

In Vitro Antioxidant, Metal-Chelating, and Enzyme-Inhibitory Potential of Hop Leaves and Cones

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ABSTRACT

Aim. Hop leaves represent an underutilised by-product of hop cultivation and a potential source of bioactive compounds. This study aimed to compare the chemical composition, antioxidant activity, metal-chelating capacity, and enzyme inhibitory potential of leaves and cones from four hop cultivars (Blisk, Nugget, Bačka, and Saphir).

Material and methods. Hop leaf and cone extracts were chemically characterised, and their antioxidant, metal-chelating, and enzyme-inhibitory activities were evaluated using *in vitro* assays. Total phenolic content (TPC) was determined spectrophotometrically, while xanthohumol and lupulone were quantified by HPLC. Chemical profiles were characterised using GC–MS. Antioxidant activity was evaluated using DPPH, ABTS,

FRAP, and CUPRAC assays. Metal-chelating activity and the inhibition of acetylcholinesterase, butyrylcholinesterase, and tyrosinase were also assessed.

Results. Cones exhibited higher TPC and greater concentrations of xanthohumol and lupulone than leaves. The highest TPC was recorded for Blisk cones (22.01 mg GAE/g), whereas leaves contained 6.54–12.70 mg GAE/g. Blisk cones contained the highest xanthohumol concentration (2.55 mg/g) and showed the strongest cholinesterase inhibition, with IC_{50} values of 15.9 mg/mL and 17.98 mg/mL against acetylcholinesterase and butyrylcholinesterase, respectively. Among the leaves, the Blisk cultivar exhibited the highest antioxidant activity and enzyme-inhibitory potential, with acetylcholinesterase inhibition (IC_{50} = 20.4 mg/mL) comparable to that of selected cone samples. In contrast, metal-chelating activity was stronger in leaves (IC_{50} = 3.17–3.64 mg/mL) than in cones (5.93–10.91 mg/mL). Principal component analysis indicated associations between phenolic content, antioxidant activity, and cholinesterase inhibition.

Conclusions. These findings demonstrate that hop leaves are a valuable source of compounds with antioxidant, metal-chelating, and enzyme-inhibitory activities that may be relevant to neuroprotection. In particular, leaves of the Blisk cultivar exhibited biological activity comparable to that of selected cone samples, supporting their further investigation for food, nutraceutical, and pharmaceutical applications.

1. Introduction

Sustainable agricultural practices are increasingly important for meeting the growing demand for food, medicine, and other plant-based products while minimising environmental impact [1]. The utilisation of plant by-products can reduce waste and improve resource efficiency, contributing to more sustainable production systems [2]. While many plants are primarily valued for their edible or medicinal parts, less-used tissues such as leaves, stems, seeds, peels, and roots often contain bioactive secondary metabolites with potential applications in the food, cosmetic, and pharmaceutical industries [3–5]. The valorisation of such materials not only creates new opportunities for their utilisation but also supports a more comprehensive use of plant resources. Integrating plant by-products into consumer products, however, requires appropriate quality control to ensure their safety [6,7].

Hops (*Humulus lupulus* L.) are widely used in the food, pharmaceutical, and cosmetic industries and represent a rich source of biologically active compounds [8–10]. Their diverse biological properties, including antioxidant, anti-inflammatory, neuroprotective, antimicrobial, and anti-cancer activities, are attributed to the presence of prenylated flavonoids, hop acids, and terpenes [11]. Among these compounds, xanthohumol has attracted particular attention due to its neuroprotective and neuroregenerative potential. Studies have shown that xanthohumol attenuates neu-

ronal damage, modulates cholinergic pathways, activates the Nrf2-ARE antioxidant pathway, and improves cognitive function in experimental models of neurodegeneration [12–15]. Additionally, hop-derived iso- α -acids have demonstrated anti-inflammatory and cognition-related effects in animal models [16,17], while β -acids such as lupulone exhibit potent *in vitro* antioxidant activity [18]. However, these findings should be interpreted with caution, as the effects of isolated hop compounds were generally observed at pharmacological concentrations, while extract-based studies often employed non-purified preparations.

Although hop cones have been extensively studied, other plant parts, particularly leaves, remain relatively underexplored. Previous studies have identified bioactive compounds in hop leaves and suggested their pharmacological potential; however, their biological activity remains insufficiently characterised. As an underutilised by-product of hop cultivation, hop leaves may represent a valuable source of antioxidant compounds and other bioactive constituents potentially relevant to neuroprotection. The four cultivars were selected to represent different geographical origins and technological types, including bitter cultivars Blisk (Slovenia) and Nugget (USA), and aroma cultivars Bačka (Serbia) and Saphir (Germany), enabling comparison between different hop types and geographical origins. Therefore, this study investigated the chemical composition and biological activ-

ity of leaves and cones from four hop cultivars (Blisk, Nugget, Bačka, and Saphir), with particular emphasis on their antioxidant, metal-chelating, and enzyme-inhibitory activities.

