

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

Rr. Eqrem Çabej, 10000 Prishtinë, Republika e Kosovës  
Tel: +383-38-249-873 • E-mail: [fshmn@uni-pr.edu](mailto:fshmn@uni-pr.edu) • [www.uni-pr.edu](http://www.uni-pr.edu)

Ref. nr. 2010 Prishtinë, Dt: 25/06/2025

**PËR:** Këshillin Mësimor të Departamentit të Biologjisë  
Këshillin e Studimeve të Doktoratës së FSHMN-së  
Këshillin e Fakultetit të Shkencave Matematiko Natyrore,

**NGA :** Msc. Bledar Pulaj

**LËNDA:** Kërkesë për formimin e komisionit për vlerësimin e dorëshkrimit të temës së doktoratës

### K Ë R K E S Ë

**Arsyetim:** Sipas Statutit të Universitetit të Prishtinës "Hasan Prishtina" dhe Rregullores ekzistuese për studime të doktoratës, i plotësoj kushtet për vlerësimin e dorëshkrimit, prandaj kërkoj nga organet e lartpërmendura të FShMN-së të më mundësojnë formimin e komisionit për vlerësim të dorëshkrimit të temës së doktoratës me titull: "VEÇORITË MORFO-ANATOMIKE, FITOKIMIKE DHE MOLEKULARE TË DISA LLOJEVE TË GJINISË *ACHILLEA* (ASTERACEAE) NË KOSOVË"

**Kërkesës ia bashkangjes:**

1. Kopjen e dorëshkrimit
2. Një punim shkencor nga lëmia e ngushtë
3. Pëlqimin e mentorit për dorëzimin e dorëshkrimit
4. Dëshmitë për pjesëmarrje në konferenca
5. Formularin F6

Prishtinë,

25.06.2025

Kandidati: Msc. Bledar Pulaj

Email: [bledar.pulaj@uni-pr.edu](mailto:bledar.pulaj@uni-pr.edu)

Tel: 044-735-321



UNIVERSITETI I PRISHTINËS  
"HASAN PRISHTINA"  
FAKULTETI I SHKENCAVE MATEMATIKE NATYRORE  
Rr. Eqrem Çabej, 10000 Prishtinë, Republika e Kosovës  
Tel: +383-38-249-873 • E-mail: [fshmn@uni-pr.edu](mailto:fshmn@uni-pr.edu) • [www.uni-pr.edu](http://www.uni-pr.edu)

Ref. nr. 2014 Prishtinë, Dt: 25/06/2025

**PËR:** Këshillin Mësimor të Departamentit të Biologjisë  
Këshillin e Studimeve të Doktoratës së FSHMN-së  
Këshillin e Fakultetit të Shkencave Matematiko Natyrore,

NGA : Prof, Dr. Behxhet Mustafa

**LËNDA:** Pëlqimi nga Mentori për dorëzimin e dorëshkrimit të temës së doktoratës me titull "Veçoritë morfo-anatomike, fitokimike dhe molekulare të disa llojeve të gjinisë *Achillea* (Asteraceae) në Kosovë" të kandidatit Msc. Bledar Pulaj

**MENDIM:**

Unë, **Prof. Dr. Behxhet Mustafa**, në cilësinë e mentorit të kandidatit MSc. **Bledar Pulaj**, deklaroj se kam ndjekur nga afër dhe në vazhdimësi hulumtimin dhe përgatitjen shkencore të temës së tij të doktoratës të tij të titulluar "Veçoritë morfo-anatomike, fitokimike dhe molekulare të disa llojeve të gjinisë *Achillea* (Asteraceae) në Kosovë"

Punimi përfaqëson një kontribut të rëndësishëm dhe të mirë strukturuar shkencërisht në fushën e botanikës, ekologjisë së bimëve, taksonomisë dhe fitokimisë, duke përfshirë analiza të thelluara të dhjetë specieve të gjinisë *Achillea* të pranishme në Kosovë.

Dorëshkrimi përmban të dhëna origjinale dhe të vlefshme nga analizat e veçorive morfo-anatomike, përbërjes fitokimike (vajrave esenciale, komponentëve fenolike, flavonoideve si dhe aktivitetin antioksidativ FRAP dhe DPPH), si dhe nga analizat molekulare filogjenetike, të cilat janë kryer me metoda bashkëkohore dhe të standardizuara në nivel ndërkombëtar. Të dhëna nga ky punim i doktoratës janë publikuar në revistë shkencore dhe janë prezantuar në disa konferenca vendore e ndërkombëtare:

➤ Pjesë të punimit ë botuara në revistë shkencore:

- **Pulaj, B., Mustafa, B., & Hajdari., A.** 2024. "Differentiation of *Achillea millefolium*, *A. crithmifolia*, and *A. nobilis* through Analysis of Volatile Constituents using HS-SPME-GC/MS and Chemometric Techniques." *Records of Natural Products* 18, no. 6 (November–December 2024): 597–609. <https://doi.org/10.25135/rnp.482.2408.3286>.

➤ Pjesë të punimit të prezantuara në konferenca vendore dhe ndërkombëtare:

- **Pulaj, B.**, Hajdari, A., Mala, X., & Mustafa, B. (2023, October). “*Chemical composition of essential oil of Achillea coarctata Poir. from Kosovo and Albania*”. PSE Natural Drug Discovery: Current Approach and Future Perspectives, 12–15 October 2023, Antalya, Türkiye.
- **Pulaj, B.**, Selmani, B., Hajdari, A., & Mustafa, B. 2022. “Antioxidant Activity of the Methanolic Extracts of *Achillea* sp. Growing Wild in Kosovo.” Presentation at the International Conference on New Achievements in Science, Technology and Arts (ICNA-STA), 14–15 April 2022, Peja, Kosovo
- **Pulaj, B.**, Hajdari, A., Mala, X., & Mustafa, B. (2021, October). “*Morpho-anatomical properties of the Balkan endemic plant Achillea ageratifolia subsp. aizoon (Griseb.) Heimerl.*” 4th International Plant Science and Technology Congress. Stamboll, Türkiye (Online).
- **Pulaj, B.** (2018, September). “Morpho-anatomical properties of the endemic plant *Achillea alexandri-regis* Bornm. & Rudsky” . Albanian Conference on Biology & Environment, Prishtinë, Kosovë

Në përfundim, hulumtimi përfaqëson një kontribut të vlefshëm shkencor, pasi siguron njohuri të reja dhe gjithëpërfshirëse mbi morfologjinë, përbërjen kimike dhe karakteristikat gjenetike të specieve të gjinisë *Achillea* në Kosovë, duke ndihmuar jo vetëm në identifikimin dhe klasifikimin taksonomik të tyre, por edhe në ruajtjen dhe përdorimin e qëndrueshëm të këtij burimi të rëndësishëm natyror në nivel kombëtar dhe ndërkombëtar

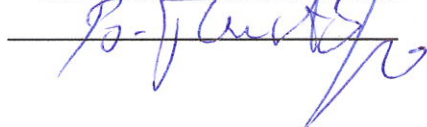
Duke u bazuar në përmbajtjen, nivelin e punës kërkimore dhe rëndësinë shkencore të rezultateve të arritura, konstatoj se tema e doktoratës së kandidatit MSc. Bledar Pulaj i plotëson të gjitha kushtet për të vazhduar me procedurat më tutje në përputhje me rregulloren për studimet e doktoratës të institucionit përkatës.

**Prishtinë:**

25.06.2025

**Mentori:**

**Prof. Dr. Behxhet Mustafa**



# UNIVERSITETI I PRISHTINËS "HASAN PRISHTINA"

F6- Paraqitja e punimit të doktoratës

PARAQITJA E PUNIMIT TË DOKTORATËS <sup>1</sup>	
TË DHËNAT E PËRGJITHSHME	
Doktoranti:	Msc. Bledar Pulaj
Adresa:	Enver Maloku, Mati 1, Bl C5, L1, nr. 5. Prishtinë, 10 000
Tel./ fax:	+383 44 735 321
E-mail:	bledar.pulaj@uni-pr.edu / bledarpulaj@hotmail.com
Emërtimi i studimit:	Biologji e organizmave dhe Ekologji, Program i Doktoratës
Udhëheqësi i studimit:	Universiteti i Prishtinës "Hasan Prishtina" (Fakulteti i Shkencave Matematiko- Natyrore)
TË DHËNAT PËR PUNIMIN E DOKTORATËS	
Titulli në gjuhën shqipe	Veçoritë morfo-anatomike, fitokimike dhe molekulare të disa llojeve të gjinisë <i>Achillea</i> (Asteraceae) në Kosovë.
Titulli në gjuhën angleze	Morpho-anatomical, Phytochemical and Molecular Properties of some <i>Achillea</i> (Asteraceae) Species in Kosovo.
Fusha e hulumtimit	Botanikë
DEKLARATA E MENTORIT/BASHKËMENTORIT	
<p>Unë, Prof. Dr. Behxhet Mustafa, në cilësinë e mentorit të kandidatit të doktoratës, MSc. <b>Bledar Pulaj</b>, deklaroj se kam ndjekur nga afër dhe në vazhdimësi përgatitjen shkencore të disertacionit të tij të titulluar si më lartë. Punimi përfaqëson një kontribut të rëndësishëm dhe të mirë strukturuar shkencërisht në fushën e Botanikës, ekologjisë së bimëve, taksonomisë dhe fitokimisë, duke përfshirë analiza të thelluara të dhjetë specieve të gjinisë <i>Achillea</i> të pranishme në Kosovë.</p> <p>Dorëshkrimi përmban të dhëna origjinale dhe të vlefshme të analizimit të veçorive morfo-anatomike, përbërjes fitokimike (të vajrave esenciale, fenolëve totale, flavonoideve totale, dhe aktivitetin antioksidativ FRAP dhe DPPH) si dhe nga analizat molekulare-filogjenetike, të cilat janë kryer me metoda bashkëkohore dhe të standardizuara në nivel ndërkombëtar. Të dhëna nga ky punim i doktoratës janë publikuar në revistë shkencore dhe janë prezantuar në disa konferenca.</p>	
<p>❖ Pjesë të punimit ë botuara në në revistë shkencore:</p> <ul style="list-style-type: none"> <li>Pulaj, B., Mustafa, B., &amp; Hajdari, A. 2024. "Differentiation of <i>Achillea millefolium</i>, <i>A. crithmifolia</i>, and <i>A. nobilis</i> through Analysis of Volatile Constituents using HS-SPME-GC/MS and Chemometric Techniques." <i>Records of Natural Products</i> 18, no. 6 (November–December 2024): 597–609. <a href="https://doi.org/10.25135/rnp.482.2408.3286">https://doi.org/10.25135/rnp.482.2408.3286</a>.</li> </ul>	
<p>❖ Pjesë të punimit të prezantuara në konferenca vendore dhe ndërkombëtare:</p> <ul style="list-style-type: none"> <li>Pulaj, B., Hajdari, A., Mala, X., &amp; Mustafa, B. (2023, October). <i>Chemical composition of essential oil of Achillea coarctata Poir. from Kosovo and Albania</i>. Paper presented at <b>PSE Natural Drug Discovery: Current Approach and Future Perspectives</b>, Antalya, Türkiye. (Online).</li> <li>Pulaj, B., Selmani, B., Hajdari, A., &amp; Mustafa, B. (2022, April 14–15). <i>Antioxidant activity of the methanolic extracts of Achillea sp. growing wild in Kosovo</i>. Paper presented at the <b>International Conference on New Achievements in Science, Technology and Arts (ICNASTA)</b>, Peja, Kosovo.</li> <li>Pulaj, B., Hajdari, A., Mala, X., &amp; Mustafa, B. (2021, October). <i>Morpho-anatomical properties of the Balkan endemic plant Achillea ageratifolia subsp. aizoon (Griseb.) Heimerl</i>. Paper presented at the <b>4th International Plant Science and Technology Congress</b>, Istanbul, Türkiye. (Online).</li> <li>Pulaj, B. (2018, September). <i>Morpho-anatomical properties of the endemic plant Achillea alexandri-regis Bornm. &amp; Rudsky</i>. Paper presented at the <b>Albanian Conference on Biology &amp; Environment</b>, Prishtinë, Kosovë.</li> </ul>	
<p>Duke u bazuar në përmbajtjen, nivelin e punës kërkimore dhe rëndësinë shkencore të rezultateve të arritura, konstatoj se tema e doktoratës e kandidatit MSc. Bledar Pulaj i plotëson të gjitha kushtet për të vazhduar me procedurat më tutje në përputhje me rregulloren për studimet e doktoratës të institucionit përkatës.</p>	
Vendi, data dhe nënshkrimi	

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

shuar me: 25/10/2025			
Org.	Numer	Sasia	Vlera
01	2012	4	-

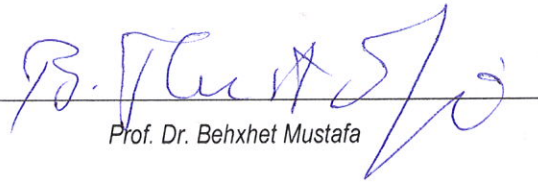
<sup>1</sup> Lutei që ta plotësoni formularin dhe ta dërgoni të nënshkruar me postë elektronike.

