UNIVERSITETI I PRISHTINËS
"HASAN PRISHTINA"
FAKULTETI I SHKENCAVE MATEMATIKE NATYRORE

Rr. Eqrem Çabej, 10000 Prishtinë, Republika e Kosovës
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FSHMN **Ref. nr.** 1955 **Prishtinë, Dt.** 23/06/2025

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Këshillin e Studimeve të Doktoratës të FSHMN-së

Këshillin e Fakultetit të Shkencave Matematike- Natyrore

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

Më: 20.06.2025 Prishtinë

Kandidatja: MSc. Blerta Salihu

BSalihu

Tel: +38349685262

Email: blerta_198@hotmail.com

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Këshillin e Fakultetit të Shkencave Matematike- Natyrore

Lënda: Pëlqimi nga Mentori për dorëzimin e doreshkrimit të temës së doktoratës me titull: "**Vlerësimi i variabilitetit brenda llojor të sumbullarit të egër (*Humulus lupulus* L.) në Kosovë bazuar në analizat kimike dhe molekulare**" për kandidaten MSc. Blerta Salihu

Mendim:

Tema e doktoratës së kandidates MSc. Blerta Salihu ofron një analizë gjithëpërfshirëse të variabilitetit gjenetik, kimik dhe aktivitetit antioksidues tek popullatat e egra të sumbullarit nga rajone të ndryshme të Kosovës. Rezultatet nga analizat gjenetike, bazuar në mikrosatelit (marker SSR), si dhe ato fitokimike të bazuara në përbërjen kimike të vajrave esenciale, fenolve dhe flavonoideve totale tregojnë një variabilitet të lartë gjenetik brenda popullatave dhe më të ulët ndërmjet tyre. Po ashtu, rezultatet tregojnë se sumbullari i egër nga Kosova ka një potencial të lartë antioksidues, duke e bërë këtë lloj bimor kandidat të vlefshëm për testime të aktiviteteve tjera biologjike.

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Prezantimi në konferenca shkencore:

1. Salihu, B., Mustafa, B., Pulaj, B., Hajdari, A. (2023). Chemical composition of the essential oil of hops (Humulus lupulus L.) growing wild in Kosovo. CMAPSEEC 2022 (11th Conference on Medicinal and Aromatic Plants of Southeast European Countries), Ohrid 6-10 October 2022
2. Salihu, B., Mustafa, B., Pulaj, B., Hajdari, A. (2023). Total Phenolic, Total Flavonoid and Antioxidant Activity of *Humulus lupulus* L. from wild populations in Kosovo. Conference: International Conference on New Achievements in Science, Technology and Arts" – ICNA-STA, Prishtina, Kosovo. University of Prishtina.

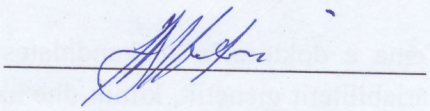
Punimi shkencor:

1. Salihu, B., Samarakoon, Th., Pulaj, B., Quave, C., Mustafa, B., Hajdari, A. (2024). Analysis of chemical and genetic variability in wild hop (*Humulus lupulus* L.) populations of Kosovo. Plant biology. ISSN 1435-8603. 10.1111/plb.13699.

Më: 20. 06. 2025, Prishtinë

Mentori:

Prof. Avni Hajdari



PARAQITJA E PUNIMIT TË DOKTORATËS [1]

TË DHËNAT E PËRGJITHSHME

Doktoranti:	MSc: Blerta Salihu
Adresa:	Rr. "Ali Zeneli" Mitrovicë
Tel./ fax:	+38349685262
E-mail:	Blerta_198@hotmail.com
Emërtimi i studimit:	Biologji e organizmave dhe ekologji
Udhëheqësi i studimit:	Universiteti i Prishtinës

TË DHËNAT PËR PUNIMIN E DOKTORATËS

Titulli në gjuhën shqipe	**Vlerësimi i variabilitetit brenda llojor të sumbullarit të egër (*Humulus lupulus* L.) në Kosovë bazuar në analizat kimike dhe molekulare**
Titulli në gjuhën angleze	**Evaluation of Intraspecific Variability of Wild Hop (*Humulus Lupulus* L.) in Kosovo Based on Chemical and Molecular Analyses**
Fusha e hulumtimit	

DEKLARATA E MENTORIT/BASHKËMENTORIT

Mendim:

Tema e doktoratës së kandidates MSc. Blerta Salihu ofron një analizë gjithëpërfshirëse të variabilitetit gjenetik, kimik dhe aktivitetit antioksidues tek popullatat e egra të sumbullarit nga rajone të ndryshme të Kosovës. Rezultatet nga analizat gjenetike, bazuar në mikrosatelit (marker SSR), si dhe ato fitokimike të bazuara në përbërjen kimike të vajrave esenciale, fenolve dhe flavonoideve totale tregojnë një variabilitet të lartë gjenetik brenda popullatave dhe më të ulët ndërmjet tyre. Po ashtu, rezultatet tregojnë se sumbullari i egër nga Kosova ka një potencial të lartë antioksidues, duke e bërë këtë lloj bimor kandidat të vlefshëm për testime të aktiviteteve tjera biologjike.

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

2. Salihu, B., Mustafa, B., Pulaj, B., Hajdari, A. (2023). Total Phenolic, Total Flavonoid and Antioxidant Activity of *Humulus lupulus* L. from wild populations in Kosovo. Conference: International Conference on New Achievements in Science, Technology and Arts" – ICNA-STA, Prishtina, Kosovo. University of Prishtina.

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Vendi, data dhe nënshkrimi

Në Prishtinë, 20.06.2026 N

Nënshkrimi _____

(Emri e mbiemri i mentorit)

Nënshkrimi _____

(Emri e mbiemri i bashkëmentorit)

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ESEARCH ARTICLE

Analysis of chemical and genetic variability in wild hop (*Humulus lupulus* L.) populations of Kosovo

B. Salihu[1], T. Samarakoon[2], B. Pulaj[1], C. L. Quave[2,3], B. Mustafa[1] & A. Hajdari[1]

1 Department of Biology, Faculty of Mathematical and Natural Science, University of Prishtina, Prishtina, Kosovo
2 Emory Herbarium, Emory University, Atlanta, GA, USA
3 Department of Dermatology, Emory University School of Medicine, Atlanta, GA, USA

Keywords
Essential oil; hops; natural variability; SSR markers.

Correspondence
A. Hajdari, Department of Biology, Faculty of Mathematical and Natural Science, University of Prishtina, Mother Theresa, St. Prishtinë 10000, Kosovo.
E-mail: avni.hajdari@uni-pr.edu

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ABSTRACT

- Hops is an economically important species due to its diverse secondary metabolites and extensive use in the brewing and medicinal industries. Although hops is widely distributed in Kosovo, the chemical composition of its essential oils and genetic variability of wild populations remain understudied. Therefore, this study aimed to evaluate the chemical and genetic variability of Kosovo's wild hop population using essential oil constituents and microsatellite (simple sequence repeat – SSR) markers.
- Female hop inflorescences were collected from 21 wild populations in Kosovo. Essential oils were extracted from the dried plant material using a Clevenger apparatus. Chemical composition of the essential oils was analysed using GC-FID-MS. DNA was extracted from dried leaves, and 15 SSR markers were used for fragment analysis.
- The main constituents of the essential oil were myrcene, α-humulene, (E)-β-farnesene, α-selinene, β-selinene, and E-caryophyllene. Statistical analyses based on chemical composition of essential oils and SSR markers highlighted the low variability among populations and high variability within populations.
- These findings provide valuable insights for developing strategies for potential use and conservation of wild hop populations in Kosovo, laying the groundwork for future research and comparison with commercial cultivars to assess their breeding potential.

INTRODUCTION

Kosovo is rich in plant diversity and represents a unique hotspot of biological richness in Europe (Mustafa & Hajdari 2014). Among the various species in Kosovo, *Humulus lupulus* L. (hops; Cannabaceae) is widespread and grows in habitats including rich deep loam soil, along riverbanks, and in clearings that can support climbing plants. Hop is a perennial climber native to the Northern Hemisphere (Patzak *et al.* 2010). Because of its economic importance, it is widely cultivated in Europe, West Asia, and North America (Awasthi *et al.* 2021). The plant is dioecious and wind-pollinated, with male and female flowers occurring on separate plants (Karlsson Strese *et al.* 2014). Female inflorescences, or strobili, are the most valuable parts of the plant because they contain bioactive chemicals in their glands (Korpelainen & Pietiläinen 2021). Among these chemicals, bitter acids (α-acid and β-acid) are the most prevalent secondary metabolites (Patzak *et al.* 2010; Mongelli *et al.* 2015; Salanta *et al.* 2015; Kondić *et al.* 2021; Hajdari *et al.* 2023; Paguet *et al.* 2023), followed by essential oils (rich in terpenes, including α-humulene, β-myrcene, trans-caryophyllene, and farnesene; Mockute *et al.* 2008; Patzak *et al.* 2010; Ligor *et al.* 2014; Riccioni *et al.* 2021; Rossini *et al.* 2021; Kondić *et al.* 2021), and flavonoids (xanthohumol; Mongelli *et al.* 2015; Maliar *et al.* 2017; Metaj *et al.* 2023; Piasecki *et al.* 2023). Most of these studies highlight variability in constituents of essential oil between populations.