2. Material and methods

2.1. Plant material

The study used four hop varieties, categorised as either aromatic or bitter types, originating from different regions worldwide: Blisk (bitter) from Slovenia, Nugget (bitter) from the USA, Bačka (aromatic) from Serbia, and Saphir (aromatic) from Germany. These hops were cultivated under natural field conditions on medium-heavy alluvial soil at the Agricultural Experimental Station of the Institute of Soil Science and Plant Cultivation in Puławy, Poland (51°24'19"N, 21°57'32"E, altitude 116 m). Further details on plant material collection and cultivation conditions are provided in the Supplementary Material.

2.2. Extraction of hop plant material

The extraction procedure was adapted from our previously reported ultrasound-assisted extraction protocol [19] with slight modifications. The hop extracts were prepared as follows: each sample, consisting of Blisk, Nugget, Bačka, and Saphir hop leaves and cones, was ground. Then, 5 g of each material was extracted with 75 mL of 70% (v/v) methanol at 50°C for 30 minutes in an ultrasonic bath (constant sonication, frequency 37 kHz, ultrasonic peak max. 800 W) (Thermo Fisher Scientific, Waltham, MA, USA). Following the initial extraction, the solid residues were filtered, re-soaked in 75 mL of fresh 70% methanol, and subjected to a second extraction under the same conditions. The resulting filtrates were combined and adjusted to a final concentration of 0.125 g of raw material per mL for each extract.

2.3. Analysis of total phenolic content

The total phenolic content (TPC) was determined using a modified Folin-Ciocalteu method as previously described [19]. Briefly, the plant extracts or gallic acid standards were mixed with the Folin-Ciocalteu reagent and sodium carbonate, incubated, and the absorbance was measured at 760 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of plant

material. TPC was performed in three independent experiments, with four technical replicates per experiment. The results are presented as the mean $\pm$ standard deviation.

2.4. High-performance liquid chromatography analysis

Xanthohumol and lupulone levels were analysed through high-performance liquid chromatography equipped with a diode array detector (HPLC-DAD) using a Shimadzu system (Kyoto, Japan). The analysis followed a previously established and validated method by Stasiłowicz-Krzemień et al. [19]. Separation was performed on a ReproShell PFP column (150 $\times$ 4.6 mm, 2.7 μ m) using 0.1% trifluoroacetic acid and methanol as the mobile phases, with gradient elution from 50% to 85% methanol over 35 min. The flow rate was 1.0 mL/min, the column temperature was 40°C, the injection volume was 10 μ L, and detection was performed at 323 nm.

2.5. Gas chromatography analysis

For gas chromatography analysis, samples were prepared in glass Petri dishes (4 cm in diameter), each filled with 10 mL of a 70% extract from hop leaves or cones and gently heated at 55°C until complete evaporation. The dry extract remaining on the dish was dissolved in methanol and filtered through a 0.20 μ m syringe filter. The filtrate was used for GC-MS analysis. The analyses were performed using a Bruker gas chromatograph Scion 436-GC with mass detection (single quadrupole (Q)) under the following parameters: electron energy of 70 eV; ion source temperature at 200°C; silica column – VF-5 ms (30 m $\times$ 0.25 mm $\times$ 0.39); df = 0.25 μ m; carrier gas – helium; gas flow rate – 1 mL/min. Temperature program: Enable coolant at 50.0°C; Coolant Timeout 20.0 min; Stabilisation Time 0.5 min; Temperature 60.0°C; Hold 3.00 min; Total 3.0 min; Temperature 280.0°C; Rate 10.0°C/min; Hold 35.0 min; Total 60.0 min. Compound identification was performed by comparing retention times and mass spectra against the NIST MS database.

2.6. Antioxidant, metal-chelating, and enzyme-inhibitory activities of hop extracts

Antioxidant activity was assessed using four complementary assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-

sulfonic acid) (ABTS), cupric reducing antioxidant capacity (CUPRAC), and ferric reducing antioxidant power (FRAP), using methods previously described in the literature [19]. The DPPH and ABTS assays evaluated the radical-scavenging activity of the extracts, whereas CUPRAC and FRAP assessed their reducing capacity through the reduction of Cu(II)–neocuproine and Fe(III)–TPTZ complexes, respectively. The absorbance was measured at 517, 734, 450, and 593 nm for the DPPH, ABTS, CUPRAC, and FRAP assays, respectively. Trolox was used as the reference standard. The results were reported as milligrams of Trolox per gram of plant material [20–22]. The ability of the extracts to chelate Fe²⁺ and their inhibitory effects on acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), and tyrosinase were tested using previously described methods [19]. Galantamine was used as a positive control in the AChE and BuChE inhibition assays, whereas azelaic acid was used as a positive control in the tyrosinase inhibition assay. All assays were performed in three independent experiments, with four technical replicates per experiment. The results are presented as the mean ± standard deviation, calculated from the three independent experiments.

2.7. Statistical analysis

Statistical analyses were conducted using Statistica 13.3 (StatSoft Poland, Krakow). The normality of the data distribution was assessed using skewness and kurtosis, while Levene's test was used to assess homogeneity of variances. Differences between groups were assessed using one-way ANOVA, and the Bonferroni post hoc test was applied to identify significant differences among extracts, with significance set at $p < 0.05$. Principal component analysis (PCA) was performed using PQStat v.1.8.4.140 (Poznań, Poland) to explore relationships between compound profiles and the biological activities of the extracts. A Pearson correlation matrix was generated using the same software. To identify the extract with the most promising combined antioxidant and enzyme-inhibitory profile, a multi-dimensional comparative analysis (MCA) was employed [23]. This approach enables the evaluation and ranking of multi-criteria objects using synthetic indicators. Technical replicates were first averaged within each independent experi-

ment. The mean values obtained from the three independent experiments for each extract were subsequently used for Pearson correlation analysis, PCA, and MCA.