UNIVERSITETII PRISHTINËS "HASAN PRISHTINA"

F6- Paraqitja e punimit të doktoratës

Në Prishtinë, 25.06.2025

Nënshkrimi \_\_\_\_\_






Prof. Dr. Behxhet Mustafa

Nënshkrimi \_\_\_\_\_

(Emri e mbiemri i bashkëmentorit)

Pranuar me: 25/10/2025			
Nj. org.	Numër	Sasia	Vlera
01	2013	4	—

## Differentiation of *Achillea millefolium*, *A. crithmifolia*, and *A. nobilis* through Analysis of Volatile Constituents using HS-SPME-GC/MS and Chemometric Techniques

Bledar Pulaj , Behxhet Mustafa  and Avni Hajdari 

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

(Received August 12, 2024; Revised October 12, 2024; Accepted October 16, 2024)

**Abstract:** *Achillea millefolium*, *A. crithmifolia* and *A. nobilis* are distinct species that share morphological similarities, making their differentiation challenging. This study utilized gas chromatography-mass spectrometry (GC/MS) combined with headspace-solid phase microextraction (HS-SPME) to analyze the volatile components of these species and employed chemometric methods for species differentiation. A total of 109 volatile compounds from the three studied species were identified. Of these compounds, 71 were identified in *A. millefolium*, 75 in *A. crithmifolia*, and 58 in *A. nobilis*. The primary volatile compounds of *A. millefolium* were germacrene D, 1,8-cineole, sabinene, and  $\beta$ -pinene; in *A. crithmifolia*, the main compounds were caryophyllene, 1,8-cineole, camphor, ascaridole, and *o*-cymene, while in the essential oil of *A. nobilis*, camphor, lavandulyl acetate, camphene, and isobornyl acetate were determined as the main volatile compounds. The study demonstrated that the HS-SPME-GC/MS techniques, combined with chemometric methods such as discriminant analysis (DA) and principal component analysis (PCA), effectively distinguished the samples of *A. millefolium*, *A. crithmifolia*, and *A. nobilis* based on the differences in the chemical composition of their essential oils.

**Keywords:** *Achillea millefolium*; *A. crithmifolia*; *A. nobilis*; HS-SPME-GC/MS; discriminant analysis (DA); principal component analysis (PCA). © 2024 ACG Publications. All rights reserved.

### 1. Introduction

The genus *Achillea* L. (Asteraceae) comprises about 130 species worldwide, distributed mainly in Europe and the temperate regions of Asia and North America [1]. Based on existing records (Krasniqi, 1972, 1987; Rexhepi, 1986, 1994, 2000), in Kosovo are present 18 *Achillea* species, including *Achillea millefolium* L., *Achillea crithmifolia* Waldst. et Kit, and *Achillea nobilis* L. [2-6]

According to Flora Europaea [7], *A. millefolium* and *A. crithmifolia* belong to the *A. millefolium* group, while *A. nobilis* belongs to the *A. nobilis* group. Although they belong to different groups, they share many similarities. On the other hand, *A. millefolium* and *A. nobilis* especially exhibit high polymorphism [7], making their identification challenging.

Comparing the botanical characteristics of these species, they all have erect stems in common. *A. millefolium* can grow up to 8-90 cm, *A. crithmifolia* 20-60 cm, while *A. nobilis* up to 10-60 cm [7]. The middle cauline leaves of *A. millefolium* measure 3-5 x 0.5-1.2 cm and are lanceolate in outline, 2

\* Corresponding author: E-Mail: [avni.hajdari@uni-pr.edu](mailto:avni.hajdari@uni-pr.edu); Phone: +383 38 249 872; Fax: +383 38 226 104

(-3) pinnatisect, and more or less pubescent; the cauline leaves of the *A. crithmifolia* are 4-6 x 0.8-2 cm, ovate to lanceolate in outline, plane, pinnatisect, and more or less pubescent, while *A. nobilis* has cauline leaves that measure 1.5 x 3 to 1-1.5 cm, which are ovate in outline, pinnatifid to pinnatisect, and more or less pubescent [7]. Additionally, the ligules of *A. millefolium* are usually white; the ligules of *A. crithmifolia* are white to pale yellow, while the ligules of *A. nobilis* are white [7]. These shared main characteristics of stem length, leaf morphology, and ligule colour make challenging the accurate identification of these species, especially for local communities who traditionally use these species and local collectors who trade them.

Despite morphological similarities, the chemical composition of the essential oil exhibits significant variability within and among these species. In this regard, the variability in the chemical composition of the essential oil of *A. millefolium* originated from different countries was reported by various authors [8-12]. Similarly, the variability reported for the chemical composition of the essential oil of *A. crithmifolia* [8,13-15] and *A. nobilis* [15-17]. So, one approach to differentiate the morphologically similar species such as *A. millefolium*, *A. crithmifolia* and *A. nobilis* is to analyze the chemical composition of the essential oils.

The diverse chemical composition of the essential oils obtained from the abovementioned species may contribute to the diverse biological activities and traditional uses associated with these species. In Kosovan traditional medicine, *A. millefolium* has been used to enhance general health and treat a variety of conditions, including respiratory inflammations, urinary system infections, gastrointestinal disorders, skin diseases, cardiovascular diseases, neuromuscular disorders, and ophthalmological infections [18-24]. Furthermore, since ancient times, it has been used to make recreational tea and prepare beverages for enjoyment [22].

On the other hand, there is no ethnobotanical evidence indicating that *A. crithmifolia* and *A. nobilis* have been traditionally used as medicine or food in Kosovo. However, despite the lack of data on their traditional use as medicine or food, these species may have been used instead of *A. millefolium* due to their morphological similarities. Therefore, these species can be considered "ethnospecies," meaning that multiple scientifically distinct species are used as a single species and used for similar purposes by the local community [25].

In Kosovo, *A. millefolium* is an economically significant medicinal and aromatic plant. Local people collect it from the wild population and export it. The exported amount of *Achillea* sp. (exact species not specified) from Kosovo in 2021 was 14 tonnes [26], while in 2022, the export was 10 tonnes [27]. However, the reports use the English name "yarrow," which is the common name for *A. millefolium*. Therefore, it is very likely that these species were included in the *A. millefolium* data because local collectors could not distinguish these taxa.

The possibility of adulteration of *A. millefolium* raw material with *A. nobilis* due to similar morphological characteristics has been previously reported [29] and references therein. The misidentification of the species may lead to replacing one species with another, which may decrease the quality of the final product [28] and might result in unintended side effects.

Furthermore, chemo-polymorphism within the species, hybridization and polyploidization, genetic polymorphism and ecological plasticity were also reported for *A. millefolium* group [30-31], which further complicates the accurate identification of this species.

Essential oils volatile constituents from *Achillea* sp. are usually extracted using the hydro distillation method. However, this method requires a lot of plant material, is labour-intensive, and is time-consuming. In this context, headspace solid-phase microextraction (HS-SPME) seems to be an alternative extraction method for analysing the volatile constituents of *Achillea* sp. This method is simple, doesn't require solvents, takes less time, and can be automated [32]. Moreover, plant species with similar morphological characteristics can be discriminated by applying chemometrics methods, including principal component analysis (PCA) and discriminant analyses (DA), by using the variation of the main volatile components of the essential oils. This approach was previously used to identify variations and commonalities among complex sample clusters [32].

There is limited data available on the chemical composition of the essential oil of *A. millefolium* from Kosovo, whereas, to the best of our knowledge, there are no previous reports on the chemical composition of the essential oil of *A. crithmifolia* and *A. nobilis*. This study aims to assess the differences in the chemical composition of essential oils volatile constituents among *A.*

*millefolium*, *A. crithmifolia*, and *A. nobilis* in Kosovo and to test analytical HS-SPME-GC/MS techniques and statistical multivariate methods for discriminating these plants.

## 2. Materials and Methods

### 2.1. Plant Materials

The inflorescences of *A. millefolium*, *A. crithmifolia* and *A. nobilis* were gathered from the natural population in Kosovo, the Novobërdë municipality (Latitude: 42°37'10"; Longitude: 21°27'80"; Altitude: 1248m), during the blooming season of 2023. The inflorescences gathered in a small area within approximately half a square kilometre, where all three species coexisted. This sampling approach was selected to observe changes that can be attributed more to the species' genetic background and minimize changes in the chemical composition of the species' essential oils that arise due to environmental impact. The plant materials were collected in eight plots in total, and due to slight morphological variability within the species, *A. millefolium* and *A. crithmifolia* were collected in more than one plot. Thus, the *A. crithmifolia* was collected in four plots, *A. millefolium* in three, and *A. nobilis* in one (Table 1). Five samples per plot were collected, each obtained from an individual plant. Plant species were identified by Dr. A. Hajdari using the local Floras and Flora Europaea [34-37]. Herbarium samples were prepared from the identified plant samples and deposited in the Herbarium of the University of Prishtina (Table 1).

The collected plant materials were dried for four days at 35°C in a dry and light-proof cabinet. The dried samples were packaged, labelled, and stored in plastic bags under a vacuum in a dry, light-proof environment.

**Table 1.** Plant species, sample code and herbarium specimens accession information

Plant species	Sample codes	Specimens accession no.
<i>Achillea nobilis</i>	<i>A. nobilis</i> (AN)	00002021
<i>Achillea crithmifolia</i>	1 <i>A. crithmifolia</i> (1AC)	00002022
	2 <i>A. crithmifolia</i> (2AC)	00002024
	3 <i>A. crithmifolia</i> (3AC)	00002025
	4 <i>A. crithmifolia</i> (4AC)	00002023
<i>Achillea millefolium</i>	1 <i>A. millefolium</i> (1AM)	00002028
	2 <i>A. millefolium</i> (2AM)	00002026
	3 <i>A. millefolium</i> (3AM)	00002027

### 2.2. SPME Extraction of the Essential Oils

The volatile components of the species were extracted using the headspace solid phase microextraction (HS-SPME) technique with an autosampler (HTA, model: HT2800T, Brescia, Italy). 0.5 g of plant material (inflorescences) was placed in a 20 mL vial, incubated for 20 minutes, and then extracted for 30 minutes at 70°C with shaking intervals of 0.1 minutes within 3 minutes. The adsorption was performed using DVB/CAR/PDMS fibre size 23 ga, followed by desorption of the absorbed compounds at 250°C for 3 minutes.

### 2.3. GC/FID and GC/MS Analyses

The volatile constituents were quantified using gas chromatography coupled with a flame ionization detector (GC/FID) (Agilent 7890A, Agilent Technologies). The separation was performed on an HP-5MS column (30m × 0.25mm, 0.25µm film thickness). Helium was used as the carrier gas with an initial flow rate of 0.6 mL/min. The front inlet was set at 250°C, while the split ratio was set at 50:1. The GC oven temperature program started at 60°C and increased to 280°C at a rate of 5°C/min. The FID detector operated at 250°C with an airflow of 350 mL/min and a hydrogen flow of 35 mL/min.

Volatile constituents of *Achillea* sp.

The qualitative analyses of the essential oils were carried out using gas chromatography coupled with mass spectrometry (GC/MS) (GC: Agilent 7890A; MS: 5975C, Agilent Technologies). The GC operation conditions were as described for the GC/FID analyses. The MS ionization energy was set to 70 eV, and the mass range was set at 40-400 *m/z*. The injection volume for each sample was 1.0  $\mu$ L.

The essential oil volatile constituents were identified by comparing their Arithmetic Retention Indices (ARI) with those reported in the literature [33], by comparing the mass spectra of each constituent with those stored in NIST 08.L and WILEY MS 9<sup>th</sup> databases and with mass spectra from the literature [33]. The percentage of oil was calculated using a normalization method without correction factors based on the GC peak areas.

#### 2.4. Statistical Analysis

The primary volatile constituents, higher than 2% and highlighted in bold in Table 2, were considered for the statistical analysis (PCA and DA). The statistical analyses were performed by XLSTAT software, Version 2023.1.2 (New York, NY, USA).

### 3. Results and Discussion

#### 3.1. Chemical Analyses of the Essential Oils

The essential oils from 40 samples were investigated. These samples were categorized into eight groups based on morphological characteristics (Table 1), each representing the average of five plants. *A. crithmifolia* comprised four groups (20 samples), *A. millefolium* comprised three groups (15 samples), and *A. nobilis* comprised the remaining group (five samples) as indicated in Table 1 and 2.