Because of the diverse chemical composition, hops have been used in brewing, medicine, and cosmetics (Korpelainen & Pietiläinen 2021; Piasecki *et al.* 2023). In traditional medicine, hops are used as a sedative against leprosy, toothache, fever, gastric problems, and anxiety, and as a preservative, deodorant, and in several foods, as well as in making fabric, paper, and as cattle fodder (Korpelainen & Pietiläinen 2021). In Kosovan traditional medicine, an infusion of hops prepared from female flowers is used as a neuro-relaxant and to treat kidney inflammation, while decoctions from aerial parts are used to treat insomnia and regulate the menstrual cycle (Mustafa *et al.* 2012). Hops also have anti-inflammatory, estrogenic, cancer chemopreventive, and anti-angiogenic properties, as well as use in obesity, type-2 diabetes and cancer treatment (Zanoli & Zavatti 2008; Chung *et al.* 2009). Other studies have demonstrated antimicrobial (Sefidgar *et al.* 2015; Cermak *et al.* 2017) and antiviral (Buckwold *et al.* 2004) activities. Because of the sedative effects, hops have been approved for treating restlessness, anxiety, and sleep disturbances by the German Commission E and the European Scientific Cooperative on Phytotherapy (Zanoli & Zavatti 2008). Beyond medicinal uses, hops are extensively used in the brewing industry to impart aroma and typical bitter taste of beer (Dresel *et al.* 2016). Phytochemicals present in hop, in addition to their flavour and fragrance properties, also have antimicrobial properties and enhance shelf life (Stewart 2016).

lops have been extensively cultivated in Europe for 000 years, and most hops raw material is currently sourced om cultivars. According to FAOSTAT, by 2021, annual global ops trade exports were worth ca. $800 million (USD), of which ca. $450 million were from Europe (FAOSTAT 2023). While several beer breweries operate in Kosovo, the country relies on imports from other countries, primarily Slovenia, to meet hop raw material requirements (Customs Kosovo 2023). In 2022, the value of imported hop raw material into Kosovo was ca. €0.5 million (Customs Kosovo 2023). Despite this, hop cultivation is not currently practised in Kosovo, and there is no tradition.

Hop cultivars are derived from hybridisation (Henning 2006), which decreases genetic variability (Peredo *et al.* 2010). However, wild hop germplasm and genetic diversity provide a genetic reservoir that can be used for crop improvement and sustainability (Mafakheri *et al.* 2020; Rossini *et al.* 2021). Since the chemical properties of hops are influenced by environmental factors, molecular DNA methods have proven effective in evaluating population variability (Patzak *et al.* 2010). Paguet *et al.* (2023) and references therein showed that simple sequence repeat (SSR) fingerprinting provides a high level of accuracy and that the results are co-dominant and multi-allelic. Therefore, SSR markers have been successfully used to evaluate genetic variability among hop populations (Jakse *et al.* 2004; Murakami *et al.* 2006; Stajner *et al.* 2008; Patzak *et al.* 2010; Koelling *et al.* 2012; Rodolfi *et al.* 2018; Mafakheri *et al.* 2020; Grdiša *et al.* 2021; Calvi *et al.* 2023). Recently, chemical and genetic variability between populations of European wild hop have been reported; however, to the best of our knowledge, apart from a preliminary presentation on chemical composition of essential oils (Salihu *et al.* 2022), there has been no research on the chemical composition of essential oils or genetic variability of wild populations of hops in Kosovo using microsatellite (SSR) markers. This study aimed to address this gap by evaluating the Kosovo wild hop genetic and chemical variability using essential oil composition and SSR markers.

MATERIAL AND METHODS

Plant material

Female flowers (inflorescences) were collected from 21 wild hop populations in Kosovo between 2 and 19 October 2021 (Figure S1, Table S1). A total of 199 individuals were collected, with ten individuals per location, apart from populations from Deçan and Orllan, where nine individuals were collected, Dragash (six individuals), and Prizren (five individuals). The flowers of each plant were saved separately. Voucher specimens from each individual in each population were deposited at the Herbarium of the Department of Biology, University of Prishtina. After collection, the plant material was dried in drying cabinets at 35 °C for 3 days. At the same time as flower collection, fresh leaves were also collected from the same populations and stored in silica gel at room temperature before DNA extraction for genomic studies.

Extraction of essential oils

Essential oil was obtained via hydro-distillation of 30 g dried flowers and distilled water (0.5 l) in a 1-l flask. The distillation rate was 3 $ml \cdot min^{-1}$, and the process was carried out for 2 h in a Clevenger apparatus. Samples were then stored in darkness at −18 °C in a freezer until further analysis.

Analyses using GC-FID and GC-MS

The GC/FID analyses were conducted using an Agilent 7890A system equipped with an HP-5MS column. Helium was used as carrier gas at 0.6 $ml \cdot min^{-1}$ flow rate, and the front inlet was set at 250 °C. The split ratio was set at 50:1, and GC oven temperature was increased from 60 to 280 °C at a 5 $°C \cdot min^{-1}$. The FID was operated at 250 °C with a hydrogen flow rate of 35 $ml \cdot min^{-1}$ and airflow of 350 $ml \cdot min^{-1}$. GC-MS analysis was performed with an Agilent 7890A system coupled with a 5975C MSD. Ionisation energy was set at 70 eV, and mass range was 40–400 m/z. The injection volume was 1.0 µl. Essential oils were identified by comparing their Arithmetic Retention Indices with those reported in the literature (Adams 2017) and from 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 oil was calculated using a normalisation method without correction based on the GC peak areas.

Extraction and analysis of DNA

The DNA was extracted from fresh leaves using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA quality was checked by agarose gel electrophoresis on 1.0% agarose gels containing 0.4 × PeqGreen (VWR, Erlangen, Germany) for 40 min at 120 V, which was documented using the microDOC system with UV transilluminator (Cleaver Scientific, Rugby, UK) at 312 nm.

Microsatellite analysis

Fifteen microsatellites (SSR) were used in this study (Table S2). Before running PCR, the primer was multiplexed using five *H. lupulus* primer pairs. Multiplex PCR amplification was optimised for a 10-µl reaction volume containing 2–10 ng genomic DNA, 2 µl Hot FIREPol Multiplex Mix, double distilled water, and the respective concentrations of forward and reverse primers (Table S2). The cycling protocol used a Thermal Controller (SensoQuest LabCycler). Before the first cycle, a prolonged denaturation step (95 °C for 12 min) was performed, followed by 35 cycles at 95 °C for 20 s, 60 °C for 50 s, and 72 °C for 120 s. The last cycle was a 5-min extension at 72 °C.

Fragment analysis was carried out using a size standard GeneScan LIZ500 (Applied Biosystems) and analysed on an Applied Biosystems 3730XL DNA analyser with the following conditions: injection time 10 s; injection voltage 1.6 kV; run time 2100 s; run voltage 15 kV; capillary length 50 cm; polymer POP7 filter Dye Set G5.

Statistical analysis

For chemical data analysis we used Principal Components Analysis (PCA) to assess variations in chemical composition among the Kosovo hop populations. Analyses included the six main essential oil constituents highlighted in bold in Table S3.

PCA was conducted using XLSTAT software (New York, NY, USA; v. 2023.1.2).

For molecular analysis with SSR markers, we utilised Cervus v. 3.0.7 software to determine allele number (N), Polymorphic Information Content (PIC), Observed Heterozygosity (Ho), and Expected Heterozygosity (H). The Shannon Diversity Index (I), Fixation Index (F), Inbreeding Coefficient (F), and Principal Coordinates Analysis (PCoA) were performed using GenAlEx software v. 6.503. To evaluate genetic structure both among and within the populations, Structure 2.3.4 software was used (Pritchard *et al.* 2000), with a 100,000 burn-in period, followed by 1,000,000 Markov Chain Monte Carlo (MCMC) with 1 to 11 (K = 1–11) clusters adjusted and 10 runs. The results of each replication were used to determine the most likely number of groups (best K value) using HARVESTER v. 0.6.92 (Earl & vonHoldt 2012).

RESULTS AND DISCUSSION

Yield and chemical composition of the essential oils

Evaluating hops resources through analysis of essential oil yield and chemical composition in wild populations is of utmost importance as it provides valuable insights into potential uses and conservation, and because yield and chemical composition of essential oils influences flavour and fragrance of the hops. In the Kosovo populations studied, the highest yield of essential oil was in the population from Lipjan (0.2–0.9% v/w DW flowers). In contrast, flowers from the population at Hani i Elezit had the lowest yields (0.07–0.2% v/w DW). It is important to highlight that variability in essential oil yield was both within and among different populations, as highlighted in Table S3.

The total oil content in our samples was within the range observed for European wild hops (0.04–0.69%; Patzak *et al.* 2010), Italian wild hops (0.2–0.66; Rodolfi *et al.* 2022), including hops from central Italy (0.10–0.44%; Riccioni *et al.* 2021) and northern Italy (0.2–0.6%; Mongelli *et al.* 2015), and Bosnia and Herzegovina (0.2–0.4%; Kondić *et al.* 2021). However, in our sample, total essential oil content was generally below that of commercial germplasms (0.17–2.30%; Mongelli *et al.* 2015). In comparison to other cultivars and varieties, Kosovo hops generally yield less essential oil. For example, Saaz produces 0.3–0.9% v/w (Nesvadba & Charvátová 2020), Saaz Brilliant 0.3–0.8%, Saaz Comfort 0.4–1.1%, and Saaz Shine 0.5–1.3% (Mikyška *et al.* 2021). Additionally, our samples typically had lower essential oil yields compared to cultivars such as Mimosa (0.5–1.2%; Nesvadba & Charvátová 2020), Nugget (1.60 ± 0.07% v/w), Columbus (1.70 ± 0.07% v/w), Chinook (1.20 ± 0.07% v/w), Magnum (1.20 ± 0.07% v/w), Cascade (0.75 ± 0.07% v/w), and Fuggle (0.90 ± 0.07% v/w) (Paniagua-García *et al.* 2023), as well as Klon 18 (0.4–0.8%), National (0.8–1.2%), Alta (1.0–2.0%), Promin (1.2–2.5%), and Xantha (1.0–1.5%) (Protsenko *et al.* 2023).