3. Results

3.1. Chemical characterisation of hop leaf and cone extracts

The quantitative results of the extracts revealed differences in the chemical composition between hop leaves and hop cones. The analysis of the TPC (see **Figure 1**) in hop plant materials reveals that the Blisk variety is the richest in polyphenols in both leaves and cones, with 12.7 mg GAE/g and 22.01 mg GAE/g, respectively. Among the remaining cultivars, Nugget cones also exhibited a high TPC (20.01 mg GAE/g), which did not differ significantly from that of Blisk cones. Saphir and Bačka cones showed lower TPC values of 16.18 and 13.69 mg GAE/g, respectively. In contrast, the leaves of Nugget, Bačka, and Saphir showed TPC values (6.54–7.77 mg GAE/g) that were similar to, and significantly lower than, those of Blisk leaves.

HPLC analysis showed that hop cones generally contained higher levels of xanthohumol and lupulone than leaves, with marked variation among cultivars (see **Table 1**). Among the leaves, Blisk had the highest xanthohumol content (25.12 µg/g), whereas Bačka had the highest lupulone content (30.79 µg/g). Among the cones, Blisk contained the highest xanthohumol content (2.55 mg/g), whereas Nugget showed the highest lupulone content (9.67 mg/g). The higher concentrations of these secondary metabolites in cones can be attributed to the presence of lupulin glands, where these compounds are synthesised [24].

The GC-MS analysis of hop leaf and cone extracts (see **Tables S1–S2**, Supplementary Materials) confirmed the presence of a wide variety of bioactive compounds, including alcohols, acids, esters, terpenes, and ketones. These findings align with the results of HPLC and TPC analyses, which showed that cones generally contain higher levels of key secondary metabolites, likely due to the presence of lupulin glands concentrated in this part of the plant. While hop cones were characterised by a high lupulin content, leaf extracts

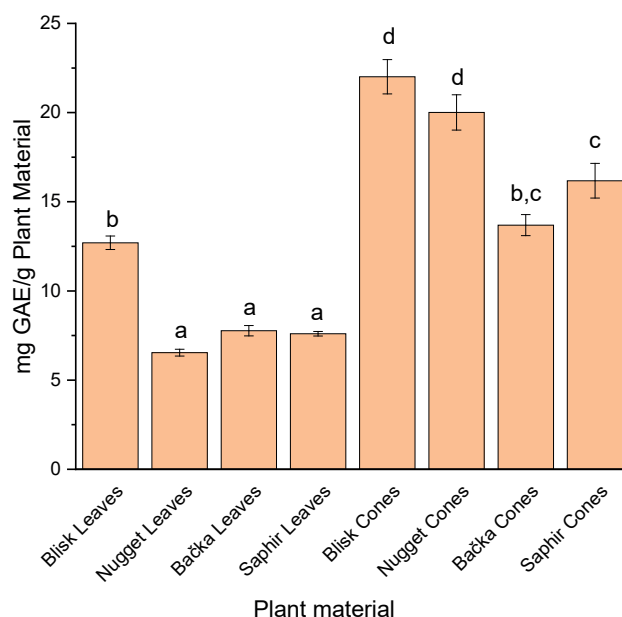


Figure 1. Total phenolic content (TPC) in the investigated hop cone and leaf extracts, expressed as mg gallic acid equivalents (GAE)/g plant material. Data are presented as mean $\pm$ SD from three independent experiments ($n = 3$), each performed with four technical replicates. Different letters (a–d) within the bars indicate statistical differences ($p < 0.05$)

Table 1. The content of xanthohumol and lupulone in extracts of leaves and cones was presented as $\mu\text{g/g}$ and mg/g of plant material, respectively

	Xanthohumol ($\mu\text{g/g}$)	Lupulone ($\mu\text{g/g}$)		Xanthohumol (mg/g)	Lupulone (mg/g)
Blisk Leaves	25.12 ± 0.86	20.22 ± 1.12	Blisk Cones	2.55 ± 0.03	2.73 ± 0.10
Nugget Leaves	7.48 ± 0.45	12.37 ± 0.49	Nugget Cones	1.45 ± 0.10	9.67 ± 0.08
Bačka Leaves	14.48 ± 0.75	30.79 ± 1.51	Bačka Cones	0.68 ± 0.03	5.56 ± 0.04
Saphir Leaves	6.41 ± 0.41	25.43 ± 1.37	Saphir Cones	0.78 ± 0.04	7.59 ± 0.12

exhibited broader chemical diversity. The composition and abundance of compounds varied notably between different hop varieties and plant parts, reflecting their distinct physiological roles and metabolic activity.