109 volatile compounds were separated from the essential oils of three analysed plant species, 71 from *A. millefolium*, 75 from *A. crithmifolia*, and 58 from *A. nobilis*. Out of the 109 volatile compounds, 106 were successfully identified.

The major components of the *A. millefolium* in the studied samples were germacrene D (11.5-25.0%), followed by 1,8-cineole (7.7-22.5%), sabinene (3.4-20.4%),  $\beta$ -pinene (12.8-18.7%) and  $\beta$ -caryophyllene (8.4-15.0%). El-Kalamouni [9] also reported that germacrene D is one of the primary volatile constituents in *A. millefolium* sample collected from France. In addition, other researchers (from France, Serbia, Romania, and Bosnia and Hercegovina) [8-12] reported similar data for 1,8-cineole in the essential oil of *A. millefolium*. However, contrary to the data in these reports, in our study, the camphor and borneol percentages were found to be very low, 0.2-1.5% and 0.2-0.3%, respectively.

1,8-cineole (27.8-40.2%), camphor (5.0-28.8%), ascaridole (7.4-15.6%) and *o*-cymene (6.6-10.7%) were the most abundant volatile compounds in the essential oil of *A. crithmifolia*. Other authors have also identified 1,8-cineole as the main compound in the essential oil of *A. crithmifolia* [8], [13-15]. Furthermore, similar to our findings, camphor was one of the main volatile constituents of the essential oils of *A. crithmifolia* [13-15]. Ascaridole, as one of the primary constituents in our samples, was reported by Stanković *et al.* [13] to have a low concentration. However, artemisia alcohol, *trans*-chrysanthemum, and artemisia ketone, which were identified as major volatile constituents in the research reports mentioned above, were not detected in our samples.

The primary volatile constituents of *A. nobilis* in our samples were camphor (45.2%), lavandulyl acetate (11.0%), camphene (5.4%) and isobornyl acetate (4.8%). However, other studies have reported different chemical profiles for the essential oil, i.e. 1,8-cineole (eucalyptol) (22.9%), chrysanthenone (14.3%), and endo-borneol (9.9%) [16]; 1,8-cineole (eucalyptol) (21.0%),  $\alpha$ -thujone (11.0%), and camphor (8.5%) [17];  $\alpha$ -thujone (25.7%), artemisia ketone (14.8%) and borneol (9.9%) (8.2%) was reported in the literature on the species [15].



Volatile constituents of *Achillea sp.*

<i>cis</i> -Linalool oxide	1170	1163	-	-	-	-	-	-	-	-	0.10
<b>Terpinen-4-ol</b>	<b>1174</b>	<b>1170</b>	<b>0.43</b>	<b>0.66</b>	<b>0.24</b>	<b>0.42</b>	<b>2.46</b>	<b>3.12</b>	<b>1.86</b>	<b>0.42</b>	
<i>ρ</i> -Cymen-8-ol	1179	1172	-	-	-	0.29	0.36	0.34	0.40	-	
<b>α-Terpineol</b>	<b>1186</b>	<b>1180</b>	<b>4.54</b>	<b>2.87</b>	<b>0.79</b>	<b>0.73</b>	<b>1.29</b>	<b>2.03</b>	<b>1.92</b>	<b>-</b>	
Myrtenol	1194	1191	0.11	t	0.24	0.29	0.70	0.15	0.26	0.12	
<i>trans</i> -Piperitol	1207	1206	-	t	-	t	0.24	0.14	0.21	-	
Verbenone	1204	1198	t	-	0.12	0.57	t	t	0.16	-	
<i>trans</i> -Carveol	1215	1210	-	t	t	0.33	t	0.20	0.16	-	
<i>neoiso</i> -Dihydro carveol	1226	1225	-	-	-	t	0.11	-	0.23	-	
<i>cis</i> -Carveol	1226	1219	-	-	-	-	-	-	-	0.11	
<b>Ascaridole</b>	<b>1234</b>	<b>1227</b>	<b>t</b>	<b>t</b>	<b>-</b>	<b>7.41</b>	<b>15.57</b>	<b>7.47</b>	<b>12.05</b>	<b>-</b>	
Carvone	1239	1234	0.21	t	t	0.18	t	0.34	0.22	-	
Piperitone	1249	1243	-	-	-	0.37	0.32	0.12	0.24	-	
Linalool acetate	1254	1251	-	-	-	-	-	-	-	t	
<i>trans</i> -Piperitone epoxide	1252	1246	-	-	-	0.11	0.27	0.18	0.19	-	
<i>cis</i> -Chrysanthenyl acetate	1261	1261	-	-	0.17	0.42	-	0.18	0.26	1.82	
<i>o</i> -Guaiacol acetate	1261	1256	-	-	-	-	-	-	-	0.33	
Perilla aldehyde	1269	1264	-	t	0.48	t	0.22	1.02	0.42	-	
<b>Isobornyl acetate</b>	<b>1283</b>	<b>1280</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>0.35</b>	<b>0.56</b>	<b>0.17</b>	<b>0.64</b>	<b>4.81</b>	
<b>Lavandulyl acetate</b>	<b>1288</b>	<b>1284</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>-</b>	<b>10.95</b>	
Thymol	1289	1284	0.19	0.15	-	0.38	0.40	0.20	0.44	0.41	
Perilla alcohol	1294	1288	-	-	-	0.14	0.23	t	0.13	-	
Carvacrol	1298	1295	-	-	-	-	-	-	-	0.66	
<i>trans</i> -Carvyl acetate	1339	1339	-	-	-	-	-	-	-	0.28	
<b>Unknown 1</b>	<b>1311</b>	<b>1311</b>	<b>-</b>	<b>-</b>	<b>0.19</b>	<b>3.12</b>	<b>3.64</b>	<b>2.80</b>	<b>3.56</b>	<b>-</b>	
Linalool propanoate	1334	1330	-	-	t	t	-	0.64	0.61	-	
<i>trans</i> -Carvyl acetate	1339	1337	-	t	t	0.40	0.17	0.16	0.27	-	
α-Cubebene	1348	1346	-	-	-	-	-	0.19	0.24	-	
α-Longipinene	1350	1345	-	-	-	-	-	-	-	0.24	
Eugenol	1356	1354	0.55	0.34	0.15	0.13	0.34	0.17	0.17	0.38	

<i>cis</i> -Carvyl acetate	1365	1364	-	-	-	-	-	-	-	1.74
Cyclosativene	1369	1370	-	-	t	t	-	0.15	0.19	-
Linalool isobutanoate	1373	1372	-	-	-	-	-	-	-	0.34
$\alpha$ -Ylangene	1373	1370	t	t	-	0.12	t	0.17	t	-
<i>trans</i> -p-Menth-6-en-2,8-diol	1371	1366	0.15	0.13	0.28	0.11	0.11	0.34	0.16	-
$\alpha$ -Copaene	1376	1374	0.61	0.56	1.90	-	-	-	-	-
<b>Geranyl acetate</b>	<b>1379</b>	<b>1375</b>	-	-	-	-	-	-	-	<b>2.72</b>
$\beta$ -Bourbonene	1387	1388	0.22	0.19	0.32	0.69	t	t	-	-
7-Epi-Sesquithujene	1391	1390	-	-	-	-	-	-	-	1.08
(Z)-Jasmone	1392	1387	0.26	0.14	0.19	1.71	1.18	0.52	1.42	0.25
<b>Methyl eugenol</b>	<b>1403</b>	<b>1401</b>	<b>2.24</b>	<b>1.32</b>	<b>t</b>	-	-	<b>t</b>	-	-
Unknown 2	1416	1416	0.16	0.14	0.34	0.12	t	-	t	0.27
<b>Caryophyllene</b>	<b>1419</b>	<b>1418</b>	<b>8.43</b>	<b>10.33</b>	<b>15.04</b>	<b>0.43</b>	<b>0.73</b>	<b>1.75</b>	<b>0.47</b>	-
Lavandulyl isobutanoate	1421	1420	-	-	-	-	-	-	-	0.12
Linalool butanoate	1423	1422	-	-	-	-	-	-	-	0.40
$\beta$ -Copaene	1430	1431	0.10	0.12	t	t	-	-	-	0.36
(Z)- $\beta$ -Farnesene	1440	1435	-	-	-	-	-	-	-	0.18
Aromadendrene	1439	1433	0.36	0.39	0.40	0.15	0.26	0.27	0.10	-
$\alpha$ -Humulene	1454	1453	1.29	1.43	1.89	t	t	0.13	t	-
(E)- $\beta$ -Farnesene	1454	1451	t	0.10	0.37	t	t	0.10	t	-
$\gamma$ -Gurjunene	1475	1472	-	-	-	-	-	-	-	0.59
<b><math>\gamma</math>-Himachalene</b>	<b>1481</b>	<b>1480</b>	<b>1.30</b>	<b>0.91</b>	<b>2.54</b>	<b>t</b>	<b>0.27</b>	<b>t</b>	<b>0.17</b>	<b>0.71</b>
<b>Germacrene D</b>	<b>1480</b>	<b>1476</b>	<b>12.16</b>	<b>11.51</b>	<b>25.00</b>	<b>0.76</b>	<b>0.87</b>	<b>1.22</b>	<b>0.63</b>	<b>3.01</b>
$\gamma$ -Curcumene	1481	1477	-	-	-	0.18	-	0.27	-	-
<b><math>\alpha</math>-Zingiberene</b>	<b>1493</b>	<b>1489</b>	<b>6.50</b>	<b>4.85</b>	<b>1.79</b>	-	-	-	-	-
$\alpha$ -Murolene	1500	1498	0.37	0.36	1.18	t	t	0.12	t	0.35
$\beta$ -Himachalene	1500	1495	t	t	-	-	0.17	-	0.37	-
Lavandulyl isovalerate	1509	1507	-	-	-	-	-	-	-	1.23
$\gamma$ -Cadinene	1513	1510	0.13	0.11	0.15	-	-	-	-	0.21
$\beta$ -Curcumene	1515	1511	0.21	0.15	0.26	t	t	t	t	-

Volatile constituents of *Achillea sp.*

<b>δ-Cadinene</b>	<b>1522</b>	<b>1520</b>	<b>2.03</b>	<b>1.58</b>	<b>0.95</b>	<b>t</b>	<b>t</b>	<b>t</b>	<b>t</b>	<b>-</b>
β-Sesquiphellandrene	1521	1516	-	-	-	-	-	-	-	0.42
<i>cis</i> -Sesquisabinene hydrate	1544	1542	-	-	-	-	-	-	-	0.17
Elemol	1548	1542	0.13	t	t	-	-	-	-	-
Unknown 3	1561	1561	-	-	-	-	-	-	-	0.15
( <i>E</i> )-Nerolidol	1561	1558	0.20	0.13	0.13	-	0.23	0.40	-	-
<i>epi</i> -Longipinanol	1562	1559	-	-	-	-	-	-	-	0.22
Spathulenol	1577	1574	t	0.10	0.14	0.17	-	-	0.14	0.79
<b>Caryophyllene oxide</b>	<b>1582</b>	<b>1579</b>	<b>1.06</b>	<b>1.42</b>	<b>2.43</b>	<b>0.69</b>	<b>1.32</b>	<b>2.83</b>	<b>0.81</b>	<b>0.27</b>
Allo-cedrol	1589	1584	t	-	t	0.25	0.15	0.26	0.11	-
Cubeban-11-ol	1595	1589	0.15	0.13	0.22	t	t	t	t	-
<i>cis</i> -Dihydro-Mayurone	1595	1590	-	-	-	-	-	-	-	0.18
Ledol	1602	1598	0.13	0.13	t	0.26	0.14	0.29	t	-
Humulene epoxide II	1608	1603	t	t	0.19	t	0.13	0.13	t	-
Caryophylla-4(12),8(13)-dien-5 $\alpha$ -ol	1639	1639	t	t	0.91	-	0.10	0.15	-	-
1,7-Diepi- $\alpha$ -Cedrenal	1639	1638	-	-	-	-	-	-	-	0.26
β-Eudesmol	1649	1645	-	-	-	0.78	0.42	0.30	0.80	-
Selin-11-en-4- $\alpha$ -o	1659	1658	0.23	0.16	0.24	-	-	-	-	1.34
<i>neo</i> -Intermedeol	1658	1653	-	-	-	-	-	-	-	0.25
Valeranone	1674	1672	0.28	0.17	t	-	-	-	-	-
Germacra-4(15),5,10(14)-trien-1- $\alpha$ -ol	1685	1681	-	-	-	-	-	-	-	0.32
		Monoterpenes	14.73	16.28	15.47	16.62	28.95	38.29	28.99	10.22
		Oxygenated monoterpenes	73.22	72.06	69.27	71.16	31.35	24.14	12.98	53.00
		Sesquiterpenes	2.78	2.75	4.61	2.45	33.92	32.65	51.96	7.14
		Oxygenated sesquiterpenes	2.23	2.59	4.44	2.07	2.38	2.46	4.56	4.86
		Others	7.05	6.21	6.07	7.55	3.40	2.47	1.29	24.79

<sup>a</sup>Compounds listed in order of elution from a HP-5MS column; <sup>b</sup>ARI - Arithmetic Retention Indices from the literature [33]; <sup>c</sup>ARI - Arithmetic Retention Indices calculated against a mixture of C9- C28n alkanes; Compounds marked in boldface (with relative concentrations higher than 2%) chosen for HCA and PCA statistical analyses; - = compound not detected; t = trace < 0.1%.

