inflorescences of *C. kosaninii* (Table 3). In the inflorescences, strong correlations were determined between DPPH antioxidant activity and TPC (r = 0.93) and TFP (r = 0.83), while moderate positive correlations were observed between TPC (r = 0.56), TFP (r = 0.57), and the FRAP assay. Similar to inflorescence samples, the leaves of *C. kosaninii* showed strong correlations between DPPH antioxidant activity and TPC (r = 0.92) and TFP (r = 0.88); however, weaker correlations were determined between the FRAP assay and TPC (r = 0.41) and TFP (r = 0.30). These results suggest a strong correlation between phenols, flavonoids, and antioxidant activity, especially in the DPPH radical scavenging activity.

Table 3
Pearson correlation matrix for the total phenolic, total flavonoid contents and antioxidant activity in leaves and inflorescence of *C. kosaninii*.

		Total Phenols	Total Flavonoids
Inflorescences	DPPH	0.929[a]	0.829[a]
	FRAP	0.564[b]	0.572[b]
Leaves	DPPH	0.920[a]	0.885[a]
	FRAP	0.413	0.303

[a] Correlation is significant at the 0.01 level (2-tailed).
[b] Correlation is significant at the 0.05 level (2-tailed).

4. Conclusions

This study provides first insights into the genetic and chemical diversity of *C. kosaninii*, revealing limited genetic variation among populations and indicating low genetic diversity within the species. Future research using classical population genetic markers may yield further insights into the genetic structure of *C. kosaninii*.

The VOCs comprise 107 distinct chemical constituents. Among these, heptacosane, hexadecanoic acid, n-tricosane, n-pentacosane, [Z,Z,Z]-9,12,15-octadecatrienoic acid methyl ester, linoleic acid, germacrene D, (E)-isovalenceno, n-tetracosane, isopimara-9(11),15-diene, and α-acorenol were identified as the primary constituents that exhibited variations among different populations. Our analyses indicated that the VOC composition of the *C. kosaninii* population from Pashtrik differs from those in Shtëpcë and Kishnicë. These differences appear to reflect variations in altitude, substrate chemical composition, and varying levels of biodiversity interactions at different geographic origins. However, we were not able to identify ten chemical compounds representing 5.8–6.4 % of the total composition, which suggests the need for further characterisation.

Significant TPC, TFC, and antioxidant activity were observed between the leaves and inflorescences of *C. kosaninii*, with TPC and TFC showing a strong correlation with DPPH radical scavenging activity. The correlations with FRAP were more moderate, suggesting that these compounds may play varied roles in different antioxidant mechanisms. Therefore, further research on the detailed profiling of phenolic and flavonoid compounds will help clarify the specific contributions of individual constituents to antioxidant properties.

Our findings also reveal a significant correlation between environmental factors, stress-related metabolites, and antioxidant activity, emphasising the role of abiotic stress in shaping the species' metabolic profile. Considering that *C. kosaninii* already produces stress-associated metabolites, such as fatty acids and methyl esters, future climatic shifts could impose additional metabolic burdens, potentially reducing overall fitness. The relatively low genetic and chemical diversity further suggests limited adaptive capacity.

To support the long-term survival of this species, conservation strategies should focus on maintaining large and contiguous habitats that facilitate natural migration in response to climate change. Protecting these habitats and ensuring their connectivity will be crucial for the resilience of *C. kosaninii* in an increasingly variable climate.

CRediT authorship contribution statement

Mimozë Buzhala: Writing – original draft, Formal analysis. **Behxhet Mustafa:** Writing – review & editing, Data curation, Conceptualization. **Bledar Pulaj:** Investigation, Formal analysis. **Xhavit Mala:** Resources, Investigation. **Kristian Peters:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Christoph Rosche:** Writing – review & editing, Visualization, Funding acquisition, Data curation. **Hazbije Sahiti:** Writing – review & editing, Formal analysis. **Avni Hajdari:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Data availability

Data supporting the findings of this study are available upon request from the corresponding author.

Ethics approval and consent to participate

This article does not contain any studies with human participants or vertebrates performed by any of the authors.

Funding

This work was supported by the Kosovo Ministry of Education, Science, Technology and Innovation (Project no. 2-814-2). CR gratefully acknowledges the support of iDiv funded by DFG (FZT 118, 202548816). No funds, grants, or other support were received during the preparation of this manuscript.