3.2. Antioxidant, metal-chelating, and enzyme-inhibitory activities of hop extracts

The antioxidant results showed that Blisk cones had the highest antioxidant activity in all assays, excelling in DPPH ($83.19 \text{ mg Trolox/g}$), FRAP ($56.39 \text{ mg Trolox/g}$), ABTS ($76.78 \text{ mg Trolox/g}$), and CUPRAC ($75.25 \text{ mg Trolox/g}$) (see **Figure 2**). The antioxidant activity differed among the studied varieties, with cones demonstrating significantly greater activity compared to leaves.

The chelating activity of hop extracts varied between leaves and cones, with the leaf variety

Bačka ($3.17 \pm 0.47 \text{ mg/mL}$) showing the lowest IC_{50} value (see **Table 2**). However, no significant differences were noted among the leaves' chelating activity. The cone extracts exhibited generally higher IC_{50} values, with Nugget cones having the lowest at $5.93 \pm 0.28 \text{ mg/mL}$.

Blisk cones exhibited the strongest cholinesterase inhibitory activity, with IC_{50} values of 15.9 mg/mL for AChE and 17.98 mg/mL for BuChE (see **Figure 3**). Blisk leaves also showed notable AChE inhibition ($\text{IC}_{50} = 20.4 \text{ mg/mL}$), exceeding that of the remaining leaf samples and the cones of Bačka and Saphir, while not differing significantly from Blisk and Nugget cones. Galantamine showed IC_{50} values of $24.4 \pm 1.2 \mu\text{g/mL}$ for AChE and $163.1 \pm 3.7 \mu\text{g/mL}$ for BuChE. Blisk leaves exhibited the strongest BuChE inhibitory activity among the leaf extracts and showed

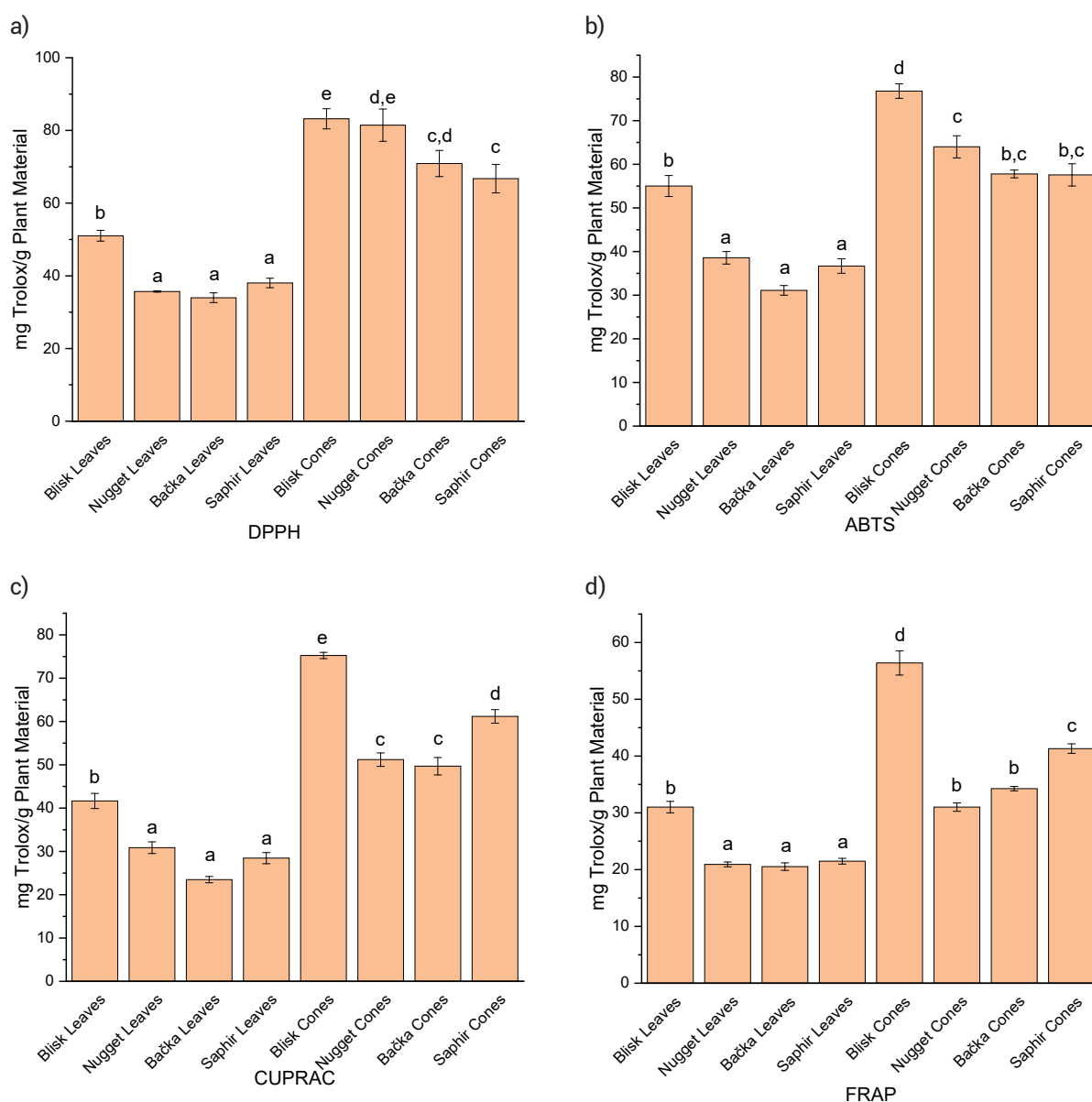


Figure 2. The antioxidant potential of hop cone and leaf extracts studied in DPPH (a), ABTS (b), CUPRAC (c), and FRAP (d) assays. Data are presented as mean $\pm$ SD from three independent experiments ($n = 3$), each performed with four technical replicates. Different letters (a–e) within the bars indicate statistical differences ($p < 0.05$)