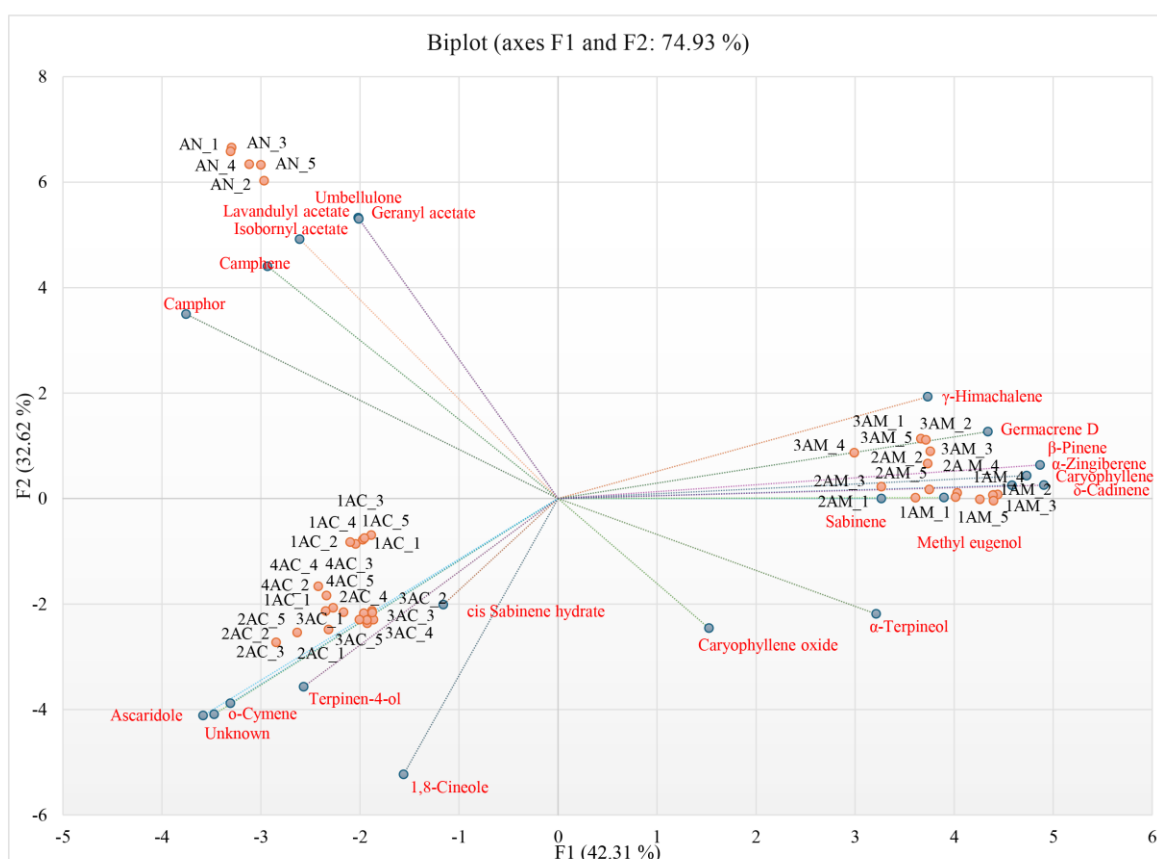
\*Species names are abbreviated according to the format used in Table 1.

Considering the main classes of volatile constituents of *A. millefolium*, the predominant constituents were found to be oxygenated monoterpenes (6.3-73.2%), followed by monoterpenes (14.7-16.3%), other volatile constituents (6.1-7.1%), oxygenated sesquiterpenes (2.2-4.4%), and sesquiterpenes (2.8-4.6%). In the essential oil of *A. crithmifolia*, the main classes of volatile constituents are oxygenated monoterpenes (13.0-71.2%), followed by sesquiterpenes (2.5-52.0%), monoterpenes (2.5-33.9%), other constituents (1.3-7.6%), and oxygenated sesquiterpenes (2.1-4.6%). Additionally, the primary main classes of volatile constituents of *A. nobilis* include oxygenated monoterpenes (53.0%), followed by other constituents (24.8%), monoterpenes (10.2%), sesquiterpenes (7.1%), and oxygenated sesquiterpenes (4.9%) [15].

The variations in the chemical composition of essential oils within the species may be due to environmental factors (climate conditions, soil characteristics, etc.), altitude, geographic isolation, habitat composition, plant growth period, harvest timing, and genetic factors like hybridization [39-43].

### 3.2. Principal Component Analysis and Discriminant Analysis

The DA and PCA were utilized to evaluate the variation in the volatile composition of *A. millefolium*, *A. crithmifolia*, and *A. nobilis*. In both cases, the localities were used as grouping variables to classify or discriminate between different species based on the main chemical constituents of the essential oils as independent variables.



**Figure 1.** PCA scatter plot of the main essential oil constituents obtained from *A. millefolium* (AM), *A. crithmifolia* (AC), and *A. nobilis* (AN).

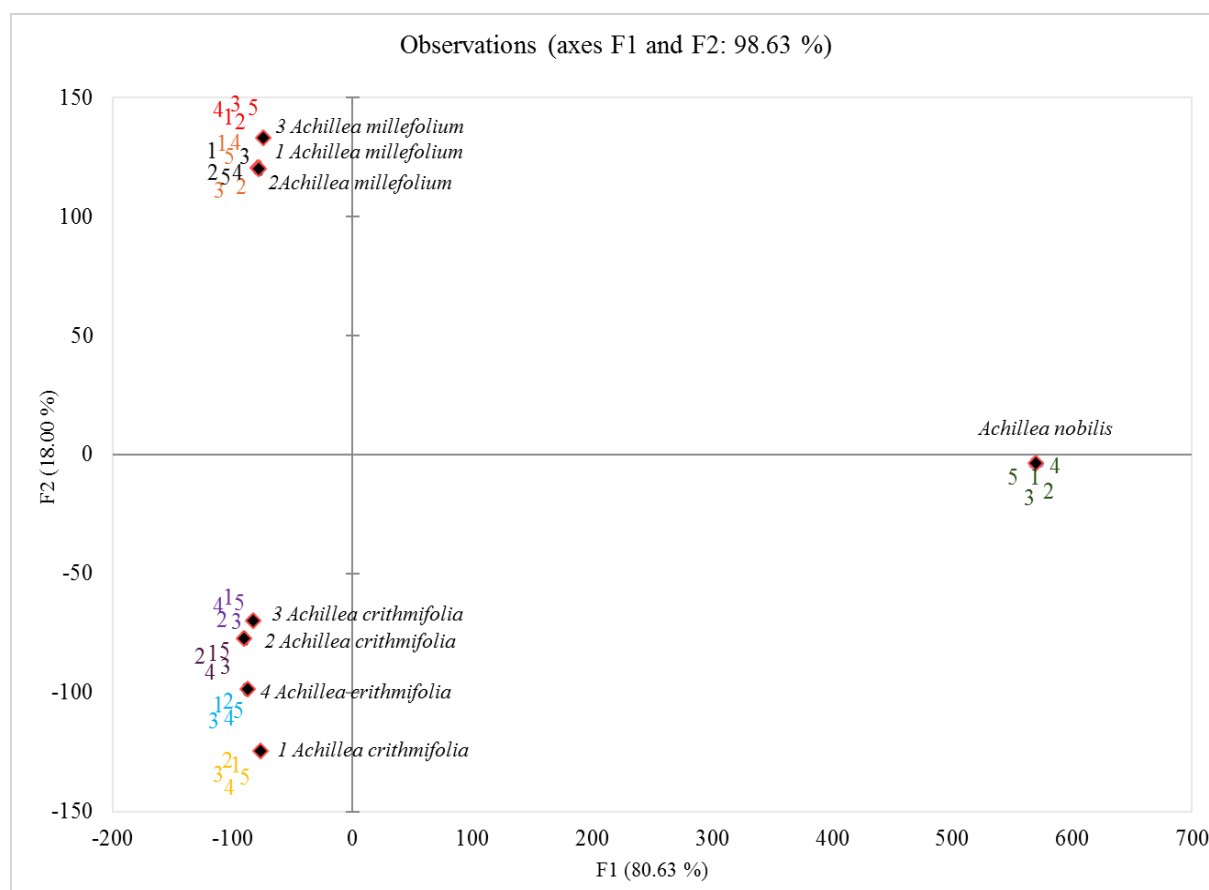
For PCA statistical analysis, 22 primary volatile constituents of the essential oils were used; the ones with a concentration of more than 2% are highlighted in bold in Table 2. The PCA revealed that

Volatile constituents of *Achillea* sp.

the first two principal components accounted for 74.9% of the total variance. The first axis, which accounts for 42.3% of the total variance (Eigenvalue variability 9.3%), demonstrates positive contributions from volatile compounds such as caryophyllene oxide,  $\alpha$ -terpineol, methyl eugenol, sabinene,  $\delta$ -cadinene, caryophyllene,  $\alpha$ -zingiberene,  $\beta$ -pinene, germacrene D, and  $\gamma$ -himachalene. Conversely, negative contributions are associated with camphene, *o*-cymene, 1,8-cineole, *cis*-sabinene hydrate, camphor, umbellulone, terpinen-4-ol, ascaridole, isobornyl acetate, lavandulyl acetate, unknown 1, and geranyl acetate. The second PCA axis accounted for 32.6% of the total variance (Eigenvalue variability 7.2%) and was primarily influenced by umbellulone, geranyl acetate, isobornyl acetate, lavandulyl acetate, camphene, camphor,  $\gamma$ -himachalene, germacrene D,  $\beta$ -pinene,  $\alpha$ -zingiberene, caryophyllene, and  $\delta$ -cadinene. Conversely, it was negatively affected by sabinene, *o*-cymene, 1,8-cineole, *cis*-sabinene hydrate, terpinen-4-ol,  $\alpha$ -terpineol, ascaridole, unknown 1, methyl eugenol, and caryophyllene oxide (Figure 1). The PCA scatter plot (Figure 1) indicates a differentiation among the studied samples based on species.

Similarly, the DA scatter plot (Figure 2) further validates the species-based discrimination of the samples. This suggests that the variation in the chemical composition of essential oils of the analyzed species may be attributed to the species' genetic background, as the plant material for all species was collected in a limited area, which minimized the impact of environmental factors like habitat composition, altitude, and microclimatic conditions.

However, in both the DA and PCA plots, intermediate samples between the species were absent, indicating a lack of hybridization among the analyzed species.



**Figure 2.** A scatter plot of discriminant analysis obtained by analyzing the essential oils *A. millefolium*, *A. crithmifolia*, and *A. nobilis*

#### 4. Conclusions

In this study, we effectively utilized the HS-SPME-GC/MS method to identify the volatile constituents from *Achillea* species. This solvent-free method, which is simple and rapid, allowed us to separate 109 volatile constituents from the essential oils of three analyzed plant species. The main volatile constituents of the essential oil of *A. millefolium* were germacrene D, followed by 1,8-cineole, sabinene,  $\beta$ -pinene, and caryophyllene. The essential oil of *A. crithmifolia* was dominated by 1,8-cineole, camphor, ascaridole, and o-cymene, while the main volatile constituents of *A. nobilis* were camphor, lavandulyl acetate, camphene, and isobornyl acetate. Chemometric tools, like DA and PCA, were successfully used to differentiate the samples of *A. millefolium*, *A. crithmifolia*, and *A. nobilis* based on their differences in volatile chemical composition. The study revealed that genetic background significantly impacted the chemical composition and variability of essential oils of the analyzed species, as also indicated by the PCA and DA statistical analyses. However, it is important to note that other factors, such as micro-environment, vegetative periods, altitude, geographic isolation, habitat composition, etc. can also influence the volatile chemical composition of the essential oil. Thus, due to the small sampling area, our data does not reflect the environmental impact, which is a limitation of this research. Therefore, further studies using more extensive samples and populations from various geographical locations supported by molecular DNA analyses are needed.