Declaration of competing interest

The authors declare that they do not have any competing financial interests or personal relationships that could have affected the work reported in this paper. They confirm adherence to all ethical guidelines and affirm that the study's integrity and transparency have been maintained throughout. No private funding or personal relationships influenced the outcomes, and all potential competing interests have been disclosed. The authors acknowledge any institutional support received without stipulations affecting the research conclusions. Furthermore, they assure that this work is original and has not been published elsewhere, nor is it under consideration for publication by any other entity.

Data availability

Data will be made available on request.

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Macedonian pharmaceutical bulletin, 68 (Suppl 2) 65 - 66 (2022)
Online ISSN 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2022.68.04.027
Short communication

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

Mimozë Buzhala*[1], Behxhet Mustafa[1], Bledar Pulaj[1], Avni Hajdari[1]

[1]*Department of Biology, Faculty of Mathematics and Natural Science, University of Prishtina, Mother Theresa St. 10000 Prishtinë. Kosovo*

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

*avni.hajdari@uni-pr.edu

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.

Acknowledgements

The authors are thankful to the Kosovo Ministry of Education, Sciences, Technology and Innovation (Project no. 2-814-2) for the provided financial support.

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INTERNATIONAL CONFERENCE ON NEW ACHIEVEMENTS IN SCIENCE, TECHNOLOGY AND ARTSICNA-STA4th - 5th May 2023, Prishtina, Kosova

Taxonomic – status of the *Centaurea melanocephala* Pančić and *C. albertii* Rexhepi (sect. *Acrocentron*, fam. *Asteraceae*)

Mimozë Buzhala1*, Behxhet Mustafa1, Bledar Pulaj1, Xhavit Mala2, Hazbije Sahiti1, AvniHajdari1

Department of Biology, Faculty of Mathematical and Natural Science, University of Prishtina"Hasan Prishtina". St. George Bush, no. 31. 10000 Prishtinë, Kosovo; Sharri National Park, Prizren, Kosovo

Corresponding author e-mail (mimoze.buzhala@student.uni-pr.edu)

Abstract

Kosovo has several endemic plant species which belong to the genus Centaurea, including C.melanocephala Pančić and C. albertii Rexhepi. C. melanocephala (syn. Centaureacandelabrum Hayek & Košanin) shares many similar morphological characteristics with C.albertii. Due to that, applying traditional methods based on morphological characteristics fortheir identification is challenging, and these species have sometimes been treated as synonymsand are often erroneously identified. Novel approaches based on chemical and molecular DNAmarkers are needed for their proper identification and classification. Plant materials werecollected from four wild populations (C. melanocephala: Bajgorë and C. albertii: Devë,QafëPrush and Golesh) in Kosovo (Jun-September 2021). Volatile compounds and DNAmarkers (ITS, trnL-trnF, rbcL, psbA-trnH and rps) are employed to evaluate their taxonomicstatus. Volatile compounds were extracted using hydro-distillation and then analyzed with gaschromatography coupled with mass spectrometer (GC–MS) and flame ionization detector(GC–FID). Plant DNA was extracted using the DNeasy Plant Mini Kit, amplified using PCRand then sequenced by a capillary DNA analyzer. The main volatile components were (E)-Caryophyllene, Germacrene D, Caryophyllene oxide, Germacra-4 (15), 5, 10 (14)-trien-1-α-ol,Hexadecanoic acid, (Z, Z)-9, 12-Octadecadienoic acid, (Z, Z, Z)-9, 12, 15-Octadecatrienoicacid, n-Tricosane, n-Tetracosane, n-Pentacosane and Heptacosane. The analyses based onvolatile chemical constituents and DNA markers did not show significant differences betweenthe analyzed species, indicating that C. albertii and C. melanocephala should not be treated asdistinct species. This work shows that volatile chemical constituents and genetic data can beused as markers to determine these species' taxonomic status. Further analyses with moreCentaurea species will be necessary to better understand the natural variability within thesetaxa. Understanding their taxonomic status is crucial in determining the

strategies for theirconservationKeywords: Centaurea albertii, C. melanocephala, phylogenetics, phytochemicals, geneticmarkers.

Acknowledgements: The authors are thankful to the Kosovo Ministry of Education, Sciences, Technology and Innovation (Project no. 2-814-2) for the provided financial support.