Table 2. The chelating activity of hop leaf and cone extracts. Data are presented as mean $\pm$ SD from three independent experiments ($n = 3$), each performed with four technical replicates. Means with different superscript letters (a–b) within the same column differ significantly ($p < 0.05$)

Plant Material	Variety	IC ₅₀ (mg/mL)
Leaves	Blisk	3.64 $\pm$ 0.11 ^a
	Nugget	3.42 $\pm$ 0.06 ^a
	Bačka	3.17 $\pm$ 0.47 ^a
	Saphir	3.39 $\pm$ 0.29 ^a
Cones	Blisk	9.13 $\pm$ 0.93 ^b
	Nugget	5.93 $\pm$ 0.28 ^a
	Bačka	8.97 $\pm$ 2.31 ^b
	Saphir	10.91 $\pm$ 0.62 ^b

activity comparable to Nugget and Bačka cones. These results highlight their potential as a source of natural cholinesterase inhibitors.

The Blisk cones exhibited the highest potency in tyrosinase inhibition, with an IC_{50} of 16.02 mg/mL (see Figure 4). Nugget cones followed with an

IC_{50} of 26.81 mg/mL. The leaf extracts showed higher IC_{50} values than the cones. Intriguingly, Blisk leaves demonstrated notable tyrosinase-inhibitory activity, with an IC_{50} of 27.29 mg/mL, statistically similar to that of Blisk and Nugget cones.

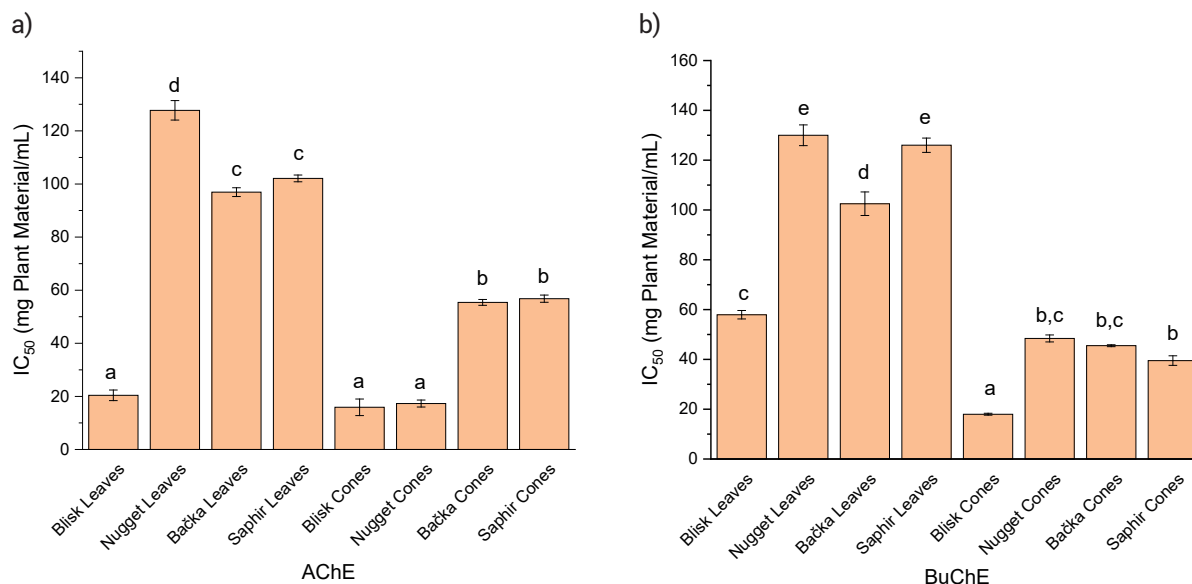


Figure 3. Inhibition of acetylcholinesterase (a) and butyrylcholinesterase (b) of extracts. Data are presented as mean $\pm$ SD from three independent experiments ($n = 3$), each performed with four technical replicates. Different letters (a–e) within the bars indicate statistical differences ($p < 0.05$)