#### ORCID

Bledar Pulaj: [0000-0003-0543-090X](https://orcid.org/0000-0003-0543-090X)

Behxhet Mustafa: [0000-0002-1052-5252](https://orcid.org/0000-0002-1052-5252)

Avni Hajdari: [0000-0001-5688-9679](https://orcid.org/0000-0001-5688-9679)

#### References

- [1] S. Saeidnia, A. Gohari, N. Mokhber-Dezfuli and F. Kiuchi (1972). A review on phytochemistry and medicinal properties of the genus *Achillea*, *Daru*. **19(3)**, 173-186.
- [2] F. Krasniqi (1972). Šumska vegetacija brdskog regiona Kosova. Zajednica naučnih ustanova Kosova studije, Kosovo.
- [3] F. Krasniqi (1987). Endemi u flori Kosova i problemi njihove zashtite. *ANU BiH Posebna knjiga*. **83**, 119-24
- [4] F. Rexhepi (1986). Flora e maleve të larta të Kosovës. Enti i Teksteve dhe Mjeteve Mësimore i KSAK, Prishtinë, Kosovë.
- [5] F. Rexhepi (1994). Vegetation of Kosova. Universiteti i Prishtinës, Prishtinë, Kosovë.
- [6] F. Rexhepi (2000). Kosovo endemics plants. University of Prishtina, Prishtinë, Kosovë.
- [7] T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters and D. A. Webb (1964-1980). *Flora Europaea*. University Press, Cambridge, UK.
- [8] A. Smelcerovic, M. Lamshoeft, N. Radulović, D. Ilic and R. Palić (2010). LC-MS Analysis of the Essential Oils of *Achillea millefolium* and *Achillea crithmifolia*, *Chromatogr.* **71(1)**, 113-116.
- [9] C. El-Kalamouni, P. R. Venskutonis, B. Zebib, O. Merah, C. Raynaud and T. Talou (2017). Antioxidant and antimicrobial activities of the essential oil of *Achillea millefolium* L. grown in France, *Medicines* **4(2)**, 30.
- [10] M. D. Berechet, E. Manaila, R. Physics, M. D. Stelescu, G. D. Craciun and R. Physics (2017). The composition of essential oils obtained from *Achillea millefolium* and *Matricaria chamomilla* L., originary from romania, *Rev. Chim.* **68(12)**, 2787-2795.
- [11] D. Vidic, S. Ćavar Zeljković, M. Dizdar and M. Maksimović (2016). Essential oil composition and antioxidant activity of four Asteraceae species from Bosnia, *J. Essen. Oil Res.* **28(5)**, 445-457.
- [12] J.P. Stojanović, G.S. Stojanović, Z.Z. Stojanović-Radić, B.K. Zlatković, J.D. Ickovski, I.G. Zlatanović, S.Č. Jovanović and Z.S. Mitić (2022). Essential oils of six *Achillea* species: chemical profiles, antimicrobial potential and toxicity toward crustaceans, *Chem. Biodivers.* **19(3)**, e202100905.
- [13] N. Stanković, T. Mihajilov-Krstev, B. Zlatković, J. Matejić, V.S. Jovanović, B. Kocić and L. Čomić (2016). Comparative study of composition, antioxidant, and antimicrobial activities of essential oils of selected aromatic plants from Balkan peninsula, *Planta Med.* **82(7)**, 650-661.

Volatile constituents of *Achillea* sp.

- [14] A. Konakchiev and A. Vitkova (2014). Essential oil composition of *Achillea crithmifolia* Waldst. et Kit, *J. Essent. Oil-Bear. Plants* **7**(1), 32–36.
- [15] R. Palić, G. Stojanović, T. Nasković and N. Ranelović (2003). Composition and antibacterial activity of *Achillea crithmifolia* and *Achillea nobilis* essential oils, *J. Essent. Oil Res.* **15**(6), 434–437.
- [16] I. Yener, M.A. Yılmaz, O.T. Olmez, M. Akdeniz, F. Tekin, N. Hasimi, M.H. Alkan, M. Ozturk and A. Ertaş (2020). A detailed biological and chemical investigation of sixteen *Achillea* species' essential oils via chemometric approach, *Chem. Biodivers.* **17**(3), e1900484.
- [17] D. A. Sampietro, A.D.L.A. Gomez, C.M. Jimenez, E.F. Lizarraga, Z.A. Ibatayev, Y.M. Suleimen and C.A. Catalán (2017). Chemical composition and antifungal activity of essential oils from medicinal plants of Kazakhstan, *Nat. Prod. Res.* **31**(12) 1464–1467.
- [18] B. Mustafa, A. Hajdari, and F. Krasniqi, E. Hoxha, H. Ademi, C.L. Quave and A. Pieroni (2012). Medical ethnobotany of the Albanian Alps in Kosovo, *J. Ethnobiol. Ethnomed.* **8**, 1-14.
- [19] B. Mustafa, A. Hajdari, Q. Pajazita, B. Sylja, C. L. Quave and A. Pieroni (2012). An ethnobotanical survey of the Gollak region, Kosovo, *Genet. Resour. Crop Evol.* **59**(5), 739-754.
- [20] A. Pieroni, R. Sökand, C. L. Quave, A. Hajdari and B. Mustafa (2017). Traditional food uses of wild plants among the Gorani of South Kosovo, *Appetite* **108**, 83–92.
- [21] B. Mustafa, A. Hajdari, A. Pieroni, B. Pulaj, X. Koro and C. L. Quave (2015). A cross-cultural comparison of folk plant uses among Albanians, Bosniaks, Gorani and Turks living in south Kosovo, *J. Ethnobiol. Ethnomed.* **11**(1), 1-26.
- [22] A. Hajdari, A. Pieroni, M. Jhaveri, B. Mustafa, and C.L. Quave (2018). Ethnomedical knowledge among Slavic speaking people in South Kosovo. *Ethnobiol. Conserv.* **7**, 1-42.
- [23] B. Mustafa, A. Hajdari, B. Pulaj, C. L. Quave and A. Pieroni (2020). Medical and food ethnobotany among Albanians and Serbs living in the Shtërpçë/Štrpçe area, South Kosovo, *J. Herb. Med.* **22**, 100344.
- [24] B. Mullalija, B. Mustafa, A. Hajdari, C. L. Quave and A. Pieroni (2021). Ethnobotany of rural and urban Albanians and Serbs in the Anadrini region, Kosovo, *Genet. Resour. Crop Evol.* **68**(5), 1825–1848.
- [25] R. Florian, N. Shahbaz, W. Ingrid, G. Sabine, S. Johannes, J. Johann and K. Wolfgang (2002). Determination of taxa of the *Achillea millefolium* group and *Achillea crithmifolia* by morphological and phytochemical methods I. Characterisation of Central European taxa, *Sci. Pharm.* **70**(2), 199–229.
- [26] Organika, (2022). Study of the non-wood forest products (NWFP) and medicinal and aromatic plants (MAP) sector 2021, Organika, Prishtinë, Kosovo.
- [27] Organika, (2023). Study of the non-wood forest products (NWFP) and medicinal and aromatic plants (MAP) sector 2022, Organika, Prishtinë, Kosovo
- [28] J. Otieno, S. Abihudi, S. Veldman, M. Nahashon, T. van Andel and H. J. de Boer (2015). Vernacular dominance in folk taxonomy: a case study of ethnospices in medicinal plant trade in Tanzania, *J. Ethnobiol. Ethnomed.* **11**(1), 1-7.
- [29] E. Marchart, A. Hattenberger, L. Krenn and B. Kopp (2003). Analysis of flavonoids in *Achillea nobilis* L. by capillary electrophoresis, *Scientia Pharmaceut.* **71**(2), 133–145.
- [30] F. Ehrendorfer and Y.-P. Guo (2006). Multidisciplinary studies on *Achillea* sensu lato (Compositae-Anthemideae): new data on systematics and phylogeography, *Willdenowia* **36**(1), 69-87.
- [31] L. Raudone, G. Vilkičkyte, M. Marksa and J. Radusiene (2024). Comparative phytoprofilng of *Achillea millefolium* morphotypes: assessing antioxidant activity, phenolic and triterpenic compounds variation across different plant parts, *Plants* **13**(7), 1043, doi:10.3390/plants13071043.
- [32] P.R.R. Mesquita, E.C. Nunes, F.N. dos Santos, L.P. Bastos, M.A. Costa, F.D.M. Rodrigues and J.B. de Andrade (2017). Discrimination of *Eugenia uniflora* L. biotypes based on volatile compounds in leaves using HS-SPME/GC–MS and chemometric analysis, *Microchem. J.* **130**, 79-87.
- [33] R.P. Adams (2017). Identification of essential oil components by gas chromatography/mass spectroscopy. Alluredpublishing Co. Carol Stream, Illinois.
- [34] K. Papanisto, J. Vangjeli, B. Ruci, A. Mullaj and X. Qosja (1988-2000). Flora e Shqipërisë. 1-4. Instituti i Kërkimeve Biologjike ASHASH, Tirana, Albania.
- [35] Q Pajazitaj (2004). Përcaktuesi i bimëve Pteridofite dhe Spermatofitite; Prishtina, Kosovo. Universiteti i Prishtinës. Prishtinë Kosovë.
- [36] M. Josifović (1970-1977). Flora SR Srbije 1-9. Srpska akademija nauka i umetnosti. Beograd, Srbija.
- [38] T. Tutin, V. Heywood, N. Burges, D. Valentine, S. Walters, D. Webb (1964) Flora Europaea. Cambridge, UK. University Press.

- [39] I. Iliev, V. Nedelchev, V. Gandova and A. Stoyanova (2023). Influence of vegetation on the composition of essential oil from (*Melissa officinalis* L.). *BIO Web Conf.* **58**, 01018. doi:10.1051/bioconf/20235801018.
- [40] A. Labhar, Y. El-Mernissi, N. Ahidar, A. Zouhri, O. Benamari, F. Siddique, M. Bashir, A. Salhi, M. Ahari, S. Ibenmoussa and M. Bourhia, (2024). Seasonal Variations in the Essential Oil Composition and Biological Activities of Wild *Lavandula dentata* L. *Nat. Prod. Commun.* **19**(2).
- [41] F.Z. Benomari, M. Sarazin, D. Chaib, A. Pichette, H. Boumghar, Y. Boumghar and N. Djabou (2023). Chemical variability and chemotype concept of essential oils from Algerian wild plants. *Mol.* **28**(11), 4439.
- [42] A. Hajdari, B. Mustafa, G. Ahmeti, B. Pulaj, B. Lukas, A. Ibraliu, G. Stefkov, C.L. Quave and J. Novak, (2015). Essential oil composition variability among natural populations of *Pinus mugo* Turra in Kosovo. *SpringerPlus* **4**, 1-13.
- [43] A. Hajdari, B. Mustafa, A. Kaçiku, X. Mala, B. Lukas, A. Ibraliu, G. Stefkov and J. Novak, (2016). Chemical composition of the essential oil, total phenolics, total flavonoids and antioxidant activity of methanolic extracts of *Satureja montana*, *Rec. Nat. Prod.* **10** (6). 750-760.

**ACG**  
**publications**

© 2024 ACG Publications

**PSE**  
Meeting  
2023

**PSE Natural Drug Discovery  
Current Approach and  
Future Perspectives**  
12-15 October 2023, Antalya, Türkiye



# **CERTIFICATE of ATTENDANCE**

**BLEDAR PULAJ**

Thank you for your attendance to the  
**PSE Natural Drug Discovery, Current Approach and Future Perspectives**  
held in Antalya, Türkiye on October 12-15, 2023

**Didem Şöhretoğlu**  
Chair



# PSE Meeting 2023

PSE Natural Drug Discovery  
Current Approach and  
Future Perspectives  
12-15 October 2023, Antalya, Türkiye

## Abstract Book

# PSE Meeting 2023

**PSE Natural Drug Discovery**  
Current Approach and  
Future Perspectives  
12-15 October 2023, Antalya, Türkiye

**Dear Colleagues,**



On behalf of the Phytochemical Society of Europe and the Organizing Committee, we are delighted to invite you to join the conference on 'Natural drug discovery: Current approach and future perspectives', which will be held in Antalya, Türkiye, 12-15 October 2023.

The conference's main objective is to bring natural product and food scientists together to share the most recent research findings, concepts, and experiences.

The congress will focus on all main topics of natural products research: Isolation and structure elucidation; omics-based approaches; ethnopharmacology and phytotherapy; biosynthesis and biotechnology of secondary metabolites; production, quality and regulatory aspects of herbal preparations, food supplements as well as functional foods; natural products in veterinary medicine, cosmetic and food area.

