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COMPARATIVE ANALYSIS OF BIOACTIVE COMPONENT CONTENT IN TWO VARIETIES OF *Humulus lupulus L.*

Matea Kuzmanoska[1], Tatjana Risteska[2], Viktorija Maksimova[1*]
[1]Faculty of Medical Sciences, Goce Delcev University, Krste Misirkov 10-A, 2000 Stip, Republic of North Macedonia
[2]Prilepska Pivarnica AD Prilep, Republic of North Macedonia

**Corresponding author: viktorija.maksimova@ugd.edu.mk*

Abstract

Humulus lupulus L. has gained strong scientific interest due to its rich phytochemical profile and diverse biological activities. Among its bioactive constituents, bitter acids have been extensively investigated for their antioxidant, anti-inflammatory, and neuroprotective effects, highlighting their potential therapeutic value in the prevention and management of various health conditions. This study aimed to perform a comparative analysis of the bioactive component content in two *Humulus lupulus L.* varieties, *Herkules* and *Aurora*, as their chemical composition represents a key determinant of the potential application not only in the brewery industry but also in herbal pharmaceutical products. The experimental analysis was conducted using an internally standardized spectrophotometric (UV/Vis spectrophotometer Cary 60 UV-Vis, Agilent Technologies, USA) and gravimetric method, routinely used as an analytical approach in the brewing industry. Diethyl ether extracts, consequently diluted in methanol, were assessed by measuring UV absorbance at wavelengths of 355 nm, 325 nm, and 275 nm. Absorbance at 355 nm predominantly reflects the presence of α-acids (humulones). Measurements at 325 nm are used to quantify oxidized bitter acid derivatives and related soft resin fractions, while absorbance at 275 nm enables the assessment of phenolic constituents and other resinous compounds that may interfere with the analysis. The combined use of these three wavelengths enables selective and more accurate spectrophotometric determination of α-acids in hop extracts. The content of total resins, soft resins, hard resins was determined gravimetrically. The results revealed differences among the analyzed samples, reflecting variability in their chemical composure, emphasizing differences in the content of bioactive components in the two examined varieties. One of the varieties exhibited a higher concentration of α-acids, suggesting a potentially greater biological activity. In conclusion, the obtained results highlight the importance of cultivar-dependent variations in *Humulus lupulus* and their implications for both phytotherapeutic and brewing applications. The present findings highlight the importance of improved characterization of the chemical profiles of hop varieties, which may support their targeted utilization and warrant further detailed research into their therapeutic potential and role in rational phytotherapy.

Key words: *bitter acids, hop extracts, phytotherapy, spectrophotometric analysis.*

INTRODUCTION

Humulus lupulus L., (common hop) is a perennial plant belonging to the Cannabaceae family, widely recognized for its significant applications in the brewing and pharmaceutical industries. One of the main botanical characteristics of this dioecious plant is the formation of its reproductive organs, as its male and female flowers are represented on separate plants. Female inflorescences are especially important because it is the place where bioactive compounds of hops are synthesized and stored in characteristic lupulin glands (lupulin), which secrete a fine, yellow, resinous powder. The whole inflorescence is a cone-like structure commonly known as a strobile. The central axis of each cone bears bracts and bracteoles, and in the basis of these bracteoles, lupulin glands are present (Kogut et al., 2026).

Throughout its use in the brewing industry, this plant has gained increasing attention for its rich content of bioactive compounds with significant pharmacological potential (Haunold et al., 1993).

Secondary metabolites in hops are classified into three main categories: resins, essential oils, and polyphenols, which together account for approximately 4–14% of the dry weight (Steenackers et al., 2015). Based on their relative solubility in organic solvents, hop resins are classified into soft resins and hard resins. Compounds that are soluble in hexane, paraffinic hydrocarbons, and petroleum ether belong to the so-called soft resins, whereas compounds that are insoluble in these solvents are classified as hard resins (Bertelli et al., 2018). Two types of bitter acids are distinguished into the soft resins fraction: α-acids (primarily humulone, and its analogues cohumulone and adhumulone) and β-acids (primarily lupulone, colupulone and adlupulone) (Karabin et al., 2016). Among the identified bitter acids, α-acids (humulones) are considered the most important bioactive components. In addition to their role in imparting bitterness and stability to beer, these compounds exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, antimicrobial, and neuroprotective effects. Recent studies have highlighted their potential role in improving cognitive function, modulating metabolic processes, and contributing to overall health (Iniguez & Zhu, 2021; Kogut et al., 2026).

Bitter acids are highly susceptible to oxidation, resulting in the formation of a mixture of poorly defined oxidation products that remain soluble in diethyl ether but are no longer soluble in hexane, commonly referred to as hard resins. The deterioration of hop quality during storage is accompanied by the development of a strong odor, which is undesirable in brewing applications (Hansel & Steinegger, 1998). To minimize these changes, hops are dried immediately after harvest, pelletized, and stored in hermetically sealed packaging, preferably under low-temperature conditions.