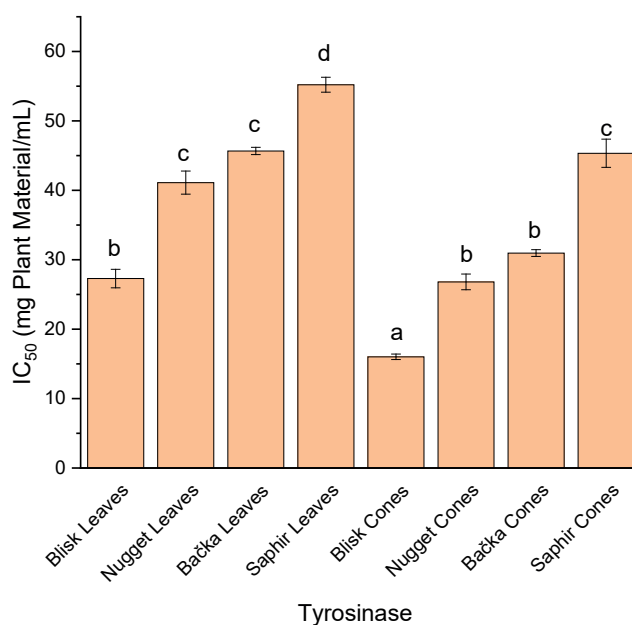


Figure 4. Inhibition of tyrosinase by hop cones and leaf extracts. Data are presented as mean $\pm$ SD from three independent experiments ($n = 3$), each performed with four technical replicates. Different letters (a–d) within the bars indicate statistical differences ($p < 0.05$)

3.3. Multivariate analysis of chemical composition and biological activities

The Multidimensional comparative analysis ranked Blisk cones as having the most potent antioxidant and enzyme inhibitory potential, followed by Nugget cones in second place, both attributed to their notably high TPC and xanthohumol levels. Blisk leaves, ranked third, exhibited the highest TPC and xanthohumol content among leaf extracts, highlighting their value for continued investigation.

PCA was conducted to explore the relationships among xanthohumol (XAN), lupulone (LUP), TPC, antioxidant activities (DPPH, ABTS, CUPRAC, and FRAP), chelating activity, and the inhibition of AChE, BuChE, and tyrosinase (TYR). Given the limited number of extracts included in the analysis, the multivariate results should be interpreted as exploratory. Factor 1 explained 76.96% of the total variance, while Factor 2 accounted for an additional 13.66%, resulting in a cumulative variance of 90.63% (see **Figure 5**). Factor 1 showed strong positive loadings for TPC (0.961), DPPH (0.969), ABTS (0.982), CUPRAC (0.975), and FRAP (0.926), indicating that antioxidant-relat-

ed parameters were the primary contributors to sample differentiation. In contrast, AChE (-0.830) and BuChE (-0.948) were negatively associated with Factor 1, reflecting the inverse relationship of IC_{50} values, where lower values indicate stronger enzyme inhibition. Factor 2 was moderately associated with secondary metabolite content and negatively related to enzyme inhibitory activity. The correlation matrix confirmed strong positive correlations between TPC and DPPH ($r = 0.964$), ABTS ($r = 0.957$), CUPRAC ($r = 0.920$), and FRAP ($r = 0.863$). AChE and BuChE were negatively correlated with TPC and antioxidant parameters, suggesting that extracts with higher antioxidant capacity exhibited stronger cholinesterase-inhibitory effects. These findings are consistent with previous reports linking phenolic constituents with both antioxidant and cholinesterase inhibitory activities [25–27]. TPC and antioxidant activity were the primary determinants of variability in the dataset. Lupulone content showed strong correlations with antioxidant activity ($r = -0.85$ to -0.82) and moderate-to-strong correlations with AChE, BuChE, and tyrosinase inhibition ($r = 0.51, 0.70$, and 0.62 , respectively). Xanthohu-

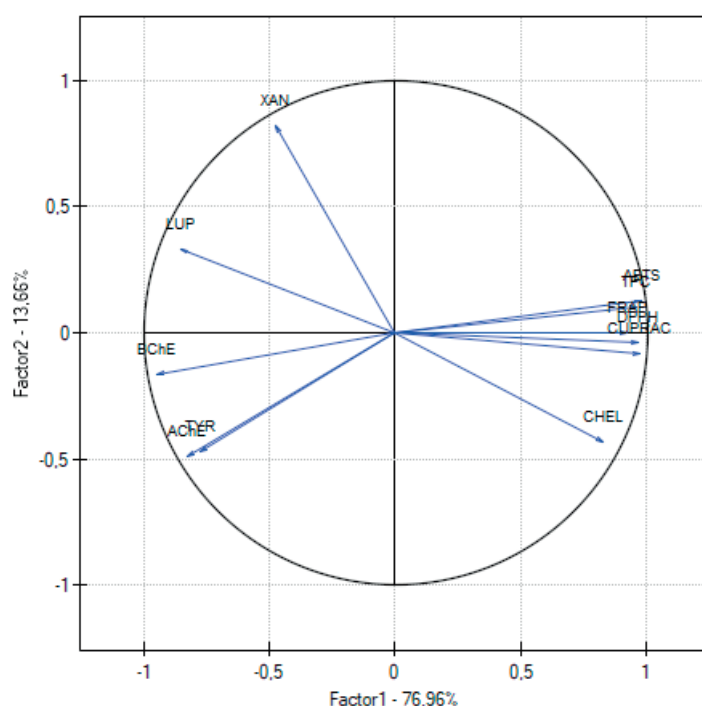


Figure 5 PCA loading plot showing the relationships among xanthohumol (XAN), lupulone (LUP), total phenolic content (TPC), antioxidant activities (DPPH, ABTS, FRAP, and CUPRAC), chelating activity, acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), and tyrosinase inhibition

mol content was moderately correlated with antioxidant activity ($r = -0.67$ to -0.40). The negative relationship between antioxidant activity and cholinesterase IC_{50} values further indicates that redox-active constituents may contribute to neuroprotective activity. The bioactivity observed in both cones and leaves highlights the potential of utilising the entire hop plant as a sustainable source of bioactive compounds for food, nutraceutical, and pharmaceutical applications.