Antalya is one of the world's foremost tourist destinations where mountains and sea meet each other. The city has many well-preserved historical sites and ancient settlements dating back thousands of years. There is history, natural beauty, and a rich variety of local lifestyles to be discovered.

We warmly invite you to this exciting conference. Please join us and have a great time in Antalya!

**Professor Didem Şöhretoğlu**  
**Chair of conference**

<b>PP-48. Phenolic Compounds Contents and Antioxidant Activity of <i>Hypericum calycinum</i> L.</b>	
Derya Çiçek Polat <sup>1</sup> , Safa Gümüşok <sup>1</sup> , Ceyda Sibel Kılıç <sup>1</sup> .....	111
<b>PP-49. Comparison of <i>Hypericum elongatum</i> Ledeb. ex. Rchb. and <i>Hypericum scabrum</i> L. in Terms of Phenolic Compounds and Antioxidant Activity</b>	
Derya Çiçek Polat <sup>1</sup> , Safa Gümüşok <sup>1</sup> , Ceyda Sibel Kılıç <sup>1</sup> .....	112
<b>PP-50. Potential Coronaviral entry Inhibitors Identified <i>In Silico</i> and <i>In Vitro</i> from a Large Natural Product Library</b>	
Nasim Shahhamzehei <sup>1</sup> , Thomas Efferth <sup>1</sup> .....	113
<b>PP-51. Neuroprotective Effects of <i>Gentiana olivieri</i>: Evaluating the Impact of Different Extracts on Immune Response</b>	
Deniz Arca Çakır <sup>1,2</sup> , Sevda Karabeyeser <sup>3</sup> , Sonia Sanajou <sup>1</sup> , Pınar Erkekoğlu <sup>1,2</sup> , Funda N. Yalçın <sup>3</sup> .....	114
<b>PP-52. The Protective Effects of <i>Polygala</i> species on Lipopolysaccharide-Induced Neurotoxicity</b>	
Gözde Işık <sup>1,2</sup> , Nadire Özenver <sup>3</sup> , Zübeyde Uğurlu Aydın <sup>4</sup> , Ali A Dönmez <sup>4</sup> , Hülya Tezel <sup>1</sup> , Pınar Erkekoğlu <sup>1,2</sup> , Funda N. Yalçın <sup>3</sup> .....	115
<b>PP-53. Targeting-Sensitive and Multi-Drug Resistant CCRF-CEM Leukemia Cells with Benzofuran-Isatin Conjugate</b>	
Chunmei Jin <sup>1</sup> , Mohamed-Elamir F. Hegazy <sup>1,2</sup> , Nadeen T. Ali <sup>1</sup> , Mahmoud A. A. Ibrahim <sup>3</sup> , Wagdy M. Eldehna <sup>4</sup> , Thomas Efferth <sup>1</sup> .....	116
<b>PP-54. On The Trial of Novel Foods: Romanian <i>Ajuga reptans</i> L. Extracts - Volatile Phytochemicals, Aromas, and Antioxidant Properties</b>	
Georgiana-Luminita Gavrila <sup>1</sup> , Magdalena Wrona <sup>2</sup> , Michał Świeca <sup>3</sup> , Cristina Nerin <sup>2</sup> .....	117
<b>PP-55. Botanical Identification of <i>Rumex</i> species: A Biosystematic Approach</b>	
Filiz Boyalı, L. Ömür Demirezer .....	118
<b>PP-56. Targeted Metabolomics Data Analysis on Different <i>Rumex</i> species Growing in Türkiye</b>	
Filiz Boyalı <sup>1</sup> , Engin Koçak <sup>2</sup> , Emirhan Nemitlu <sup>3</sup> , L. Ömür Demirezer <sup>1</sup> .....	119
<b>PP-57. Chemical Composition of Essential Oil of <i>Achillea coarctata</i> Poir. from Kosovo and Albania</b>	
Bledar Pulaj <sup>1</sup> , Behxhet Mustafa <sup>1</sup> , Genista Mustafa-Çerkini <sup>2</sup> , Avni Hajdari <sup>1</sup> .....	120
<b>PP-58. Identification of Narciclasine as <i>in vitro</i> Anti-inflammatory Component of <i>Cyrtanthus contractus</i> by Correlation-based Metabolomics</b>	
Lucie Rárová <sup>1</sup> , Bhukumthetho Ncube <sup>2</sup> , Johannes Van Staden <sup>2</sup> , Robert Fürst <sup>3</sup> , Miroslav Strnad <sup>1</sup> , Jiri Gruz .....	121

**PP-57. Chemical Composition of Essential Oil of *Achillea coarctata* Poir. from Kosovo and Albania**

**Bledar Pulaj<sup>1</sup>, Behxhet Mustafa<sup>1</sup>, Genista Mustafa-Çerkini<sup>2</sup>, Avni Hajdari<sup>1</sup>**

<sup>1</sup>Department of Biology, Faculty of Mathematical and Natural Science, University of Prishtina "Hasan Prishtina", Mother Theresa St. 10000 Prishtinë, Kosovo

<sup>2</sup>Alma Mater Europaea Campus College Rezonanca, Veternik, 10000 Prishtinë, Kosovo

Corresponding author: [bledar.pulaj@uni-pr.edu](mailto:bledar.pulaj@uni-pr.edu)

The genus *Achillea* comprises approximately 130 species distributed throughout Europe, Asia, and other regions. Several species of this genus are also prevalent in Kosovo and Albania, which remain understudied regarding their phytochemical properties. *A. coarctata* Poir. in particular, stands out among those species. This study was carried out to analyse the chemical composition of essential oils (EO) obtained from *A. coarctata* growing in the wild populations of Albania and Kosovo. The plant material was collected in Mokna Mountain (Kosovo) and Surroj, Kukes (Albania) in 2018 and 2019. The EO was obtained by the hydrodistillation method using a Clevenger apparatus and stored in the freezer until further analysis. A GC coupled with FID and MS detectors was used to determine the EO's chemical composition. The main identified constituents in both populations were: Hexadecanoic acid, Viridiflorol, Caryophyllene oxide, Camphor, 1,10-di-epi-Cubenol, Khusilol, 1,8-Cineole, Camphene, Borneol, (2E,6E) Farnesyl acetone,  $\beta$ -pinene etc. However, the concentration of the main constituents varied depending on the origin of the plant material. For example, hexadecanoic acid was a major constituent EO originating from Mokna and ranged from 6.75 to 10.4%, while it was completely missing in EO obtained from the plant collected in Surroi. The Viridiflorol concentration in EO from Mokna ranged from 6.25 to 10.43%, while it was slightly higher (7.54-11.02%) in EO originating from and in Surroj. 1,8-Cineole was the primary constituent in the EO from Surroj (11.5 to 17.3%), but in EO originated from Mokna, it was found in minimal concentration (1.41-3.1%). Additionally, in EO arising from Mokna, the Borneol (6.26-11.0%) and  $\beta$ -pinene (6-11.2%) were found in higher concentrations than in Surroj (1.01-1.8%) and (2.1-2.9), respectively. Two distinct chemotypes were observed based on the differing constituents identified through the analysis. This variation between the two populations may be due to the different environmental conditions under which they grow.

# PSE Natural Drug Discovery Current Approach and Future Perspectives

12-15 October 2023, Antalya, Türkiye

# PSE Meeting 2023



# CERTIFICATE OF PARTICIPATION

This certificate is proudly presented to

*Bledar Pulaj*

has participated in **International Conference on New Achievements in Science, Technology and Arts - ICNA-STA**,  
organized by the Haxhi Zeka University and a consortium of 14 researchcult partners, on 14 & 15 April 2022, in Peja, Kosovo

with the abstract entitled

**Antioxidant Activity Of The Methanolic Extracts Of Achillea Sp. Growing Wild In Kosovo**




Prof. ass. dr. Alma Shehu Lokaj  
Conference Chairperson

# Book of Abstracts

**INTERNATIONAL CONFERENCE ON NEW ACHIEVEMENTS IN  
SCIENCE, TECHNOLOGY AND ARTS  
ICNA-STA**

**4 – 5 May 2023, Prishtina, Kosova**



## Antioxidant Activity of the Methanolic Extracts of *Achillea* sp. Growing Wild in Kosovo

Bledar Pulaj, Bardhë Selmani, Avni Hajdari\*, Behxhet Mustafa

*Department of Biology, Faculty of Mathematics and Natural Science, University of Prishtina, Prishtinë Kosovo.*

\* *Corresponding author e-mail (avni.hajdari@un-pr.edu)*

### Abstract

The genus *Achillea* (Asteraceae) has several native species in Kosovo, growing in different habitats. Their populations represent an important source of secondary metabolites, including antioxidants. Due to that, the interest in *Achillea* sp. is increasing rapidly, making them important species in terms of economic values. The *A. millefolium*, is an exemplary species collected in Kosovo and exported to different EU countries. Aiming to assess the antioxidant activity of methanolic extracts, plant material of 10 plant species (*A. ageratifolia*, *A. abrotanoides*, *A. alexandri regis*, *A. millefolium*, *A. tanacetifolia*, *A. atrata*, *A. chrysocoma*, *A. lingulata*, *A. clypeolata* and *A. nobilis*) were collected in different growing wild populations in Kosovo.

Antioxidant activities (DPPH and FRAP) were determined using spectrophotometric methods. According to the DPPH assay method, the antioxidant activity of the analysed species ranged from 62.6 to 255.4 mg TE/g dm. While the antioxidant activity of the studied plant extracts using the FRAP assay method ranged from 104.2 to 388.2 mg TE/g dm. The highest antioxidant activity (determined by DPPH and FRAP antioxidative systems) was found in *A. lingulata*, while the lowest was found in *A. ageratifolia* plant extract. Statistical analysis revealed that both antioxidative systems are positively correlated with the total phenolics and flavonoids. Thus, the interspecific antioxidative variability reflects the species' phytochemical background, which was also impacted by environmental factors. Further investigation and corroboration with detailed chemical analyses are needed to confirm the correlation between different classes of secondary metabolites with antioxidative activities.

**Keywords:** *Antioxidant activity, DPPH, FRAP, Achillea, Kosovo.*

**INTERNATIONAL CONFERENCE ON NEW ACHIEVEMENTS IN SCIENCE, TECHNOLOGY AND ARTS  
 ICNA-STA  
 14th -15th April 2022, Peja, Kosovo**

---

**KEY SPEAKERS**

Curtis R. Young	<i>Iowa State University</i>
Rifat Latifi	<i>Minister of Health, Kosovo / Adjunct Professor of surgery at the University of Arizona</i>
Fiorenza Deriu	<i>University of Sapienza</i>
Diana Kopeva	<i>University of National World Economy</i>
Lidija Ćurković	<i>University of Zagreb</i>
Mihone Kerolli	<i>International Business College Mitrovica</i>
Nikša Alfirević	<i>University of Split – Faculty of Economics, Business and Tourism, Split, Croatia / Editor, Journal of Contemporary Management Issues</i>
Krzysztof Szymanski	<i>SGH Warsaw School of Economics</i>
Besnik Krasniqi	<i>University of Prishtina</i>

**SCIENTIFIC COMMITTEE**

Adrian Besimi	South East European University
Agim Rexhepi	University of Prishtina "Hasan Prishtina"
Armand Krasniqi	University "Haxhi Zeka" in Peja
Agron Bajraktari	University of Applied Sciences in Ferizaj
Aida Alla	AAB College
Aida Goga	University of Tirana
Alush Musaj	University of Mitrovica "Isa Boletini"
Ani Arutunyan	University of National and World Economy



# CERTIFICATE OF ATTENDANCE

This is to certify that

**BLEDAR PULAJ**

has attended the **"4th International Congress of Plant Science and Technology"** on 29.10.2021 - 31.10.2021

A handwritten signature in blue ink, consisting of several loops and a horizontal line at the bottom.