Regarding chemical composition, wild hop essential oil from the Kosovo populations was dominated by myrcene, with levels from 22.48% (population from Suhareka) to 40.72% (Lipjan). The second most abundant compound in the oil was α-humulene, with levels from 1.14% (in Podujevë) to 18.79% (in Prizren), followed by (E)-β-farnesene, α-selinene, β-selinene, and E-caryophyllene. The concentrations of these constituents varied among populations, with (E)-β-farnesene from 0.34% (in Prizren) to 14.18% (in Drenas), α-selinene from 0.95% (in Viti) to 10.26% (in Gjilan), β-selinene from 1.21% (in Prishtinë) to 7.84% (in Gjilan), and E-caryophyllene from 2.75% (in Poslisht) to 6.99% (in Kamenice) (Table S3). According to McAdam *et al.* (2013) and references within, typically 90% of essential oils are terpenoids, dominated by myrcene, humulene, caryophyllene, and farnesene, which aligns with our observations. Similar to our findings, the main constituents of essential oils from different European wild hops were myrcene (8.7–42.5%), caryophyllene (4.2–45.2%), humulene (2.1–36.7%), farnesene (0–29.0%), and selinene (0.2–28.4%) (Patzak *et al.* 2010). The main essential oils identified in wild hops from Italy were myrcene (10.16–31.48%), farnesene (trace to 15.89%), α-selinene (1.14–20.13%), β-selinene (0.06–27.61%), humulene (0.54–34.75%), and caryophyllene (2.06–18.07%) (Rodolfi *et al.* 2022). The main constituents of essential oils in wild hops from Corsica were: caryophyllene (0.3–30.1%), humulene (0.1–29.1%), myrcene (trace–28.3%), farnesene (trace–26.6%), β-selinene (0.5–26.6%), α-selinene (trace–23.6%) and zingiberene (trace–22.1%) (Dabbous-Wach *et al.* 2023). In central Italy, the essential oil predominantly consisted of α-humulene (2.14–45.42%), β--farnesene (13.10–32.56%), myrcene (2.19–11.76%), β--caryophyllene (11.16–21.13%), α-selinene (0.68–35.56%), and β-selinene (1.19–34.90%) (Riccioni *et al.* 2021). In northern Italy, main constituents were myrcene (0–29.68%), α-humulene (1.05–46.91%), α-selinene (0.88–23.19%), β-selinene (0.61–30.27%), and β-caryophyllene (0.1–13.21%) (Mongelli *et al.* 2015). Moreover, α-humulene (1.1–22.7%), myrcene (0.4–18.9%), β-caryophyllene (2.2–10.6%), and farnesene (0.7–21.1%) were the main constituents of essential oils in hops from Bosnia and Herzegovina (Kondić *et al.* 2021). In Lithuania, essential oils were α-humulene (11.1–33.4%), myrcene (1.5–21.1%), elemene (up to 14%), caryophyllene (5.1–14.5%), (E)-β-farnesene (0–10.4%), γ-curcumene (0–15.8%), and β--bisabolol (0–13.5%) (Mockute *et al.* 2008). Most studies highlight variations in essential oil constituents across different populations. Nevertheless, the essential oils from Kosovo generally were little different from those of other European countries. Kosovo's wild hops share a similar myrcene content to Cascade (62.04 ± 1.33%), Chinook (41.89 ± 2.29%), and Comet (61.76 ± 2.25%) (Gresta *et al.* 2023). However, myrcene levels in the Kosovo populations are generally lower compared to Promin and Ruslan, where myrcene levels were 40% to 60% (Protsenko *et al.* 2023). The α-humulene levels in Kosovo wild hops are comparable to Magnum (14.29%) and Fuggle (25.74%) (Paniagua-García *et al.* 2023), and the Saaz varieties (14–39%) (Mikyška *et al.* 2021). The (E)-β-farnesene content in Kosovo wild hops was similar to Saaz (8–20%) and Saaz Brilliant (7–17%) cultivars (Nesvadba & Charvátová 2020; Mikyška *et al.* 2021). Comparative data also indicate that E-caryophyllene levels in Kosovo wild hops are comparable with Magnum (5.96%), Chinook (10.39%) (Paniagua-García *et al.* 2023), and Saaz (5–12%) (Nesvadba & Charvátová 2020).

Considering the main classes of essential oils, sesquiterpenes dominated our samples, with concentrations from 36.41% in Lipjan to 51.32% in Hani i Elezit, followed by monoterpenes, from 23.89% in Deçan to 42.58% in Lipjan, oxygenated sesquiterpenes between 9.05% in Orllan and 15.71% in Deçan, and oxygenated monoterpenes from 2.58% in Deçan to 4.36% in

an (Table S3). Other compounds labelled 'others' ranged m 5.19% in Gjilan to 12.45% in Deçan.

Principal components analysis of hops samples

A PCA was used to evaluate variability in chemical composition across the selected hop populations. Six primary constituents of the essential oils were selected: myrcene, E-caryophyllene, α-humulene, (E)-β-farnesene, β-selinene, and α-selinene (in bold in Table S3). The first two principal components accounted for 85.4% of the total variance. The first axis (PC1), representing 63.46% of total variance, indicated positive contributions from E-caryophyllene, α-humulene, (E)-β-farnesene, β-selinene, and α-selinene. In contrast, PC2 contributed 21.8% of total variance, mainly influenced by myrcene (Fig. 1). The scatter plot generated from the PCA results (Fig. 1) indicates that the samples were not differentiated based on geographic origin, suggesting low variability among populations. However, the plot revealed high variability among individuals within the same population (intra-population variability) as individuals of the same population were not grouped closely together. A similar grouping in PCA scatter plot of individuals within the same population was reported by Hajdari *et al.* (2023) based on xanthohumol, α-acids, β-acids, total phenolic content, total flavonoid content, and antioxidant activity (DPPH and FRAP).

Microsatellite diversity

Genetic variability among the 21 wild populations of *H. lupulus* in Kosovo was assessed using 15 SSR markers (loci) (Table 1). These markers were selected from a set of microsatellites identified by Stajner *et al.* (2005) and developed by (Jakše et al. 2002; Jakse *et al.* 2008) and Stajner *et al.* (2005). SSR selection criteria were based on quality of amplification products, locus complexity, polymorphic index content and distance in the gene map found in previous studies (Jakse *et al.* 2011). We also selected non-overlapping loci by size to facilitate multiplexing, ensuring that homogeneous annealing temperatures were considered. This approach allowed simultaneous analysis of various loci in multiplex PCR. SSR markers yielded 167 alleles, averaging 11.13 alleles per locus. The loci *GT1-K1-4* and *GA7-I6-16* showed the highest diversity, with 28 and 26 alleles, respectively. However, loci *ACA1-L4-3. Hl-GA9* and *GT4-F23-14* were monomorphic, with only one allele and hence were not useful for assessing genetic diversity among the hops (Table 1). These monomorphic loci were excluded from further analyses. Similar results on genetic diversity of wild populations were reported in Croatia (Grdiša *et al.* 2021), with 101 individuals analysed and 152 alleles identified across 12 loci, with an average of 12.67 alleles per locus. However, the more diverse loci *GT1-K1-4* and *GA7-I6-16* were less diverse in

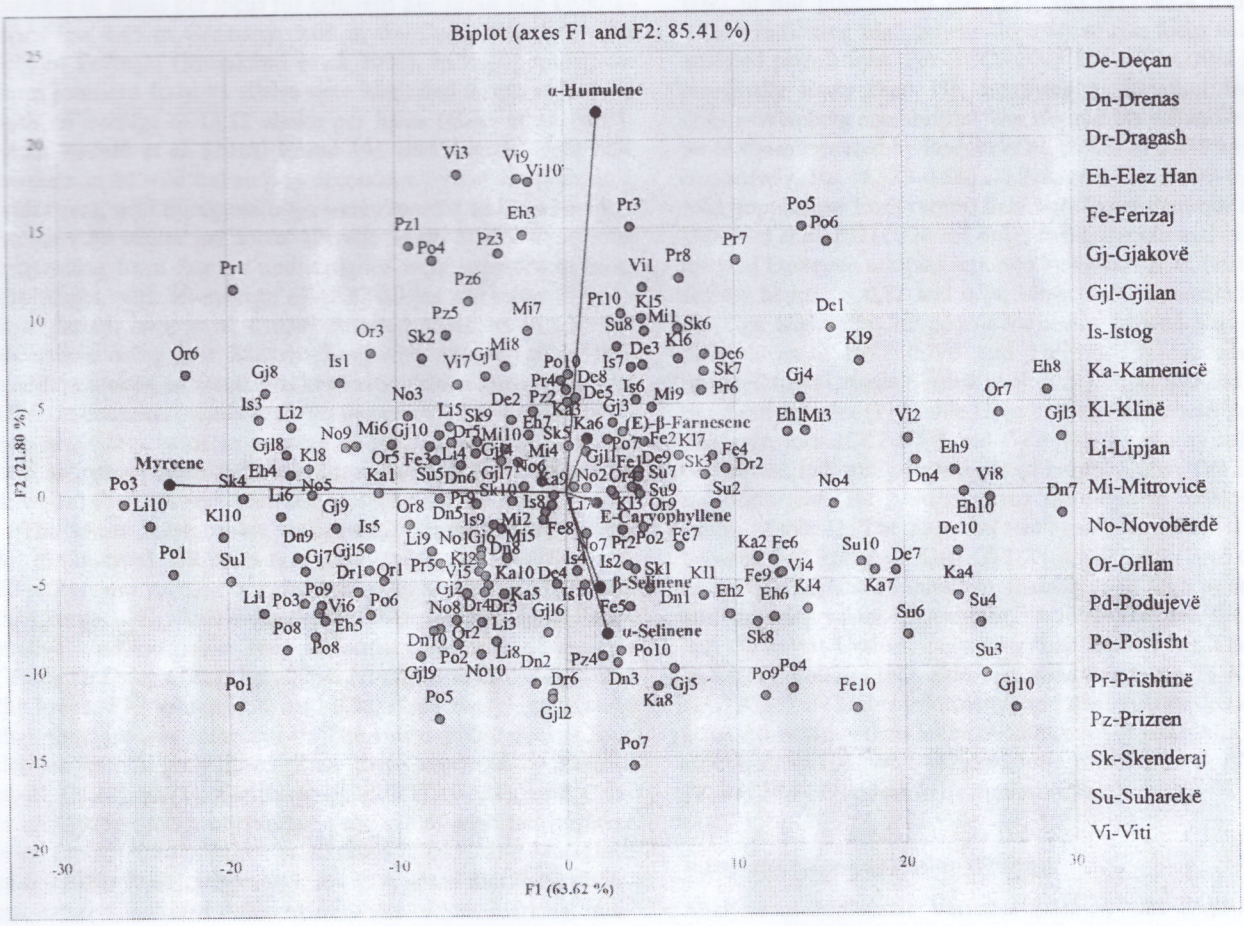

Fig. 1. Principal components analysis of oil constituents in hop populations.