Polyphenols can be further classified into flavonols (quercetin and kaempferol), flavan-3-ols (mainly catechin, epicatechin, and proanthocyanidins), phenolic carboxylic acids (e.g., ferulic acid), and, in smaller amounts, prenylated flavonoids such as xanthohumol (0.1–1% of dry weight) and isoxanthohumol (Roehrer et al., 2018). Despite their relatively low concentrations, these compounds may still exert significant biological activity. Isoxanthohumol is converted in the intestine into 8-prenylnaringenin, a phytoestrogen that is absorbed by intestinal cells. Depending on the composition of the individual gut microbiota, it is estimated that approximately one-third of the population can produce sufficiently high levels of this metabolite to reach biologically active concentrations (Possemiers et al., 2006).

Among the identified constituents, there are more than 1000 different constituents of the essential oil of hop that significantly contribute to the flavor profile of the beer. Dried hop cones contain approximately 0.5–3.0% essential oils (Olšovská et al., 2016; Bocquet et al., 2018). The hydrocarbons present in hop essential oils are classified into three groups: (1) aliphatic hydrocarbons (isoprene), (2) monoterpenes, consisting of two isoprene units (myrcene, ocimene, pinene, limonene, and cymene), and (3) sesquiterpenes, consisting of three isoprene units (humulene, caryophyllene, and farnesene) (Rutnik et al., 2021). Among these hydrocarbons, myrcene is the most abundant and significant monoterpene in hop essential oil, accounting for approximately 30–60% of the total oil content in the cone. It is primarily responsible for the sharp, green, and herbaceous aroma characteristic of fresh hop cones.

The chemical composition of *Humulus lupulus L.* varies depending on several factors, including plant variety, cultivation conditions, and processing methods (Almaguer et al., 2014).

The concentration and distribution of these compounds may vary considerably between hop varieties and directly influence their therapeutic potential and industrial application. Given the increasing interest in the therapeutic potential of hop-derived compounds, it is essential to evaluate and compare different varieties in terms of their phytochemical composition. Therefore, this study aimed to perform a comparative analysis of the content of bioactive components in two varieties of *Humulus lupulus L.*, namely Herkules and Aurora, by emphasizing the content of α-acids using a spectrophotometric method. These hop varieties were selected because they represent two commercially important cultivars with contrasting chemical profiles, particularly regarding α-acid content and essential oil composition. Additionally, both varieties can be successfully grown in North Macedonia. The climatic conditions in several regions of the country, including moderate temperatures, sufficient sunshine, and suitable soil characteristics, are favorable for the cultivation of the selected varieties.

MATERIAL AND METHODS

Study design

The study was designed as a laboratory experimental investigation focused on the quantitative analysis of bioactive components in hop (*Humulus lupulus L.*) extracts. A comparative approach was applied to evaluate phytochemical differences between two varieties.

Plant material

Two commercially available hop varieties, Humulus lupulus L. `Herkules` and *Humulus lupulus L.* `Aurora`, in pellet form, were used as plant materials. The samples were selected based on their industrial relevance and phytotherapeutic potential.

Sample preparation and extraction

Before analysis, the commercially obtained dry hop pellets (T90 pellets) supplied by BarthHaas GmbH, Germany, were subjected to extraction to isolate resinous bioactive compounds. The extraction procedure was carried out using methanol and diethyl ether as solvents, which are suitable for efficient extraction of bitter acids and related components. Methanol and diethyl ether were selected based on the recommendations of the Analytica-EBC Method (European Brewery Convention, 1998), which is widely recognized as the reference analytical procedure for the determination of hop bitter acids and resins. Diethyl ether is considered particularly suitable for the extraction of hop soft resins, including α- and β-acids, due to its high affinity for these relatively non-polar compounds, while methanol facilitates their dissolution and quantitative transfer during sample preparation. The combination of these solvents ensures efficient extraction, high recovery rates, and reproducible determination of hop bitter acids. The process was performed under controlled laboratory conditions to ensure consistency and reproducibility of the results.

Hop samples were ground using a laboratory mill (Bühler, Germany), and 5 g of each sample was accurately weighed. The samples were extracted with 50 mL of diethyl ether and placed on a mechanical shaker (HS 250 basic, IKA Labortechnik, Germany) for 2.5 hours, at room temperature, to ensure efficient extraction of total resins.

Following extraction, the samples were stored overnight at 4°C to allow phase stabilization. The solution was transferred into a separatory funnel and left undisturbed for 30 minutes to enable phase separation. Subsequently, 25 mL of the organic layer was carefully collected and transferred into a flask.

The solvent was evaporated on a water bath, resulting in the isolation of total hop resins. The obtained residue was dissolved in methanol, and the extract was quantitatively filtered through a fine filter into a 25 mL volumetric flask, which was then filled to the mark with methanol.