**Prof. Adel Dabbagh Mohammadi Nassab**  
Chair of Congress



*IPSAT*





# 4th International Plant Science and Technology Congress

30 OCTOBER 2021  
ONLINE

ABSTRACT BOOK

E-ISBN: 978-605-71081-1-1



# IPSAT

## 4<sup>th</sup> International Plant Science and Technology Congress 30 October, 2021, Online

### CONTENS

ORGANISING COMMITTEE .....	6
SCIENTIFIC COMMITTEE.....	7
PREFACE .....	8
IPSAT 2021 – FINAL PROGRAM.....	9
CONTRIBUTIONS TO THE EXPLORATION OF THE ALBANIAN FLORA.....	15
NOTES ON THE PECULIARITIES OF THE FLORA AND VEGETATION OF THE REPUBLIC OF KOSOVO .....	16
PLANT PROGRAMMED CELL DEATH IN PLANTS .....	17
PERFORMANCE AND ADAPTABILITY OF NEW DIVERSE COTTON CULTIVARS EVALUATION FOR FIBER QUALITY AND YIELD IMPROVEMENT, A REVIEW .....	18
HIGHLY THREATENED VEGETATION AND HABITATS OF EUROPEAN SIGNIFICANCE IN VJOSA RIVERSCAPE (ALBANIA) .....	19
EFFECT OF UV-C APPLICATIONS COMBINED WITH COLD MACERATION AND THERMOVINIFICATION TECHNIQUES ON <i>TRANS</i> -RESVERATROL LEVELS IN CABERNET SAUVIGNON WINES .....	21
LAND COVER DATA ASSESSMENT IN ALBANIA.....	22
MORPHO- ANATOMICAL PROPERTIES OF THE BALKAN ENDEMIC PLANT <i>ACHILLEA</i> <i>AGERATIFOLIA</i> SUBSP. <i>AIZOON</i> (GRISEB.) HEIMERL.....	23
VARIABILITY OF THE CHEMICAL COMPOSITION OF ESSENTIAL OIL OF <i>ACHILLEA NOBILIS</i> FROM KOSOVO.....	24

# IPSAT

4<sup>th</sup> International  
Plant Science and Technology Congress  
30 October, 2021, Online



E-ISBN: 978-605-71081-1-1

**Abstract Book**

# IPSAT

## 4<sup>th</sup> International Plant Science and Technology Congress 30 October, 2021, Online

### Oral Presentation

#### **Morpho- Anatomical Properties of the Balkan Endemic Plant *Achillea Ageratifolia* Subsp. *Aizoon* (Griseb.) Heimerl**

**Bledar PULAJ<sup>1</sup>, Avni HAJDARI<sup>1</sup>, Xhavit MALAJ<sup>2</sup>, Behxhet MUSTAFA<sup>1</sup>**

<sup>1</sup>Department of Biology, Faculty of Mathematical and Natural Science, University of Prishtina, Mother Theresa St. 10000 Prishtinë, Kosovo

<sup>2</sup>Sharri National Park, Petrovë, 20000 Prizren, Kosovo  
[bledar.pulaj@uni-pr.edu](mailto:bledar.pulaj@uni-pr.edu)

#### **Abstract**

*Achillea ageratifolia* (Sibth. & Sm.) Benth. & Hook.f. in Kosovo is represented with two subspecies, ssp. *aizoon* (Griseb.) Heimerl and ssp. *serbica* (Nyman) Heimerl, respectively. Both subspecies and nine other *Achillea* species in Kosovo are strictly protected (Administrative instruction of MESPI no. 12/2020). This research aims to identify morpho-anatomical characteristics that could be used as markers to accurately identify *A. ageratifolia* ssp. *aizoon*. Morpho-anatomical characteristics were investigated in 10 individuals, collected in Novobërdë (Kosovo), in June 2018. Leaves were cut into small pieces (approximately 1cm<sup>2</sup>) and bleached with hydrogen peroxide and saturated chloral hydrate solution. Microscopic analyses were carried using the Zeiss (Axiostar Plus) microscope. Microscopic images were acquired with a DP700 camera, using an Olympus Vanox microscope. The following morphological characteristics were analyzed: plant height (10.29±cm), phyllotaxy (alternate), length (1.59±0.15cm) and width (0.4±0.13cm) of stem leaves, length (2.83±0.54cm) and width (0.45±0.08cm) of the basal leaves, their shape (spathulate, entire or crenulate, rarely pectinate-pinnatifid at the base), inflorescence type (solitary capitulum), and the color of the flower (white). Except for the morphological characteristics, the anatomical structures were analyzed too. In leaves were analyzed following anatomical characteristics: the number of stomas (119.97±33.19/mm<sup>2</sup> in upper epidermis (UE) and 77.57±19.89/mm<sup>2</sup> in lower epidermis (LE)), stomas length (26.49±2.23µm in UE and 26.94±2.2µm in the LE), stomas width (23.68±3.10µm in UE and 24.24±2.13µm in the LE), the density of glandular trichomes (16.54±9.10/mm<sup>2</sup> in UE and 13.02±6.21/mm<sup>2</sup> in LE), the density of non-glandular trichomes (13.34±11.53/mm<sup>2</sup> in UE and 10.07±11.79/mm<sup>2</sup> in LE). Furthermore, the thickness of the upper (23.53±2.68µm) and lower (13.00±3.59µm) epidermis, the thickness of the palisade parenchyma (dorsal: 25.3±4.77µm and ventral: 92.60±29.12µm) and spongy parenchyma (81.72±23.46µm) were also analyzed too. While on the stem, the number (14.00±2.21), the type (collateral type) of vascular bundles and thickness of the stems (1154.30±265.15µm) stems were analyzed.

**Keywords:** *Achillea ageratifolia*, morphological-anatomical characteristics, Balkan endemic plant



# 4th International Plant Science and Technology Congress

30 OCTOBER 2021  
ONLINE

ABSTRACT BOOK

E-ISBN: 978-605-71081-1-1

 **ANT ACADEMY**





**Konferenca Shqiptare  
e Biologjisë & Mjedisit**



**Albanian Conference of  
Biology & Environment**

**KShBM – ACBE 2018**

*Sjellim afër shkencëtarët Shqiptarë  
Bringing together Albanian scientists*

**Organizuar nga Universiteti i Prishtinës “Hasan Prishtina”, Universiteti i Tiranës dhe Universiteti i Tetovës**

## **CERTIFIKATË**

**Kjo certifikatë i jepet Bledar Pulaj për pjesëmarrje në Konferencën Shqiptare të Biologjisë dhe Mjedisit (KShBM 2018)**

**28-29 Shtator 2018, Prishtinë**

**Prof. dr. Aleko Miho**

**Koordinator i konferencës**

**Prof. dr. Tahir Arbneshi**

**Dekan i FSHMN-së, Universiteti i Prishtinës**



**KShBM – ACBE 2018**  
Sjellim afër shkencëtarët Shqiptarë  
Bringing together Albanian scientists

**KSHBM / ACBE 2018:**

**Libri i abstrakteve / Book of abstracts**

Qëllimi i Konferencës është të mbledhë bashkë shkencëtarët nga rajoni dhe më gjerë, për të diskutuar punët e tyre dhe për të nxitur bashkëpunimin në fushat përkatëse. Kjo jo vetëm në frymën e fuqizimit të shkencave biologjike në rajon, në fushat themelore, apo në ato praktike, por dhe për më shumë ndërgjegjësim se sëbashku mund të ecim më mirë, dhe më larg.

\*\*\*

Aim of the Conference is to bring together the scientists from the region and beyond, to steer up the scientific dialogue and the cooperation in related fields. It aims not only strengthening the biological sciences, in basic and practical topics, but also increasing the awareness that together we can go well and far.

**KShBM / ACBE 2018**

**LIBRI I ABSTRAKTEVE / ABSTRACT BOOK**

**Konferenca Shqiptare  
e Biologjisë & Mjedisit**



**Albanian Conference on  
Biology & Environment**

**KShBM – ACBE 2018**

*Sjellim afër shkencëtarët Shqiptarë / Bringing together Albanian scientists*

# **LIBRI I ABSTRAKTEVE / ABSTRACT BOOK**

**Prishtinë / Prishtina 2018**

**ISBN:**





***Konferenca Shqiptare e Biologjisë & Mjedisit /  
Albanian Conference of Biology and Environment  
(KShBM / ACBE 2018)***

***Mbajtur në Universitetin e Prishtinës në 28-29 Shtator 2018 /  
Held in University of Prishtina in: 28-29 September 2018.***

*Me Ekskursion në Malet e Sharrit në 30 Shtator 2018 /  
With Excursion in Sharri Mountains in: 30 September 2018*

***LIBRI I ABSTRAKTEVE / ABSTRACT BOOK***

***Versioni / Version on line***

***<http://www.fshn.edu.al/>***

***Prishtinë / Prishtina 2018***

## **KShBM / ACBE 2018: Libri i abstrakteve / Book of abstracts**

### **Organizuar nga / Organized by:**

- Universiteti i Prishtinës / University of Prishtina  
([www.fshmn.uni-pr.edu](http://www.fshmn.uni-pr.edu))
- Universiteti i Tiranës / University of Tirana  
([www.fshn.edu.al](http://www.fshn.edu.al))
- Universiteti i Tetovës / University of Tetova  
([www.unite.edu.mk/](http://www.unite.edu.mk/))

### **Mbështetur nga: / Sponsored by:**

- Universiteti i Prishtinës / University of Prishtina;
- Universiteti i Tiranës / University of Tirana;
- Universiteti i Tetovës / University of Tetova;
- ERAMED, Prishtinë / Prishtina;
- VITECH sh.p.k. , Prishtinë / Prishtina;
- Dreni Pharm N.Sh., Veternik Prishtinë / Prishtina.

### **Botuar nga: / Edited by:**

Aleko Miho, Avni Hajdari, Zeqir Veselaj, Dashnor Nebija, Jani Marka,  
Merita Xhetani, Besnik Rexhepi, Gazmend Iseni, Agon Memeti.

**Shtypur në Republikën e Kosovës / Printed in the Republik of Kosovo**

*Shtypshkronja / Printing house Office Printy*

---

**Tirazhi: 200 kopje**

**Formati: 70 x 100/16**

*Sëbashku mund të ecim në mirë, dhe në larg ...  
| Together we can go well and far ...*

## Komiteti organizator / Organizing committee

- **Prof. Aleko Miho**, Departamenti i Biologjisë, Fakulteti i Shkencave të Natyrës, Universiteti i Tiranës / Department of Biology, Faculty of Natural Sciences, University of Tirana. mob. +355(0)68 2707208; e-mail: [aleko.miho@fshn.edu.al](mailto:aleko.miho@fshn.edu.al);
- **Prof. Asoc. Avni Hajdari**, Departamenti i Biologjisë, Fakultetit i Shkencave Matematike dhe Natyrore, Universiteti i Prishtinës / Department of Biology, Faculty of Mathematics - Natural Sciences, University of Prishtina, tel: +381 38 249 872; mob: +377 44 600 629; e-mail: [avni.hajdari@uni-pr.edu](mailto:avni.hajdari@uni-pr.edu);
- **Prof. Asoc. Zeqir Veselaj**, Departamenti i Mësimdhënies së Biologjisë, Fakulteti i Edukimit, Universiteti i Prishtinës / Department of Biology Teaching, Faculty of Education, University of Prishtina, e-mail: [zeqir.veselaj@uni-pr.edu](mailto:zeqir.veselaj@uni-pr.edu);
- **Prof. Asoc. Dashnor Nebija**, Departamenti i Farmacisë, Fakulteti i Mjekësisë, Universiteti i Prishtinës / Department of Pharmacy, Faculty of Medicine, University of Prishtina. e-mail: [dashnor.nebija@uni-pr.edu](mailto:dashnor.nebija@uni-pr.edu);
- **Dr. Jani Marka**, Departamenti i Biologjisë, Fakulteti i Shkencave të Natyrës, Universiteti i Tiranës / Department of Biology, Faculty of Natural Sciences, University of Tirana, e-mail: [jani.marka@fshn.edu.al](mailto:jani.marka@fshn.edu.al);
- **Dr. Merita Xhetani**, Departamenti i Biologjisë, Fakulteti i Shkencave të Natyrës, Universiteti i Tiranës / Department of Biology, Faculty of Natural Sciences, University of Tirana. e-mail: [merita.xhetani@fshn.edu.al](mailto:merita.xhetani@fshn.edu.al);
- **MSc. Besnik Rexhepi**, Departamenti i Biologjisë, Fakulteti i Shkencave Matematike-Natyrore, Universiteti i Tetovës / Department of Biology, Faculty of Mathematics - Natural Sciences, University of Tetova, e-mail: [besnik.rexhepi@unite.edu.mk](mailto:besnik.rexhepi@unite.edu.mk);
- **Dr. Gazmend Iseni**, Departamenti i Biologjisë, Fakulteti i Shkencave Matematike-Natyrore, Universiteti i Tetovës / Department of Biology, Faculty of Mathematics - Natural Sciences, University of Tetova, e-mail [gazmend.iseni@unite.edu.mk](mailto:gazmend.iseni@unite.edu.mk);
- **Prof.Asst. Agon Memeti**, Departamenti i Informatikës, Fakulteti i Shkencave Matematike-Natyrore, Universiteti i Tetovës / Department of Informatics, Faculty of Mathematics - Natural Sciences, University of Tetova, e-mail [agon.memeti@unite.edu.mk](mailto:agon.memeti@unite.edu.mk).