4. Discussion

TPC results showed that polyphenol content was higher in hop cones than in leaves, aligning with the findings of Keskin et al., who reported that the TPC of methanol extracts was higher in hop cones (7.12 ± 0.09 mg GAE/g) than in hop leaves (6.86 ± 0.05 mg GAE/g) [28]. TPC may vary not only with plant material but also with factors such as variety, cultivation methods, and the type and parameters of extraction. Lyu et al. found that among the hop strobile varieties studied (Magnum, Cascade, Saaz1, Saaz2, Cluster, El Dorado, Calypso), the richest extract in polyphenols was from the El Dorado variety, with the highest TPC in ethanol extracts, reaching 81.90 mg GAE/g; water extracts were poorer in these compounds [29]. Kowalczyk et al. compared the TPC of hop extracts from cultivars Magnum and Marynka, using water, 50% methanol, and 50% ethanol as extraction solvents [30]. Hydroalcoholic extracts had significantly higher TPC levels (38.74 – 73.49 mg GAE/g) than aqueous extracts (16.24 – 25.55 mg GAE/g), with 50% ethanol yielding the highest TPC, while Marynka exhibited higher TPC than Magnum in water extractions. Significant differences in TPC between hop varieties were also noted by Gorjanovic et al., as the results varied across Saaz, Spalter, Spalter Select, Magnum, Bačka, Aroma, and Robusta varieties. The hop variety with the highest TPC was Spalter Select, at 190.8 mg GAE/g [31]. In another study, it was also observed that TPC varied significantly among different genotypes (Gianni and Cascade) and plant materials, with the Cascade genotype generally exhibiting higher TPC values than Gianni [32]. Specifically, the TPC ranged from 3.92 mg GAE/g in Gianni roots to 8.00 mg GAE/g in Gianni leaves, while Cascade roots had 5.25 mg GAE/g

and Cascade leaves showed 7.94 mg GAE/g, highlighting the influence of both genotype and plant part on phenolic levels. A similar plant-part-dependent pattern was observed in the present study, with consistently higher TPC in cones (13.69–22.01 mg GAE/g) than in leaves (6.54–12.70 mg GAE/g). Variation in absolute TPC values among studies may reflect differences in cultivar, geographical origin, cultivation conditions, and extraction methodology.

The results of Stanius et al. confirm the higher xanthohumol content in cones compared to leaves and the variation in content among different varieties [33]. Among 'Raudoniai', 'Fredos taurieji', 'Fredos derlingieji', 'Kauno ankstyvieji', and 'Kauno gražieji' varieties, the highest content of xanthohumol was in the cones of 'Kauno gražieji' (0.2136%) and the lowest in the leaves of 'Raudoniai' (0.0014%). The study also confirms that hop leaves contain minimal amounts of bitter β -acids, while the cones are the primary source of these acids in hops. Another study also investigated different hop varieties that differ in xanthohumol and lupulone content. The xanthohumol content in pellets of the six hop varieties (Nugget, Columbus, Chinook, Magnum, Cascade, and Fuggle) ranged from 0.35% (Fuggle) to 0.76% (Nugget), while the lupulone content ranged from 1.54% (Fuggle) to 2.53% (Cascade) [34]. Consistent with these reports, the present study showed substantially lower lupulone concentrations in hop leaves (12.37–30.79 μ g/g) than in cones (2.73–9.67 mg/g). As lupulone is one of the principal bitter β -acids in hops, its lower abundance in leaves may contribute to a milder bitter profile, potentially facilitating the incorporation of leaf-derived ingredients into food and nutraceutical formulations; however, this potential sensory advantage requires experimental confirmation.

The antioxidant activity of the leaves from the studied cultivars was higher than that of the Cascade cultivar leaves, which demonstrated 8.4 ± 0.1 mg TEAC/g for the DPPH assay, 6.7 ± 1.1 mg TEAC/g for the ABTS assay, and 20.7 ± 0.3 mg TEAC/g for the FRAP assay, using a solvent mixture of ethanol and water (80:20) for the extraction process [35]. Abram et al. confirmed that the cones exhibit higher antioxidant activity than the leaves, reporting that the DPPH radical scavenging IC_{50} values were approximately 0.020 mg/mL for hop leaf extracts and between