Table 1. Informativeness of 15 SSR (simple sequence repeats) markers used in molecular analyses of 21 wild hop populations.

no.	locus	size of bp	N	PIC	Ho	He	I	F	FIS
1	*ACC2-D3-3*	199–208	13	0.84	0.01	0.86	1.48	0.99	0.99
2	*GA7-I6-16*	209–242	26	0.83	0.82	0.85	1.80	−0.04	−0.04
3	*GA7-L7-16*	146	9	0.36	0.38	0.38	0.69	−0.06	−0.06
4	*GA8-K15-4*	218–242	14	0.68	1.00	0.71	1.41	−0.47	−0.47
5	*GT1-A5-1*	164–174	16	0.83	1.00	0.85	1.82	−0.24	−0.24
6	*GT1-K1-4*	158–185	28	0.92	0.92	0.93	2.10	−0.06	−0.06
7	*GT2-O10-8*	160–175	9	0.66	0.01	0.69	1.09	0.99	0.99
8	*Hl-GT12*	200–207	11	0.72	0.86	0.75	1.44	−0.21	−0.21
9	*Hl-GT6*	119–140	2	0.28	0.31	0.33	0.46	−0.02	0.00
10	*Hl-AGA1*	251–288	14	0.84	0.85	0.86	1.76	−0.07	−0.07
11	*Hl-AGA3*	208–235	10	0.60	0.65	0.66	1.10	−0.05	−0.06
12	*Hl-AGA5*	209–228	12	0.80	0.76	0.83	1.61	−0.01	−0.01
13	*ACA1-L4-3*	163–164	1	n/a	n/a	n/a	n/a	n/a	n/a
14	*GT4-F23-14*	137–138	1	n/a	n/a	n/a	n/a	n/a	n/a
15	*Hl-GA9*	200–207	1	n/a	n/a	n/a	n/a	n/a	n/a
	Average		11.13	0.70	0.63	0.72	1.40	0.06	0.06

bp = base pair; N = Number of alleles; PIC = Polymorphic Information Content; Ho = Observed Heterozygosity; He = Expected Heterozygosity; I = Shannon's Information Index; F = Fixation index; FIS = Inbreeding Coefficient.

Croatian populations: *GA7-I6-16* had 14 alleles, while *GT1-K1-4* had 20 alleles (Grdiša *et al.* 2021), which could be related to the smaller population (101 individuals) analysed. The average number of alleles per locus for different European hop populations was 4.45 in Germany, 5.00 in the Czech Republic, and 3.18 in Portugal (Murakami *et al.* 2006). In hop populations from southern Italy, 81 alleles were identified across eight loci, with an average of 10.12 alleles per locus (Calvi *et al.* 2023), while Rodolfi *et al.* (2018) found 104 alleles across eight SSR markers in 80 wild Italian hop accessions. When considering a wider area, wild European hops were reported to have an average of 9.85 alleles per locus (Peredo *et al.* 2010), accessions originating from Europe and America were reported to have 116 alleles, with an average of 16.57 alleles per locus (Peredo *et al.* 2010). Jakse *et al.* (2004) detected 63 alleles in 124 hos accessions using four SSR markers, with an average of 15.7 alleles per locus, in accessions originating from Europe and the USA. In contrast, Stajner *et al.* (2008) reported 314 alleles in 67 hop accessions, with an average of 10.83 alleles per locus, in hop accessions from different European and Asian countries, as well as the USA and Australia, using 29 SSR markers.

The mean Polymorphic Information Content (PIC) value for the analysed SSR primers was 0.70, with the loci *GT1-K1-4*, *Hl-AGA1* and *ACC2-D3-3* displaying high values of 0.92 and 0.84, respectively. This indicates that these loci are highly informative and valuable for molecular population analysis (Table 1). On the other hand, loci *Hl-GT6* and *GA7-L7-16* had the lowest PIC values (0.28 and 0.36, respectively), suggesting that they are less informative. The mean PIC values in our dataset were slightly lower than those reported by Rodolfi *et al.* (2018) (0.71), Grdiša *et al.* (2021) (0.731), and Calvi *et al.* (2023) (0.79), but higher than values reported by Jakse *et al.* (2004) (0.64), Stajner *et al.* (2008) (0.61), and Mafakheri *et al.* (2020) (0.64); however, it must be noted that these different datasets included different numbers of SSR markers, individuals, and populations.

The average observed heterozygosity (Ho) in our populations was 0.63, which was higher for *GA8-K15-4*, *GT1-K1-4*, and *GT1-A5-1* than for other loci. The average expected heterozygosity (He) in our populations was 0.72; loci *GT1-K1-4* had higher values, indicating high genetic diversity of this locus within the analysed populations. For *ACC2-D3-3* and *GT2-O10-8* Ho was marginally lower than He, signifying a deviation from the Hardy–Weinberg equilibrium. The Ho and He values were similar to those reported by Rodolfi *et al.* (2018) at 0.646 and 0.718, respectively. Ho (0.522–0.656), 0.718, and He (0.505–0.654) in wild populations from central Italy were lower than our findings (Riccioni *et al.* 2021). On the other hand, the Ho and He values for wild European samples reported by Peredo *et al.* (2010) were slightly higher, at 0.72 and 0.74, respectively, compared to our findings. Slightly higher genetic variability in wild hop populations (average Ho = 0.770 and He = 0.759) has also been reported from Croatia (Grdiša *et al.* 2021). Most loci had a negative fixation index (F) (Table 1), indicating excess heterozygosity. However, loci *ACC2-D3-3* and *GT2-O10-8* had a positive fixation index, indicating prevalence of homozygotes. The inbreeding coefficient (Fis) values mirror the negative fixation index values (Table 1). The Shannon's information index (I) ranged between 0.46 and 2.10. Loci *GT1-K1-4*, *GT1-A5-1* and GA7-I6-16 had the highest I values, emphasising their high information and diversity values. In contrast, loci *Hl-GT6* and *GA7-L7-16* had the lowest I values, suggesting their limited use. The results in Table 1 indicate that, although some loci (i.e. *GT1-K1-4* and *GA7-I6-16*) are highly informative and demonstrate considerable genetic diversity, others are more conserved or less useful for specific analyses (i.e., *Hl-GT6*). In contrast, *ACA1-L4-3*, *GT4-F23-14*, and *Hl-GA9* appear to be monomorphic.

Intra- and inter-population diversity

Analysis of Molecular Variance (AMOVA) was conducted to assess genetic variability within and among populations. The

le 2. Analysis of molecular variation (AMOVA) within and between analyzed hop populations.

ource	df	SS	MS	Est. Var.	%	sH	%	exp (sH)
Among populations	20	131.15	6.56	0.10	2%	0.54	31.18	1.71
Among individuals	178	839.50	4.72	0.47	11%	1.18	68.82	3.26
Within individuals	199	753.00	3.78	3.79	87%	n/a	n/a	n/a

Df = Degrees Freedom; S = Sum of Squares; MS = Mean Square; Est. Var. = Estimated Variance; sH = Shannon Information; _% = Percentage of Total Information; exp(sH) = Diversity Estimate.

results revealed that 87% of genetic variation was within individual plants, only 11% was among individual plants within populations, and 2% among different populations (Table 2). On the other hand, the Shannon Information diversity statistics (Table 2) indicated that 31.2% of total genetic information or diversity was attributed to differences between populations, while 68.8% was found within individual populations. The Diversity Estimate (exp(sH)) indicated that diversity between populations was significantly higher (3.26) than within-population diversity (1.71). This suggests that most of the genetic diversity is found within populations and that each population has a rich genetic reservoir. These findings indicate that every plant possesses a distinct genetic makeup and has its own value for conservation and breeding programmes. Thus, preserving genetic diversity within populations must be considered to ensure long-term survival of the species.

Within-population diversity

The genetic variability of the 21 distinct hop populations was analysed using 12 allelic parameters (Table 3). The data revealed that the Gjakovë, Mitrovicë, Prishtinë, Hani i Elezit, Klinë, Ferizaj, and Podujevë populations had the highest genetic diversity, while populations from Dragash and Prizren had lower levels of genetic diversity (Table 3). The average number of alleles (Na) was 5.67, with the Dragash population having the lowest value (4.33), indicating a lower level of genetic diversity than in other populations. Conversely, Gjakovë, Mitrovicë and Prishtinë populations had the highest number of alleles (Table 3), suggesting that these populations possess a higher level of genetic diversity. The average effective number of alleles (Ne) was 3.75. Populations from Gjakovë (4.42) and Hani i Elezit (4.15) had the highest values, indicating relatively even allele distribution. Conversely, Dragash (3.14) had the lowest Ne values, suggesting a less balanced distribution of allele frequencies (Table 3). The Shannon's information index (I) was used to evaluate allelic diversity and richness within the populations. The overall average I was 1.34, with the Gjakovë and Mitorvicë populations having the highest values, suggesting these populations may possess the highest allelic diversity. In contrast, the Dragash and Istog populations had the lowest values, indicating lower allelic diversity (Table 3).

The number of private alleles was calculated to identify alleles unique to specific populations. The average value was 0.09, with the Suharekë and Mitrovicë populations being highest (0.25), suggesting unique genetic variation within this group. However, the overall private allele numbers were relatively low across all populations surveyed (Table 3). In contrast, large numbers of private alleles have been detected in populations in Croatia (Grdiša *et al.* 2021), northern Italy (Rodolfi *et al.* 2018), central Italy (Riccioni *et al.* 2021), Europe and North America (Jakse *et al.* 2004). Thus, private alleles can serve as tools for effective population discrimination (Peredo *et al.* 2005). The average expected heterozygosity (He) was 0.66, with Orllan, Novoberdë, Mitrovicë, and Lipajn populations having the highest values, indicating that they may possess highest genetic diversity among the groups surveyed. Nonetheless, the differences in He values between populations were small (Table 3).

Table 3. Genetic diversity metrics across populations in Kosovo.

population	Na	Ne	I	no. private alleles	He
Deçan	5.67	3.75	1.34	0.08	0.64
Dragash	4.33	3.14	1.16	0.08	0.59
Drenas	5.58	3.79	1.36	0.00	0.65
Ferizaj	6.17	3.95	1.40	0.00	0.64
Gjakovë	6.58	4.42	1.51	0.08	0.69
Gjilan	5.50	3.57	1.33	0.00	0.64
Han i Elez	6.08	4.15	1.44	0.00	0.67
Istog	5.67	3.36	1.28	0.17	0.61
Kamenicë	5.67	3.96	1.39	0.17	0.66
Klinë	6.08	3.78	1.41	0.08	0.66
Lipajn	5.92	4.12	1.46	0.00	0.70
Mitrovicë	6.33	4.17	1.50	0.25	0.70
Novoberdë	5.75	3.89	1.45	0.17	0.70
Orllan	5.67	3.94	1.46	0.08	0.71
Podujevë	6.25	4.22	1.47	0.17	0.67
Poslisht	5.83	4.07	1.42	0.17	0.67
Prishtinë	6.33	4.23	1.49	0.00	0.69
Prizren	4.67	3.60	1.33	0.00	0.67
Skenderaj	5.83	3.96	1.42	0.00	0.68
Suharekë	5.83	4.21	1.43	0.25	0.68
Viti	5.08	3.37	1.29	0.17	0.64
Average	5.67	3.75	1.34	0.09	0.64

Na = Number of Different Alleles; Ne = Number of Effective Alleles; I = Shannon's Information Index; No. Private Alleles = Number of Alleles Unique to a Single Population; He = Expected Heterozygosity.