(PDF) Book of Abstracts INTERNATIONAL CONFERENCE ON NEW ACHIEVEMENTS IN SCIENCE, TECHNOLOGY AND ARTS ICNA-STA 4 -5 May 2023, Prishtina, Kosova. Available from: https://www.researchgate.net/publication/370602616_Book_of_Abstracts_INTERNATIONAL_CONFERENCE_ON_NEW_ACHIEVEMENTS_IN_SCIENCE_TECHNOLOGY_AND_ARTS_ICNA-STA_4_-5_May_2023_Prishtina_Kosova

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Mimoze Buzhala

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at **The International Conference on New Achievements in Science, Technology, and Arts (ICNA-STA)**, hosted by University of Prishtina "Hasan Prishtina",
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PARAQITJA E PUNIMIT TË DOKTORATËS[1]

TË DHËNAT E PËRGJITHSHME

Doktoranti:	Mimozë Buzhala
Adresa:	Rr. Ilaz Kodra, Su7/7, Dardani, Prishtinë, 10 000
Tel./ fax:	+383 44 687-950
E-mail:	mimoza_buzhala@hotmail.com / buzhalamimoze@gmail.com
Emërtimi i studimit:	Biologji e Organizmave me Ekologji
Udhëheqësi i studimit:	Universiteti i Prishtinës

TË DHËNAT PËR PUNIMIN E DOKTORATËS

Titulli në gjuhën shqipe	Analizë filogjenetike e disa llojeve të gjinisë *Centaurea* (sect. *Acrocentron, Asteraceae*) në Kosovë bazuar në sekuenca të ADN-së dhe në veçori fitokimike
Titulli në gjuhën angleze	Phylogenetic analysis of some species of the *Centaurea* genus (sect. *Acrocentron, Asteraceae*) in Kosovo based on DNA sequences and phytochemical feature
Fusha e hulumtimit	Botanikë

DEKLARATA E MENTORIT/BASHKËMENTORIT

Tema e doktoratës së kandidates MSc. Mimoze Buzhala ofron një analizë gjithëpërfshirëse të variabilitetit gjenetik, kimik dhe aktivitetit antioksidues te disa lloje te përzgjedhura te gjinisë *Centaurea* (sekti *Acrocentron*) nga lokalitete të ndryshme të Kosovës. Rezultatet nga analizat gjenetike, bazuar në markeret e ADN-se se bërthamës dhe plastideve, si dhe veçoritë fitokimike (përbërja kimike e vajrave esenciale, fenoleve dhe flavonoideve totale) japin të dhënat të rëndësishme për veçoritë molekulare dhe fitokimike të këtyre llojeve. Po ashtu, rezultatet tregojnë se lloje e gjinisë *Centaurea* nga Kosova kane potencial të lartë antioksidues.
Ky punim kontribuon në qartësimin e taksonomisë së një prej grupeve më problematike të llojeve të gjinisë *Centaurea* dhe ofron bazë shkencore për rishikim në të ardhmen të taksonomis dhe ekologjisë së këtyre llojeve, në përputhje me qasjet moderne të integruara shkencore. Rezultatet e këtij hulumtimi janë prezantuar në dy konferenca dhe janë publikuar në një punim shkencor, si më poshtë:

Prezantimi në konferenca shkencore:

1. Mimoze Buzhala, Avni Hajdari, Behxhet Mustafa, Bledar Pulaj (2023). Taxonomic – status of the Centaurea melanocephala Pančić and C. alberti Rexhepi (sect. Acrocentron, fam. Asteraceae). International Conference on New Achievements in Science, Technology and Arts" – ICNA-STA, At: 4-5 May 2023, Prishtina, Kosovo,
2. Mimozë Buzhala, Behxhet Mustafa, Bledar Pulaj, Avni Hajdari. (2022). Total phenolic, total flavonoid and anti-oxidant activity of methanolic extracts of some Centaurea species from Kosovo. Macedonian Pharmaceutical Association, Faculty of Pharmacy, Ss 'Cyril and Methodius' University in Skopje, and Association for Medicinal and Aromatic plants of Southeast European Countries (AMAPSEEC). https://bulletin.mfd.org.mk/volumes/Volume%2068_4/68_4_027.pdf

Punimi shkencor:

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[1] Luteni që ta plotësoni formularin dhe ta dërgoni të nënshkruar me postë elektronike.

Vendi, data dhe nënshkrimi
Në Prishtinë, 11 . 03 . '26 Nënshkrimi Avni Hoxha (Emri e mbiemri i mentorit)
Nënshkrimi _____ (Emri e mbiemri i bashkëmentorit)