## VEÇORITË MORFO-ANATOMIKE TË BIMËS ENDEMIKE *ACHILLEA ALEXANDRI-REGIS* BORN. & RUDSKY

**Bledar Pulaj**

*Departamenti i Biologjisë, Fakulteti i Shkencave Matematiko-Natyrore,  
Universiteti 'Hasan Prishtina', Prishtinë.*

*Kontakt: [bledar.pulaj@uni-pr.edu](mailto:bledar.pulaj@uni-pr.edu)*

Gjinia *Achillea* ka përhapje të gjerë në Kosovë, ku deri tani janë njohur 18 lloje prej të cilave 10 janë lloje rreptësisht të mbrojtura (Udhëzimi administrativ i MMPH-së me nr. 12/2017), përfshirë llojin *Achillea alexandri-regis* Bornm. & Rudsky, i përhapur vetëm në Oshlak (Malet e Sharrit, Kosovë). Qëllimi i këtij hulumtimi ka qenë njohja e veçorive morfo-anatomike, të cilat mund të përdoren si shenja për njohjen e saktë të llojit *A. alexandri-regis*. Veçoritë morfo-anatomike janë analizuar në 10 individë, të mbledhur në Oshlak në muajin Korrik 2017. Për njohjen me veçoritë anatomike të gjethes, ato janë prerë në pjesë të vogla (afërsisht 1 cm<sup>2</sup>), ndërsa pigmentet janë larguar paraprakisht me peroksid hidrogjeni dhe tretësirë të ngopur të kloral hidratit. Analizat mikroskopike janë bërë me mikroskop Zeiss axiostar plus. Janë analizuar veçoritë morfologjike si në vijim: lartësia e bimës (20-40 cm), fillotaksia (e këmbyer), gjatësia (3.05±0.63 cm) dhe gjerësia (0.75±0.22 cm) e gjethëve të kërcellit, gjatësia (7.05±1.63 cm) dhe gjerësia (1.72±0.31cm) e gjethëve fundore, forma e tyre (gjatore deri heshtore), tipi i lulesës (vastak), numri i luleve në lulesë (16.75±6.66), ngjyra e luleve (e verdhë), etj. Strukturat anatomike të analizuar në gjethë janë: numri i gojzave në epidermë (98.4±16.87/mm<sup>2</sup> sipër dhe 102.9±17.91/mm<sup>2</sup> poshtë), përmasat e gojzave (gjatësia: 33.7±2.22 µm sipër dhe 33.2±2.22 µm poshtë); gjerësia: (26.2±1.10 µm sipër dhe 25.7±0.99 µm poshtë), numri i trikomeve pronditëse në epidermë (3.64±5.27/mm<sup>2</sup> sipër dhe 1.57±2.16/mm<sup>2</sup> poshtë), numri i trikomeve mbrojtëse (21.71±8.21/mm<sup>2</sup> sipër dhe 20.86±4.26/mm<sup>2</sup> poshtë). Gjithashtu, janë hulumtuar edhe trashësia e epidermës së sipërme (27.07±1.06 µm) dhe të poshtme (23.02±0.88 µm), trashësia e indit gardhor (99.8±4.56 µm) dhe indit sfungjeror (84.5±6.74 µm). Në kërcell janë hulumtuar numri i tufave përçuese (22.6±1.89) dhe tipi i tyre (kolaterale e mbyllur).

***Fjalëkyçet:*** *Achillea alexandri-regis*, veçoritë morfo-anatomike, endemike e Kosovës, lloje strikte.

## MORPHO-ANATOMICAL PROPERTIES OF THE ENDEMIC PLANT *ACHILLEA ALEXANDRI-REGIS* BORNM. & RUDSKY

**Bledar Pulaj**

Department of Biology, Faculty of Mathematics and Natural Sciences, University 'Hasan Prishtina', Prishtina.

Contact: [bledar.pulaj@uni-pr.edu](mailto:bledar.pulaj@uni-pr.edu)

Genus *Achillea* is widespread in Kosovo; so far 18 species have been recorded, of which 10 are strictly protected species (Administrative directive of MESP no. 12/2017), including *Achillea alexandri-regis* Bornm. & Rudsky, spread only in Oshlak (Sharri Mountains, Kosovo). The purpose of this research was to identify morpho-anatomical characteristics that could be used as markers for the accurate identification of *A. alexandri-regis*. Morpho-anatomical characteristics were analyzed in 10 individuals, collected in Oshlak, during the July 2017. For identification of anatomical characteristics, the leaves were cut into small pieces (approximately 1cm<sup>2</sup>), while pigments were removed in advance with hydrogen peroxide and saturated chloral hydrate solution. Microscopic analyzes were made with the Zeiss axiostar plus microscope. The following morphological characteristics were analyzed: plant height (20-40 cm), phyllotaxy (alternate), length (3.05±0.63 cm) and width (0.75±0.22 cm) of stem leaves, length (7.05±1.63 cm) and width (1.72±0.31 cm) of the basal leaves, their shape (linear to cuneate), inflorescence type (corymb), the number of flowers in the inflorescence (16.75±6.66), the color of the flowers (yellow), etc. While the anatomical structures analyzed in leaves were: number of stomas (98.4±16.87/mm<sup>2</sup> in upper epidermis and 102.9±17.91/mm<sup>2</sup> in lower epidermis), stomas dimension (length: 33.7±2.22 µm in upper epidermis and 33.2±2.22 µm in the lower epidermis; width: 26.2±1.10 µm in upper epidermis and 25.7±0.99 µm in lower epidermis), the number of glandular trichomes (3.64 ±5.27/mm<sup>2</sup> in upper epidermis and 1.57±2.16/mm<sup>2</sup> in lower epidermis), the number of trichomes (21.71±8.21/mm<sup>2</sup> in upper epidermis and 20.86±4.26/mm<sup>2</sup> in lower epidermis). Furthermore, the thickness of the upper (27.07±1.06 µm) and lower (23.02±0.88 µm) epidermis, the thickness of the palisade (99.8±4.56 µm) and spongy parenchyma (84.5±6.74 µm) were also analyzed too. On the stems were analyzed the number (22.6±1.89) and the type of vascular bundles (closed collateral type).

**Keywords:** *Achillea alexandri-regis*, morpho-anatomical properties, endemic, strict protected species.

UNIVERSITETI I PRISHTINËS  
FAKULTETI I SHKENCAVE MATEMATIKO NATYRORE  
DEPARTAMENTI I BIOLOGJISË



Msc. Bledar R. Pulaj

**VEÇORITË MORFO-ANATOMIKE,  
FITOKIMIKE DHE MOLEKULARE TË DISA  
LLOJEVE TË GJINISË *ACHILLEA*  
(ASTERACEAE) NË KOSOVË**

PUNIMI I DOKTORATËS

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

Pranuar me. 25/06/2025  
Nj. org. Numër Sasia Vlera  
01 2010 3 -

Prishtinë, 2025

UNIVERSITY OF PRISHTINA  
FACULTY OF MATHEMATICS AND NATURAL SCIENCES  
DEPARTMENT OF BIOLOGY



Msc. Bledar R. Pulaj

**MORPHO-ANATOMICAL,  
PHYTOCHEMICAL AND MOLECULAR  
PROPERTIES OF SOME *ACHILLEA*  
(ASTERACEAE) SPECIES IN KOSOVO**

DOCTORAL THESIS

Prishtinë, 2025

UNIVERSITETI I PRISHTINËS  
FAKULTETI I SHKENCAVE MATEMATIKO NATYRORE  
DEPARTAMENTI I BIOLOGJISË



Msc. Bledar R. Pulaj

**VEÇORITË MORFO-ANATOMIKE,  
FITOKIMIKE DHE MOLEKULARE TË DISA  
LLOJEVE TË GJINISË *ACHILLEA*  
(ASTERACEAE) NË KOSOVË**

PUNIMI I DOKTORATËS

Mentori:

Prof. Dr. Behxhet Mustafa

Prishtinë, 2025

## Rezyme

Gjinia *Achillea* L. (Asteraceae) përfshin rreth 130 specie në nivel botëror, prej të cilave 18 janë të dokumentuara në territorin e Kosovës. Këto bimë janë të njohura për diversitetin e tyre të lartë fitokimik, veçanërisht për përmbajtjen e vajrave esenciale, flavonoidëve dhe komponimeve fenolike, që ndërlidhen me përdorimin tradicional mjekësor, si dhe me rëndësinë e tyre farmakologjike dhe ekologjike. Megjithatë, prania e polimorfizmit të theksuar morfologjik dhe kimik, në kombinim me kompleksitetin taksonomik, e bën identifikimin dhe konservimin e specieve një sfidë shkencore, sidomos për taksonet endemike dhe të rrezikuara lokalë.

Ky disertacion trajton studimin e dhjetë specieve të gjinisë *Achillea* të pranishme në Kosovë (*A. millefolium*, *A. ageratifolia*, *A. tanacetifolia*, *A. nobilis*, *A. alexandri-regis*, *A. atrata*, *A. lingulata*, *A. chrysocoma*, *A. abrotanoides*, *A. clypeolata*) përmes një qasjeje integrale që përfshin analiza morfologjike, anatomike, fitokimike dhe molekulare. Mostrat u grumbulluan gjatë viteve 2014–2018. Tiparet morfologjike u ekzaminuan në mënyrë mikroskopike, ndërsa vajrat esenciale u ekstraktuan me hidrodistilim, mikrodistilim dhe HS-SPME. Përbërja kimike e vajrave u analizua me kromatografi gazi (GC/FID dhe GC/MS). Përmbajtja totale e fenoleve dhe flavonoidëve u mat përmes spektrofotometrisë, ndërsa aktiviteti antioksidues u vlerësua me metoda DPPH dhe FRAP. Për analizën filogjenetike molekulare u përdorën sekuenca të regjioneve gjenetike ITS, rbcL, trnL-F dhe psbA-trnH.

Rezultatet treguan variacion të theksuar ndërmjet specieve në aspektin morfologjik dhe anatomik të organeve bimore, si dhe diversitet të lartë kimik në profilin e vajrave esenciale. *A. lingulata* dhe *A. alexandri-regis* u veçuan për aktivitet të lartë antioksidues dhe përmbajtje të konsiderueshme të flavonoidëve dhe fenoleve totale, duke dëshmuar potencial të lartë bioaktiv. Të dhënat molekulare konfirmuan veçantinë taksonomike të disa specieve dhe mbështetën hipotezat e fundit për diversifikimin e gjinisë *Achillea* në Gadishullin Ballkanik. Këto gjetje janë të rëndësishme për hartimin e strategjive të ardhshme të konservimit, për përdorim të qëndrueshëm dhe për qartësimin taksonomik të gjinisë *Achillea* në Evropën Juglindore.

**Fjalët kyçe:** *Achillea* spp., karakteristikat morfo-anatomike, karakteristikat fitokimike, karakteristikat molekulare,

## Summary

The genus *Achillea* L. (Asteraceae) includes approximately 130 species worldwide, of which 18 are recorded in Kosovo. These species are known for their high phytochemical diversity, particularly in essential oils, flavonoids, and phenolic compounds, which underpins their traditional use in medicine, as well as their pharmacological and ecological importance. However, significant morphological and chemical polymorphism, combined with taxonomic complexity, has made species identification and conservation a scientific challenge, particularly for locally endemic and threatened taxa.

This doctoral study investigates ten *Achillea* species present in Kosovo (*A. millefolium*, *A. ageratifolia*, *A. tanacetifolia*, *A. nobilis*, *A. alexandri-regis*, *A. atrata*, *A. lingulata*, *A. chrysocoma*, *A. abrotanoides*, *A. clypeolata*) through an integrative approach combining morphological, anatomical, phytochemical, and molecular analyses. Field collections were conducted from 2014 to 2018, with morphological traits examined via microscopy, and essential oils extracted using hydrodistillation, microdistillation, and HS-SPME. The chemical composition of oils was determined using GC/FID and GC/MS. Total phenolic and flavonoid contents were quantified via spectrophotometry, and antioxidant activity was assessed using DPPH and FRAP assays. Molecular phylogenetic analysis was conducted using DNA sequencing of ITS, *rbcL*, *trnL-F*, and *psbA-trnH* regions.

Results revealed significant interspecific variation in morphological and anatomical features of different plant organs, as well as high chemical diversity in essential oil profiles. *A. lingulata* and *A. alexandri-regis* exhibited notably high antioxidant activity and total flavonoid and phenolic content, highlighting their bioactive potential. Molecular data confirmed the taxonomic distinctiveness of several species and supported recent evolutionary hypotheses regarding *Achillea*'s diversification in the Balkans. These findings are relevant for future conservation strategies, sustainable use, and taxonomic clarification of the genus *Achillea* in southeastern Europe.

**Key words:** *Achillea* spp., morpho-anatomical features, phytochemical features, molecular features,