Between population diversity

In addition to the AMOVA, which indicated 2% variability among populations, PCoA and genetic clustering based on Structure were employed to evaluate diversity among populations. In the PCoA, the first two principal axes accounted for 13.84% of total variance, with the first axis explaining 7.85%

and the second explaining 5.99%. Furthermore, genetic structure of the hop populations was resolved using Structure (Fig. S3), with the most likely number of groups (K) being K = 4 based on the estimated ΔK (Figure S2).

The PCoA biplot (Fig. 2) and Structure results (Figs S3 and S4) suggest that the samples did not separate according to geographic origin, indicating limited genetic variability between populations. However, there was large variability among individuals within the same population. These findings are consistent with results on variations in chemical compositions of essential oils, with a similar clustering of individuals from the same population (Fig. 1). Additionally, comparable clustering of individuals within the same population has been found based on xanthohumol, α-acids, β-acids, total phenolic content, total flavonoid content, and antioxidant activity (DPPH and FRAP) (Hajdari *et al.* 2023). Similarly, hops from Croatia exhibited low genetic differentiation among populations, with no evidence of geographic structuring across the analysed populations (Grdiša *et al.* 2021). In contrast to our findings, other authors have reported population differentiation and strong population structure. Thus, distinct hop gene pools have been reported in central Italy (Riccioni *et al.* 2021), the Calabrias in south Italy (Calvi *et al.* 2023) and northern Iran (Mafakheri *et al.* 2020). Furthermore, genetic differentiation has been reported for wild hop accessions from Canada, the Caucasus, Europe, and North America (Patzak *et al.* 2010), as well as North America and Euro-Asia (Murakami *et al.* 2006).

The relatively low genetic variability observed within hop populations can be attributed to the high level of gene flow between populations. As hop is anemophilous, it relies on wind for pollen distribution, which can result in widespread pollen dispersal and increased gene flow. Additionally, the low geographic isolation between study populations further facilitated exchange of genetic material, leading to more uniform genetic diversity among populations.

CONCLUSION

This study provides a comprehensive analysis of genetic and chemical variability of wild hop populations from Kosovo. The essential oils extracted from these populations contained myrcene, α-humulene, (E)-β-farnesene, α-selinene, β-selinene, and E-caryophyllene as main chemical constituents. There was limited variation in both essential oil yield and its chemical composition among populations; however, more variation was recorded within a population. In addition to chemical analysis, genetic analysis using SSR markers showed high genetic

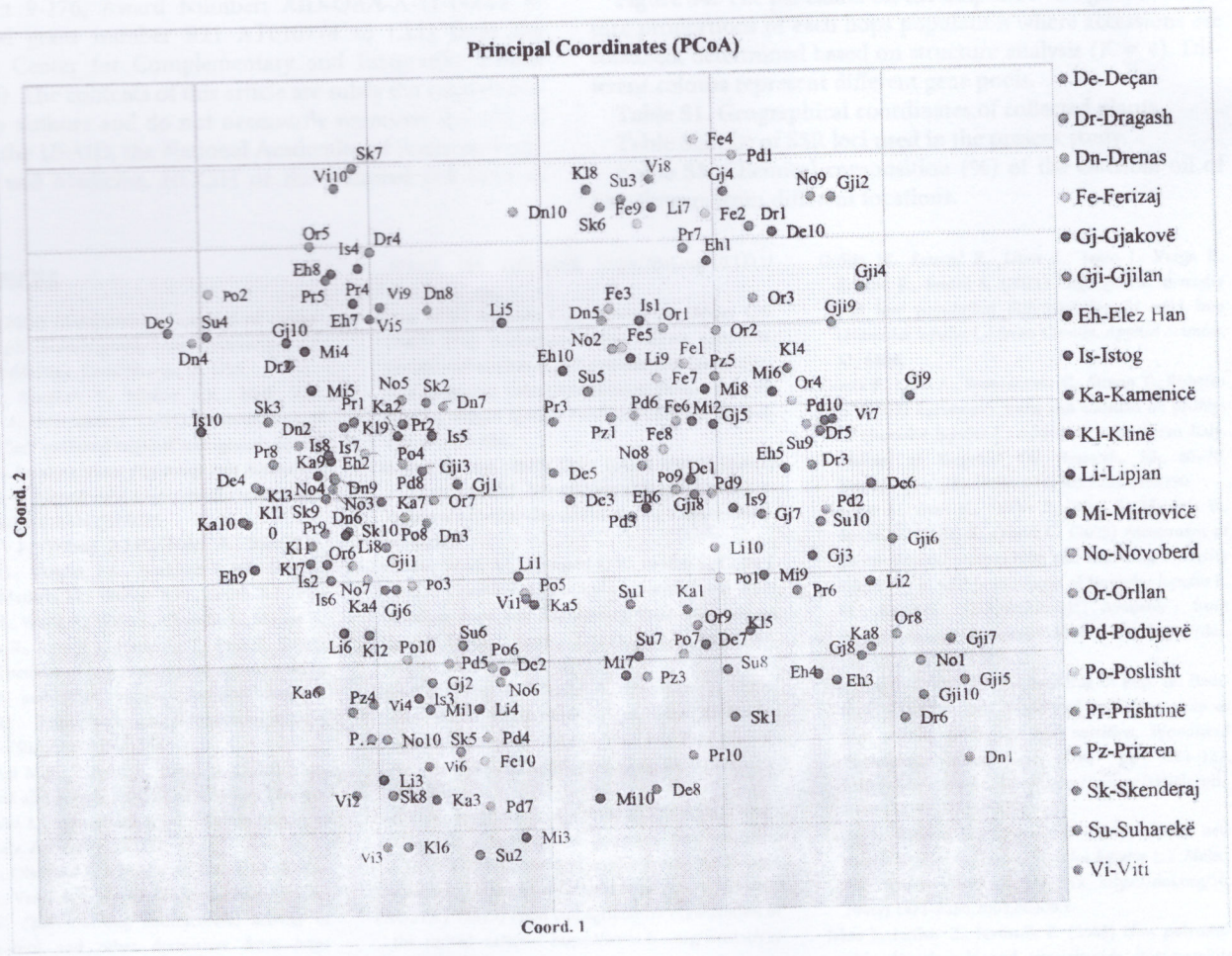

Fig. 2. The two-dimensional plot of Principal Coordinates Analysis (PCoA) based on Fst genetic distance.

ersity within wild hop populations and low genetic variabil- among populations. This is consistent with the findings on hemical composition analysis and underscores the need for conservation efforts that prioritise maintaining genetic diversity within populations. Based on analysis of genetic makeup and chemical constituents, this study emphasises the potential of Kosovan hop germplasm for conservation and utilisation. These findings provide valuable insights for developing strategies for potential hop use, conservation, and breeding programmes to improve quality of the hop crop.

AUTHOR CONTRIBUTIONS

BS carried out chemical analyses of essential oils and DNA analysis, as well as contributing to data processing and writing the manuscript; TS helped select SSR markers, reviewed molecular results and revised the manuscript; BP extracted the DNA; CLQ reviewed the results and revised the manuscript; BM helped design the research and revised the manuscript; AH contributed to design and implementation of the study, analysed results, writing, and supervising the project.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests or personal relationships that could have influenced the work reported here.

DATA AVAILABILITY STATEMENT

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

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Map of the locations where hop plant material was collected.

Figure S2. Relationship between the number of determined groups (K) and estimated ΔK.

Figure S3. STRUCTURE-based genetic clustering (K = 4) of 199 hop individuals grouped according to population (21 population), constructed using 15 SSR marker data. Each vertical bar represents an individual within the population, divided into four segments, coloured proportionally according to genetic group. Different colours represent different gene pools.

Figure S4. The pie charts on the map show the genetic structure proportions of each hops population where accessions ere collected, determined based on structure analysis (K = 4). Different colours represent different gene pools.

Table S1. Geographical coordinates of collected plants.

Table S2. List of SSR loci used in the present study.

Table S3. Chemical composition (%) of the essential oil of hop flowers from different locations.

REFERENCES

Adams R. (2017) *Identification of essential oil components by gas chromatography/mass spectroscopy, 4.1.* Allured Publishing, Carol Stream, IL, USA.

Awasthi P., Kocábek T., Mishra A.K., Nath V.S., Shrestha A., Matoušek J. (2021) Establishment of CRISPR/Cas9 mediated targeted mutagenesis in hop (*Humulus lupulus*). *Plant Physiology and Biochemistry* **160**:1–7. https://www.sciencedirect.com/science/article/pii/S0981942821000085

Buckwold V.E., Wilson R.J.H., Nalca A., Beer B.B., Voss T.G., Turpin J.A., Buckheit R.W., Wei J., Wenzel-Mathers M., Walton E.M., Smith R.J., Pallansch M., Ward P., Wells J., Chuvala L., Sloane S., Paulman R., Russell J., Hartman T., Ptak R. (2004) Antiviral activity of hop constituents against a series of DNA and RNA viruses. *Antiviral Research* **61**:57–62. https://www.sciencedirect.com/science/article/pii/S0166354203001554

Calvi A., Aci M.M., Lupini A., Preiti G. (2023) Morphological and genetic analysis of wild hop (*Humulus lupulus* L.) germplasm from Calabria region in South Italy. *Agronomy*, **13**, 352.

Cermak P., Olsovska J., Mikyska A., Dusek M., Kadleckova Z., Vanicek J., Nyc O., Sigler K., Bostikova V., Bostik P. (2017) Strong antimicrobial activity of xanthohumol and other derivatives from hops (*Humulus lupulus* L.) on gut anaerobic bacteria. *APMIS*, **125**, 1033–1038. https://doi.org/10.1111/apm.12747

Chung W.G., Miranda C.L., Stevens J.F., Maier C.S. (2009) Hop proanthocyanidins induce apoptosis, protein carbonylation, and cytoskeleton disorganization in human colorectal adenocarcinoma cells via reactive oxygen species. *Food and Chemical Toxicology*, **47**, 827–836.