0.005 and 0.010 mg/mL for hop cone extracts from the cultivars 'Aurora' and 'Hallertauer Magnum,' which were collected from four regions: Žalec (Slovenia), Leutschach (Austria), Hüll (Germany), and Žatec (Czech Republic) [36]. The antioxidant activity of hop cone extracts from 14 different hop genotypes ranged from 63.5 to 101.6 μ mol Trolox equivalents/g dry weight in the FRAP assay, highlighting variability in their antioxidant potential [37]. The antioxidant potential also depends on extraction parameters. Muzykiewicz et al. reported that antioxidant activity varied significantly with extraction parameters, with 98% (v/v) methanol yielding the highest DPPH activity (up to 3.67 mg Trolox/g raw material) and TPC (up to 8.55 mg GA/g raw material) [38]. Smith et al. also showed that TPC in hop extracts significantly contributes to their antioxidant properties, with higher TPC levels correlating with increased radical scavenging activity [39]. Consistent with previous reports, cones exhibited higher antioxidant activity than leaves in all four assays used in the present study. The strong positive correlations between TPC and DPPH, ABTS, CUPRAC, and FRAP values further indicate a close association between phenolic content and the extracts' antioxidant capacity. Alcohol extracts also exhibit superior chelating activity, as water extracts demonstrated lower chelating ability than ethanol extracts; among the examined hop cone extracts from the Magnum, Marynka, and Lubelski cultivars, the Magnum cultivar exhibited the highest iron ion chelation ability [40]. Chelating activity contributes to antioxidant defence by sequestering metal ions and reducing oxidative stress. Although hop leaves exhibited lower antioxidant activity than cones, they showed stronger metal-chelating capacity, as indicated by lower IC_{50} values. This highlights their potential as a valuable source of bioactive compounds despite being an underutilised by-product of hop cultivation. Interestingly, despite their lower TPC and antioxidant activity, the leaves exhibited a stronger metal-chelating capacity than the cones. This finding suggests that chelating activity may depend not only on the total concentration of phenolic compounds but also on the qualitative composition of the extracts.

The cones and leaves could inhibit enzymes such as AChE and BuChE. Other studies have also examined *H. lupulus* and the enzyme-inhibi-

tory potential of its constituents. Kobus-Cisowska et al. examined cholinesterase inhibition in hop cone extracts from three cultivars: Magnum, Lubelski, and Marynka [40]. Water extracts from Magnum showed the highest AChE inhibition, while ethanol extracts from Magnum were most effective against BuChE. Xanthohumol from *H. lupulus* cones exhibited anticholinesterase activity; its inhibition constants were 95.5 nM for AChE and 124.9 nM for BuChE [41]. In another study, hop tannins prepared using a 70% acetone-water extraction method, followed by purification on a Sephadex LH-20 column, effectively inhibited tyrosinase, with an IC_{50} of 76.52 ± 6.56 μ M, comparable to that of kojic acid [42]. A study identified the ethyl acetate-soluble fraction of *Humulus japonicus* as a promising tyrosinase inhibitor [43]. The major compounds, trans-N-coumaroyltyramine and cis-N-coumaroyltyramine, showed effective tyrosinase inhibition with IC_{50} values of 40.6 μ M and 36.4 μ M, respectively. Kim et al. evaluated various *Humulus lupulus* extracts for tyrosinase inhibition, finding that the methanol extract showed the most significant inhibition, with an IC_{50} of 61.2 mg/mL, whereas the ethyl acetate extract showed lower inhibition (IC_{50} of 131.2 mg/mL) [44]. 50% methanol extract showed moderate activity with an IC_{50} of 87.4 mg/mL. The antityrosinase potential observed in the present study is consistent with reports for other agricultural by-products, such as elderberry leaves [45]. In the present study, Blisk cones showed the strongest inhibition of AChE, BuChE, and tyrosinase, whereas Blisk leaves were the most active among the leaf extracts. This cultivar-dependent pattern is consistent with previous reports showing substantial variation in enzyme inhibition among hop cultivars and extract types. However, direct quantitative comparisons remain limited by differences in extraction procedures and assay conditions. Taken together, these findings highlight the novelty of the present study by providing an integrated comparison of the antioxidant, metal-chelating, and enzyme-inhibitory activities of hop leaves and cones across four cultivars, revealing the bioactive potential of underutilised hop leaves.

This study has several limitations. The findings are based exclusively on *in vitro* antioxidant, metal-chelating, and enzyme-inhibition assays and therefore do not demonstrate biological effi-

cacy in cellular or *in vivo* systems. Furthermore, only four hop cultivars were examined, which limits the generalisability of the results to other cultivars. Further studies using neuronal cell and *in vivo* models, as well as a broader range of cultivars, are required to confirm these findings. Future research should further investigate hop leaves, an underutilised by-product of hop cultivation with significant bioactive potential. Although hop cones remain the primary focus of research, hop leaves contain secondary metabolites that warrant further attention. Studies using cellular and *in vivo* models are needed to evaluate their efficacy, bioavailability, and safety. Their incorporation into functional foods and nutraceuticals may represent a sustainable approach to the valorisation of hop by-products and the development of value-added products.

5. Conclusions

This study demonstrates that hop leaves, an underutilised by-product of hop cultivation, contain compounds exhibiting *in vitro* antioxidant, metal-chelating, and enzyme-inhibitory activities potentially relevant to neuroprotection. Among the leaf extracts investigated, Blisk showed the most promising biological activity. The lower lupulone content in leaves may be associated with a milder bitter profile; however, this potential sensory advantage requires experimental confirmation. These findings support further investigation of hop leaves as a source of bioactive compounds and as a potential material for the valorisation of hop by-products. Their prospective applications in food, nutraceutical, and pharmaceutical formulations require confirmation through cellular and *in vivo* studies, as well as further assessment of bioavailability, safety, and sensory properties.

Statements

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Conflict of interest statement

The authors declare no conflict of interest.

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