

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

Blerta Salihu<sup>\*1</sup>, Bexhet Mustafa<sup>1</sup>, Bledar Pulaj<sup>1</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

### 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 ( $\alpha$ -acid) and lupulone ( $\beta$ -acid), and terpenes (essential oils), e.g. myrcene and linalool,  $\beta$ -caryophyllene, humulene, linalool, and  $\alpha$ -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](mailto: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  $\alpha$ -humulene (1.9-28.1%),  $\alpha$ -selinene (0.6-8.7%),  $\beta$ -bisabolol (0.4-8.0%),  $\beta$ -selinene (0.7-5.5%), (*E*)- $\beta$ -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  $\alpha$ -humulene (6.5-24.4%), (*E*)- $\beta$ -farnesene (3.3-13.3%),  $\alpha$ -selinene (0.9-9.9%), trans-caryophyllene (2.2-7.6%), selina-3,7(11)-diene (0.6-4.6%),  $\alpha$ -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  $\alpha$ -humulene. The content of  $\alpha$ -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  $\alpha$ -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 chemopolymorphism among wild populations of this species in Kosovo.

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