Customs Kosovo (2023) Open DATA Import January – December 2022. https://dogana.rks-gov.net/en/per-doganen/statistikat-dhe-arritjet/trading-balance-based-on-tariffs/

Dabbous-Wach A., Lorenzetti J.-V., Paolini J., Costa J. (2023) Chemical variability of essential oils from corsican hops and highlighting their influence on hops' Aroma. *Foods*, **12**(13), 2613. https://doi.org/10.3390/foods12132613

Dresel M., Vogt C., Dunkel A., Hofmann T. (2016) The bitter Chemodiversity of hops (*Humulus lupulus* L.). *Journal of Agricultural and Food Chemistry*, **64**, 7789–7799. https://doi.org/10.1021/acs.jafc.6b03933

Earl D.A., vonHoldt B.M. (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, **4**, 359–361.

FAOSTAT (2023) Food and agriculture organization of the united nations. https://www.fao.org/faostat/en/#data/TCL

Grdiša M., Šatović Z., Liber Z., Jakše J., Varga F., Erhatić R., Srečec S. (2021) High genetic diversity and low population differentiation in wild hop (*Humulus lupulus* L.) from Croatia. *Applied Sciences*, **11**, 6484.

Gresta F., Calvi A., Santonoceto C., Strano T., Ruberto G. (2023) Agronomic traits and essential oil profiles of *Humulus lupulus* L. cultivated in southern Italy. *Journal of Essential Oil Research*, **35**, 60–70. https://doi.org/10.1080/10412905.2022.2103190

Hajdari A., Gash K., Salihu B., Woo S., Mustafa B., Spahiu A., Pulaj B., Quave C. (2023) Assessment of phytochemical composition and antioxidant activity variability in wild populations of *Humulus lupulus* L. in Kosovo. (Unpublished). Available from https://ssrn.com/abstract=4589167 or https://doi.org/10.2139/ssrn.4589167

Henning J. (2006) 5 – the breeding of hop. In: Bamforth CWBT-B (ed) *Woodhead publishing series in food science, technology and nutrition.* Woodhead Publishing, Cambridge, UK, pp 102–122. https://www.sciencedirect.com/science/article/pii/B9781845690038500058

Jakše J., Bandelj D., Javornik B. (2002) Eleven new microsatellites for hop (*Humulus lupulus* L.). *Molecular Ecology Notes*, **2**, 544–546. https://doi.org/10.1046/j.1471-8286.2002.00309.x

Jakse J., Luthar Z., Javornik B. (2008) New polymorphic dinucleotide and trinucleotide microsatellite

loci for hop *Humulus lupulus* L. *Molecular Ecology Resources*, **8**, 769–772. https://doi.org/10.1111/j.1755-0998.2007.02053.x

Jakse J., Satovic Z., Javornik B. (2004) Microsatellite variability among wild and cultivated hops (*Humulus lupulus* L.). *Genome*, **47**, 889–899. https://doi.org/10.1139/g04-054

Jakse J., Stajner N., Luthar Z., Jeltsch J.-M., Javornik B. (2011) Development of transcript-associated microsatellite markers for diversity and linkage mapping studies in hop (*Humulus lupulus* L.). *Molecular Breeding*, **28**, 227–239. https://doi.org/10.1007/s11032-010-9476-3

Karlsson Strese E.-M., Lundström M., Hagenblad J., Leino M.W. (2014) Genetic diversity in remnant Swedish hop (*Humulus lupulus* L.) yards from the 15th to 18th century. *Economic Botany*, **68**, 231–245. https://doi.org/10.1007/s12231-014-9273-8

Koelling J., Coles M.C., Matthews P.D., Schwekendiek A. (2012) Development of new microsatellite markers (SSRs) for *Humulus lupulus*. *Molecular Breeding*, **30**, 479–484. https://doi.org/10.1007/s11032-011-9637-z

Kondić D., Čerenak A., Košir I.J., Ocvirk M., Krmpot T., Đurić G. (2021) Morphological and biochemical characterization of wild hop (*Humulus lupulus* L.) populations from Banja Luka area (Bosnia and Herzegovina). *Agronomy* **11**:239. https://www.mdpi.com/2073-4395/11/2/239 (accessed 1 July 2023).

Korpelainen H., Pietiläinen M. (2021) Hop (*Humulus lupulus* L.): traditional and present use, and future potential. *Economic Botany*, **75**, 302–322. https://doi.org/10.1007/s12231-021-09528-1

Ligor M., Stankevičius M., Wenda-Piesik A., Obelevičius K., Ragažinskienė O., Stanius Ž., Maruška A., Buszewski B. (2014) Comparative gas chromatographic–mass spectrometric evaluation of hop (*Humulus lupulus* L.) essential oils and extracts obtained using different sample preparation methods. *Food Analytical Methods*, **7**, 1433–1442. https://doi.org/10.1007/s12161-013-9767-5

Mafakheri M., Kordrostami M., Rahimi M., Matthews P.D. (2020) Evaluating genetic diversity and structure of a wild hop (*Humulus lupulus* L.) germplasm using morphological and molecular characteristics. *Euphytica*, **216**, 58.

Maliar T., Nemeček P., Ürgeová E., Maliarová M., Nesvadba V., Krofta K., Vulganová K., Krošlák E., Kraic J. (2017) Secondary metabolites, antioxidant and anti-proteinase activities of methanolic extracts from cones of hop (*Humulus lupulus* L.) cultivars. *Chemical Papers*, **71**, 41–48. https://doi.org/10.1007/s11696-016-0034-2

McAdam E.L., Freeman J.S., Whittock S.P., Buck E.J., Jakse J., Cerenak A., Javornik B., Kilian A., Wang C.-H., Andersen D., Vaillancourt R.E., Carling J., Beatson R., Graham L., Graham D., Darby P., Koutoulis A. (2013) Quantitative trait loci in hop (*Humulus lupulus* L.) reveal complex genetic architecture underlying variation in sex, yield and cone chemistry. *BMC Genomics*, **14**, 360.

Metaj L., Hajdini D., Gliha K., Košir I.J., Ocvirk M., Kolar M., Cerar J. (2023) Extraction of polyphenols from Slovenian hop (*Humulus lupulus* L.) aurora variety using deep eutectic solvents: choice of the extraction method vs. structure of the solvent. *Plants*, **12**, 2890.

Mikyška A., Štěrba K., Slabý M., Nesvadba V., Charvátová J. (2021) Brewing tests of new fine aroma hop varieties (*Humulus lupulus* L.) Saaz Brilliant, Saaz comfort and Saaz Shine. *KVASNY PRUMYSL* **67**:464–473. https://kvasnyprumysl.eu/index.php/kp/article/view/247

Mockute D., Bernotiene G., Nivinskiene O., Butkiene R. (2008) Variability of volatiles of wild hops (*Humulus lupulus* L.) growing in eastern Lithuania. *Journal of Essential Oil Research*, **20**, 96–101. https://doi.org/10.1080/10412905.2008.9699963

Mongelli A., Rodolfi M., Ganino T., Marieschi M., Dall'Asta C., Bruni R. (2015) Italian hop germplasm: characterization of wild *Humulus lupulus* L. genotypes from northern Italy by means of phytochemical, morphological traits and multivariate data analysis. *Industrial Crops and Products* **70**:16–27. https://www.sciencedirect.com/science/article/pii/S0926669015001314

Murakami A., Darby P., Javornik B., Pais M.S.S., Seigner E., Lutz A., Svoboda P. (2006) Microsatellite DNA analysis of wild hops, *Humulus lupulus* L. *Genetic Resources and Crop Evolution*, **53**, 1553–1562. https://doi.org/10.1007/s10722-005-7765-1

Mustafa B., Hajdari A. (2014) Medical ethnobotanical studies in Kosovo, *Ethnobotany and biocultural diversities in the Balkans: Perspectives on sustainable rural development and reconciliation*. Springer, New York, USA. https://doi.org/10.1007/978-1-4939-1492-0_7

Mustafa B., Hajdari A., Krasniqi F., Hoxha E., Ademi H., Quave C.L., Pieroni A. (2012) Medical ethnobotany of the Albanian Alps in Kosovo. *Journal of Ethnobiology and Ethnomedicine*, **8**, 6.

Nesvadba V., Charvátová J. (2020) New fine aroma varieties of hops (*Humulus lupulus* L.) Saaz Brilliant, Saaz Comfort, Saaz Shine and Mimosa. *KVASNY PRUMYSL* **66**:320–330. https://www.kvasnyprumysl.eu/index.php/kp/article/view/224

Paguet A.-S., Siah A., Lefèvre G., Moureu S., Cadalen T., Samaillie J., Michels F., Deracinois B., Flahaut C., Alves Dos Santos H., Etienne-Debaecker A., Rambaud C., Chollet S., Molinié R., Fontaine J.-X., Waterlot C., Fauconnier M.-L., Sahpaz S., Rivière C. (2023) Multivariate analysis of chemical and genetic diversity of wild *Humulus lupulus* L. (hop) collected in situ in northern France. *Phytochemistry* **205**:113508. https://www.sciencedirect.com/science/article/pii/S0031942222004241

Paniagua-García A.I., Ruano-Rosa D., Díez-Antolínez R. (2023) Fractionation of high-value compounds from hops using an optimised sequential extraction procedure. *Antioxidants (Basel)*, **13**, 45.

Patzak J., Nesvadba V., Henychová A., Krofta K. (2010) Assessment of the genetic diversity of wild hops (*Humulus lupulus* L.) in Europe using chemical and molecular analyses. *Biochemical Systematics and Ecology* **38**:136–145. https://www.sciencedirect.com/science/article/pii/S0305197809001938

Peredo E.L., Ángeles Revilla M., Reed B.M., Javornik B., Cires E., Prieto J.A.F., Arroyo-García R. (2010) The influence of European and American wild germplasm in hop (*Humulus lupulus* L.) cultivars. *Genetic Resources and Crop Evolution*, 57, 575–586. https://doi.org/10.1007/s10722-009-9495-2

Peredo E.L., Arroyo-García R., Martínez-Zapater J.M., Revilla M.Á. (2005) Evaluation of microsatellite detection using autoradiography and capillary electrophoresis in hops. *Journal of the American Society of Brewing Chemists*, **63**, 57–62.

Piasecki B., Biernasiuk A., Ludwiczuk A. (2023) Anticoccal activity and composition of the essential oils and methanolic extracts obtained from brewing quality *Humulus lupulus* L. hop pellets. *Pharmaceuticals*, **16**, 1098.

Pritchard J.K., Stephens M., Donnelly P. (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.

Protsenko L., Janse L., Koshytska N., Ryzhuk S., Liashenko M., Bober A., Sergiy G. (2023) Variability and stability of essential oil composition in Ukrainian hop varieties. *KVASNY PRUMYSL* **69**:811–821. https://www.kvasnyprumysl.eu/index.php/kp/article/view/298

Riccioni C., Belfiori B., Sileoni V., Marconi O., Perretti G., Bellucci M., Rubini A. (2021) High genetic and chemical diversity of wild hop populations from Central Italy with signals of a genetic structure influenced by both sexual and asexual reproduction. *Plant Science*, **304**, 110794.

Rodolfi M., Marieschi M., Chiancone B., Ganino T. (2022) Assessment of the genetic and phytochemical variability of Italian wild hop: a route to biodiversity preservation. *Applied Sciences*, **12**, 5751.

Rodolfi M., Silvanini A., Chiancone B., Marieschi M., Fabbri A., Bruni R., Ganino T. (2018) Identification and genetic structure of wild Italian *Humulus lupulus* L. and comparison with European and American hop cultivars using nuclear microsatellite markers. *Genetic Resources and Crop Evolution*, **65**, 1405–1422. https://doi.org/10.1007/s10722-018-0622-9

Rossini F., Virga G., Loreti P., Iacuzzi N., Ruggeri R., Provenzano M.E. (2021) Hops (*Humulus lupulus* L.) as a novel multipurpose crop for the Mediterranean region of Europe: challenges and opportunities of their cultivation. *Agriculture*, **11**, 484.

Salanta L.-C., Tofana M., Socaci S., Mudura E., Farcas A., Pop C., Pop A., Odagiu A. (2015) Characterisation of hop varieties grown in Romania based on their contents of bitter acids by HPLC in combination with chemometrics approach. *Czech Journal of Food Sciences* **33**:148–155. https://cjfs.agriculturejournals.cz/artkey/cjf-201502-0008.php

Salihu B., Mustafa B., Pulaj B., Hajdari A. (2022) Chemical composition of the essential oil of hops (*Humulus lupulus* L.) growing wild in Kosovo. *Macedonian Pharmaceutical Bulletin*, **68**, 67–68.

Sefidgar S.A.A., Taghizadeh Armaki M., Pournajaf A., Ardebili A., Omidi S., Abdian Asl A. (2015) Evaluation of antimicrobial activity of alcoholic and aqueous extracts from common hop (*Humulus lupulus*) and oak (*Quercus castaneifolia*). *Journal of Arak University of Medical Sciences* **17**:39–46. http://jams.arakmu.ac.ir/article-1-3208-en.html

Stajner N., Jakse J., Kozjak P., Javornik B. (2005) The isolation and characterisation of microsatellites in hop (*Humulus lupulus* L.). *Plant Science* **168**:213–221. https://www.sciencedirect.com/science/article/pii/S0168945204003681

Stajner N., Satovic Z., Cerenak A., Javornik B. (2008) Genetic structure and differentiation in hop (*Humulus lupulus* L.) as inferred from microsatellites. *Euphytica*, **161**, 301–311. https://doi.org/10.1007/s10681-007-9429-z

Stewart G.G. (2016) Beer shelf life and stability. In: *the stability and shelf life of Food*, Subramaniam P. (ed), 2nd ed., pp 293–309 Elsevier, Amsterdam, Netherlands. https://www.sciencedirect.com/science/article/pii/B9780081004357000101

Zanoli P., Zavatti M. (2008) Pharmacognostic and pharmacological profile of *Humulus lupulus* L. *Journal of Ethnopharmacology* **116**:383–396. https://www.sciencedirect.com/science/article/pii/S0378874108000391

UNIVERSITETI I PRISHTINËS

FAKULTETI I SHKENCAVE MATMATIKE-NATYRORE

DREJTIMI: BIOLOGJI E ORGANIZMAVE DHE EKOLOGJI

Msc. Blerta Salihu

Vlerësimi i variabilitetit brendallojor të sumbullarit të egër (*Humulus lupulus* L.) në Kosovë bazuar në analizat kimike dhe molekulare

PUNIMI I DOKTORATËS

Prishtinë, 2025

UNIVERSITY OF PRISHTINA

FACULTY OF MATHEMATICS AND NATURAL SCIENCES

FIELD: BIOLOGY OF ORGANISMS AND ECOLOGY

Msc. Blerta Salihu

Evaluation of Intraspecific Variability of Wild Hop (*Humulus Lupulus* L.) in Kosovo Based on Chemical and Molecular Analyses

DOCTORAL THESIS

Prishtina, 2025

UNIVERSITETI I PRISHTINËS

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PUNIMI I DOKTORATËS

Mentori: Prof. Avni Hajdari

Prishtinë, 2025

REZYME

Kosova karakterizohet nga një diversitet i lartë i botës bimore, ku ndër llojet e shumta të pranishme gjendet edhe sumbullari i egër (*Humulus lupulus* L.). Ky lloj bimor ka rëndësi të madhe ekonomike për shkak të metabolitëve sekondarë që posedon dhe përdorimit të gjerë që ka në industrinë farmaceutike, ushqimore dhe atë të birrës. Megjithëse ka rëndësi ekonomike, mjekësore dhe është i përhapur gjerësisht në Kosovë, të dhënat për përbërjen kimike të vajrave esenciale, diversitetit gjenetik dhe aktivitetit antioksidativ të popullatave të egra të këtij lloji në Kosovë ende mungojnë. Ky studim ka për qëllim të analizojë variabilitetin kimik, gjenetik dhe aktivitetin antioksidativ të sumbullarit të egër në Kosovë. Lulesat femërore (konet) të sumbullarit u mblodhën nga 21 popullata të egra në zona të ndryshme të Kosovës. Vajrat esencialë u ekstraktuan nga lulesat e thara, me metodën e hidrodistilimit duke përdorur aparatin Clevenger dhe u analizuan me GC-FID-MS. ADN-ja u ekstraktua nga gjethet e reja dhe të thara, dhe për analizën e variabilitetit gjenetik u përdorën 15 SSR markues. Aktiviteti antioksidativ u përcaktua me metodën spektrofotometrike nga ekstraktet metanolike të lulesave të sumbullarit. Rezultatet treguan se sasia e vajrave esenciale në lulesat e sumbullarit varionte nga 0.07% deri në 0.9%. Përbërësit kryesorë të identifikuar ishin: mirceni (22.5% - 40.7%), α-humuleni (1.1% - 18.8%), (E) -β-farneseni (0.3% - 14.2%), α-selineni (1.0% - 10.3%), β-selineni (1.2% - 7.8%) dhe E-kariofileni (2.8% - 7.0%). Analizat gjenetike treguan praninë e 167 aleleve në popullatat e analizuara, me një mesatare prej 11.13 alelesh për lokus, duke evidentuar një diversitet të lartë gjenetik brenda popullatave dhe një diversitet të ulët gjenetik ndërmjet popullatave të sumbullarit të egër. Përmbajtja e fenoleve totale varionte nga 31.4 deri në 54.2 mg CAE/100g, ndërsa ajo e flavonoideve nga 3.1 deri në 6.5 mg CE/100g. Vlerat e aktivitetit antioksidativ të përcaktuara me metodën FRAP varionin nga 7.4 deri në 13.1 mg TE/100g, ndërsa ato të përcaktuara me analizën DPPH nga 25.1 deri në 55.3 mg TE/100g. Rezultatet e këtij hulumtimi ofrojnë njohuri të rëndësishme, të cilat mund të përdoren për zhvillimin e strategjive për shfrytëzimin e qëndrueshëm dhe ruajtjen e popullatave të egra të sumbullarit në Kosovë. Përveç kësaj, këto të dhëna paraqesin një bazë të rëndësishme për hulumtime të mëtejshme, duke mundësuar vlerësimin e potencialit gjenetik të popullatave të egra në krahasim me kultivarët komercialë, çka do të krijonte mundësi për zhvillimin dhe përmirësimin e kultivarëve të rinj të sumbullarit, me ndikim të mundshëm në zhvillimin socio-ekonomik të Kosovës.

Fjalët kyçe: *Humulus lupulus, analiza kimike, analiza molekulare, aktivitet antioksidativ*

ABSTRACT

Kosovo is characterised by high plant diversity, including wild hop (*Humulus lupulus* L.). This species is economically important due to its diverse secondary metabolites and extensive use in the pharmaceutical, food, and brewing industries. Despite its importance and widely distribution in Kosovo, there is limited data on the chemical composition of its essential oils, genetic diversity, and antioxidant activity of wild hop populations in the region. This study aims to analyze the chemical variability, genetic diversity, and antioxidant activity of wild hop in Kosovo. Female inflorescences (cones) were collected from 21 wild populations across different regions of Kosovo. Essential oils were extracted from the dried inflorescences using the hydro distillation method with a Clevenger apparatus, and the samples were analyzed by GC-FID-MS. DNA was extracted from young and dried leaves, and genetic variability was assessed using 15 SSR markers. Antioxidant activity was determined spectrophotometrically from methanolic extracts of the hop inflorescences. The results indicated that the essential oil content in the hop inflorescences ranged from 0.07% to 0.9%. The main identified components included myrcene (22.5% – 40.7%), α-humulene (1.1% – 18.8%), (E) -β-farnesene (0.3% – 14.2%), α-selinene (1.0% – 10.3%), β-selinene (1.2% – 7.8%), and E-caryophyllene (2.8% – 7.0%). Genetic analyses revealed a total of 167 alleles in the analyzed populations, with an average of 11.13 alleles per locus, signifying high genetic diversity within populations but low genetic diversity between different wild hop populations. The total phenolic content ranged from 31.4 to 54.2 mg CAE/100g, while the flavonoid content varied between 3.1 and 6.5 mg CE/100g. Antioxidant activity measured using the FRAP method ranged from 7.4 to 13.1 mg TE/100g, and those determined using the DPPH assay ranged from 25.1 to 55.3 mg TE/100g. The findings of this study provide valuable insights for developing strategies for the sustainable use and conservation of wild hop populations in Kosovo. Additionally, these findings provide valuable insights for developing strategies for potential use and conservation of wild hop populations in Kosovo, laying the groundwork for future research and comparison with commercial cultivars to assess their breeding potential, which could facilitate developing and improving new hop cultivars, contributing to Kosovo's socio-economic development.

Key words: *Humulus lupulus, chemical analysis, molecular analysis, antioxidant activity*

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Total Phenolic, Total Flavonoid and Antioxidant Activity of *Humulus lupulus* L. from wild populations in Kosovo

Blerta Salihu, Behxhet Mustafa, Bledar Pulaj, Avni Hajdari

Department of Biology, Faculty of Mathematical and Natural Science, University of Prishtina "Hasan Prishtina". Mother Theresa St. 10000 Prishtinë, Kosovo.
Email: blerta.salihu8@student.uni-pr.edu

Abstract

Hop (*Humulus lupulus* L., fam. Cannabaceae) is an economically important plant species used in traditional and modern medicine and in food industry. It has diverse secondary plant metabolites, including phenolic and flavonoid compounds, demonstrating different biological activities. To assess the natural variation of the total flavonoids, total phenolics and antioxidant activity among wild populations, the female flowers were collected from 21 wild populations in Kosovo. Plant material was dried, and then 150 mg of the grounded female flowers were extracted with 25 ml of 50% MeOH for 30 min. in an ultrasonic bath. Total phenolics, flavonoids, and antioxidant activities (DPPH and FRAP) were determined using spectrophotometric methods.

The total phenolic ($\bar{X}$ and SD) ranged from 31.40 ± 7.04 to 54.15 ± 9.76 mg CAE/100g. The lowest content was recorded in the Deçan locality, while the highest was in Suhareka. Total flavonoid content ranged from 3.10 ± 0.86 (Deçan) to 6.49 ± 1.47 mg CE/100g (Kamenica). According to the FRAP assay, the lowest antioxidant activity was recorded in Deçan (7.36 ± 1.45 mg TE/100g), while the highest was in Kamenica (13.05 ± 3.28). The lowest antioxidant activity was recorded in Deçan (25.13 ± 5.98 mg TE/100g) according to the DPPH assay test, too, while the highest activity was in Lipjan (55.25 ± 14.15). One-way analysis of variance (ANOVA) shows no significant differences in total phenolics, total flavonoids and antioxidant activities (DPPH and FRAP) among the wild population. The Pearson correlation coefficient between total phenolic and total flavonoids and both antioxidant tests shows a significantly positive correlation ($p < 0.01$).

Further investigations based on molecular analysis are needed to assess the natural variability among wild populations of this species in Kosovo. Moreover, profiling and screening of the phenolic and flavonoid compounds are required to evaluate the correlation between specific chemical constituents with their antioxidant activity.

Key words: *Hop, antioxidants activity, phenolic, flavonoids.*

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Short communication

Chemical composition of the essential oil of hops (*Humulus lupulus* L.) growing wild in Kosovo

Blerta Salihu*[1], Bexhet Mustafa[1], Bledar Pulaj[1], Avni Hajdari[1]

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

Introduction

Humulus lupulus L. (Cannabaceae) is an economically important plant species used in the pharmaceutical and food industries. In Kosovo, it is widespread in rich deep loam soil. It is a dioecious plant (with male and female reproductive organs in separate individuals) and pollinated by wind. Fragrant female hops flowers, also known as cones, are primarily used for commercial purposes, making this species of economic interest. Female flowers contain glandular trichomes which accumulate secondary metabolites, such: as the bitter acids, e.g. humulone (α-acid) and lupulone (β-acid), and terpenes (essential oils), e.g. myrcene and linalool, β-caryophyllene, humulene, linalool, and α-pinene, etc. (Astray et al., 2020).

Due to the rich content of secondary plant metabolites, hops were traditionally used in herbal medicines as a sedative, to relieve gastrointestinal complaints, pain, sleep aid, fatigue, rheumatism, etc., and as food ingredients. In Kosovan traditional medicine, the infusion was prepared from female flowers and used as a neuro-relaxant and to treat kidney inflammations, while decoctions from areal parts were used to treat insomnia and regulate the menstrual cycle (Mustafa et al., 2014). Recent research reports shows that hop has demonstrated to exhibit various pharmacological activities such as anti-inflammatory, estrogenic, cancer chemopreventive, and anti-angiogenic properties, as well as helping to counteract obesity, diabetes type-2, and cancer (Chung et al., 2009; Natsume et al., 2015).

Compared to medicinal uses, hop has more extensive use in the beer industry. Its phytochemicals, except flavours and fragrances, provide foam stability to beer and, due to their antimicrobial activity, increase the shelf-life of the final beer (Korpelainen and Pietiläinen, 2021).

Although the hop is widespread in Kosovo and has economic potential, information about its chemical composition of the essential oils and its variability among wild populations are missing. Thus, this work aims to assess the chemical composition of the essential oils and evaluate their natural variability among wild populations of *H. lupulus* in Kosovo.

Materials and methods

Plant Materials

Plant material was collected in October 2021 in the location Poslisht (Prizren municipality, coordinates: 20°39'56"E, 42°11'37"N) and Barilevë (Pristina municipality, coordinates: 21°7'20"E, 42°44'47"N). Female flowers were collected from 20 individuals (10 individuals per location). Voucher specimens of each population 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 three days.

Distillation of Plant Materials

The essential oil was obtained by hydro-distillation (30 g of grounded plant material, 0.5 L of distilled water added in a one-liter flask) at a distillation rate of 3 ml/min in a Clevenger apparatus for two hours. The samples were stored in the dark at -18 °C until further analysis.

GC and GC-MS Analyses

*avni.hajdari@uni-pr.edu

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

GC/MS analyses were performed using a GC system coupled to a 5975C MSD (Agilent 7890A). The ionization energy was 70 eV with a mass range of 40-400 m/z. The injection volume was 1.0 µl.

The identification of the components was performed by comparing their Kovats retention indices with those in the literature (Adams, 2017) and by comparing the mass spectra of each constituent with those stored in the NIST 08 and WILEY MS 9th databases and with mass spectra from the literature (Adams, 2017). Some of the main peaks were identified by comparing the retention times and mass spectra with those of authentic constituents. The percentage of the oils was calculated using the normalization method from the GC peak areas without correction factors.

Results and discussion

The essential oil yield is expressed as the volume percentage of the dry weight of the dried plant material (%v/w DW). The yield of the essential oil depended on the origin of plant material, thus in the plants originating from the Poslisht locality, the yield ranged from 0.2 to 0.7% v/w DW, while in the Barilavë locality, it ranged from 0.2 to 0.6% v/w DW.

In the locality, Poslisht total of seventy-nine compounds were identified. The main constituent were myrcene (15.7-55.9%), followed by α-humulene (1.9-28.1%), α-selinene (0.6-8.7%), β-bisabolol (0.4-8.0%), β-selinene (0.7-5.5%), (*E*)-β-farnesene (0.4-5.3%), trans-caryophyllene (1.4-4.7%), selina-3,7(11)-diene (0.4-3.7%), etc.. In the locality Barilevë, totally eighty-eight compounds identified, of them myrcene (21.0-52.5%) were the main constituents, followed by α-humulene (6.5-24.4%), (*E*)-β-farnesene (3.3-13.3%), α-selinene (0.9-9.9%), trans-caryophyllene (2.2-7.6%), selina-3,7(11)-diene (0.6-4.6%), α-cadinene (0.5-4.1%), 2-undecanone (0.5-3.0%), etc. Differences in the essential oil's chemical composition were recorded, especially in the content of the main compounds myrcene and α-humulene. The content of α-humulene in two samples originated from the location Poslisht was significantly lower than its content in other samples from the same locality and the samples originating from the Barilevë locality.

Conclusion

This work studied the chemical composition of volatile constituents obtained from female hop flowers from two localities in Kosovo. Regarding the volatile components, the most prominent compounds were myrcene and α-humulene. The constitutes concentration differed among the population, as well as among the individuals of the same location too. The variability in the chemical composition of essential oils among populations reflects the environmental impact, which is influenced by differences in habitat composition and microclimatic conditions.

Further investigation including a higher number of the localities and corroborated molecular analysis are needed to confirm the natural variability and chemo-polymorphism among wild populations of this species in Kosovo.

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References

Adams, R.P., 2017. Identification of essential oil components by gas chromatography/mass spectroscopy, 4.1. ed. Allured publishing Co. Carol Stream, Illinois.

Astray, G., Gullón, P., Gullón, B., Munekata, P.E., Lorenzo, J.M., 2020. *Humulus lupulus* L. as a natural source of functional biomolecules. Appl. Sci. 10(15), 5074. https://doi.org/10.3390/app10155074.

Chung, W.G., Miranda, C.L., Stevens, J.F., Maier, C.S., 2009. Hop proanthocyanidins induce apoptosis, protein carbonylation, and cytoskeleton disorganisation in human colorectal adenocarcinoma cells via reactive oxygen species. Food and Chem. Toxicol. 47(4), 827-836. https://doi.org/10.1016/j.fct.2009.01.015.

Korpelainen, H., Pietiläinen, M., 2021. Hop (*Humulus lupulus* L.): Traditional and Present Use, and Future Potential. Econ. Bot. 75, 302–322. https://doi.org/10.1007/s12231-021-09528-1.

Mustafa, B., Hajdari, A., Krasniqi, F., Hoxha, E., Ademi, H., Quave, C.L., Pieroni, A., 2012. Medical ethnobotany of the Albanian Alps in Kosovo. J. Ethnobiol. Ethnomedicine. 8(1), 1-14. https://doi.org/10.1186/1746-4269-8-6.

Natsume, S., Takagi, H., Shiraishi, A., Murata, J., Toyonaga, H., Patzak, J., Takagi, M., Yaegashi, H., Uemura, A., Mitsuoka, C., Yoshida, K., 2015. The draft genome of hop (*Humulus lupulus*), an essence for brewing. Plant and cell physiol. 56(3), 428-441. https://doi.org/10.1093/pcp/pcu169.