Determination of total resins

An aliquot of 10 mL of methanolic extract was transferred into a pre-weighed flask. The flask was placed in a water bath and heated at 70–80°C until complete evaporation of methanol.

The residue containing total resins was further dried at 50°C for 10 minutes and then cooled in a desiccator to room temperature. The flask was weighed, and the total resin content was determined gravimetrically.

Determination of soft resins

A volume of 7.5 mL of the methanolic extract was transferred into a separatory vessel, followed by the addition of 25 mL of n-hexane and 12.5 mL of distilled water. The mixture was shaken for 20 minutes using a mechanical shaker.

After extraction, the solution was transferred into a separatory funnel and allowed to stand for 30 minutes to achieve phase separation. The organic phase containing the extracted soft resins was collected (20 mL), filtered through a fine filter, and transferred into a flask.

The solvent was evaporated to dryness in a water bath. The residue was dried at 50°C for 10 minutes, cooled in a desiccator until a constant mass was achieved, and weighed to determine the soft resin content.

Determination of α-acids

For the determination of α-acids, 1 mL of the methanolic extract was transferred into a 25 mL volumetric flask and diluted with methanol. From this solution, 1 mL was further transferred into another 25 mL volumetric flask and diluted with alkaline methanol.

The absorbance of the final solution was measured at wavelengths of 355 nm, 325 nm, and 275 nm using a UV-Vis spectrophotometer, with alkaline methanol used as a blank. Measurements were performed in a 10 mm quartz cuvette.

Samples analyzed

The procedures described were applied on two hop varieties: *Humulus lupulus L.* \`Herkules\` and *Humulus lupulus L.* \`Aurora\`. Each analysis was performed in duplicate to ensure the reliability of the results.

Spectrophotometric analysis

The determination of bioactive components was performed using a spectrophotometric method (UV/Vis spectrophotometer Cary 60 UV-Vis, Agilent Technologies, USA) in accordance with standardized analytical procedures commonly applied in the brewing industry (European Brewery Convention, 1998). Absorbance measurements were carried out at wavelengths of 355 nm, 325 nm, and 275 nm, enabling selective quantification of different fractions of bitter substances. The applied analytical procedures were internally standardized laboratory methods routinely used in the brewing industry and performed in accordance with the Analytica-EBC Method 7.7 for spectrophotometric determination of hop bitter acids (European Brewery Convention, 1998).

The analysis included the determination of total resins, soft resins, hard resins, and α-acids. Each parameter was calculated based on the absorbance values obtained at the specified wavelengths.

Data processing and calculations

The data obtained were processed using standard calculation procedures for quantitative determination of resin fractions and α-acid content. Calculations were performed separately for each hop variety, allowing comparative evaluation of their phytochemical composition. The results were expressed in accordance with established analytical protocols used in routine laboratory practice.

The content of total resins, soft resins, hard resins, and α-acids was calculated using standard analytical equations based on gravimetric and spectrophotometric measurements.

The percentage of total resins and soft resins was determined gravimetrically using the following general equation:

$$\frac{\%\ \text{total resins (d.m.)}}{100-\text{W}} \cdot 100 = \%\ \text{total resins d. m.}$$

$$\%\ \text{total resins} = (\text{G}_2 - \text{G}_1) \cdot 102 = \%\ \text{total resins d.m.}$$

where:

G_1 is the weight of the empty flask (g),
G_2 is the weight of the flask with residue (g),
102 is a conversion factor depending on the method and sample preparation.

$$\frac{\%\ \text{soft resins (d.m.)}}{100-\text{W}} \cdot 100 = \%\ \text{soft resins d. m.}$$

$$\%\ \text{soft resins} = (\text{G}_2 - \text{G}_1) \cdot 85 = \%\ \text{soft resins d.m.}$$

The content of hard resins was calculated as the difference between total and soft resins:

hard resins % = total resins % - soft resins %

The content of α-acids was determined spectrophotometrically according to the following equation:

$$\textit{hard resins}\ \% = \textit{total resins}\ \% - \textit{soft resins}\ \%$$

$$\alpha\text{-acids}\ \% = 0{,}625(-51{,}56 \cdot \mathrm{A}_{355} + 73{,}79 \cdot \mathrm{A}_{325} - 19{,}07 \cdot \mathrm{A}_{275})\%$$

where:

A_{355}, A_{325}, A_{275} represent the absorbance values measured at corresponding wavelengths.

All calculations were performed in accordance with standard methods recommended by the *European Brewery Convention (EBC)*.

RESULTS AND DISCUSSION

The results obtained from the analysis of two *Humulus lupulus L.* varieties, *Herkules* and *Aurora*, are presented in the given tables. Table 1 represents the results describing the regular quality assessments of hop pellets, while Table 2 represents the results of spectrophotometric analysis of hop extracts.

Table 1. Quality characteristics of hop pellets.

Parameter	Acceptable limits	Unit	Herkules	Aurora
Moisture	6 – 11	% d.m.	7.19	9.00
Odor	Characteristic	/	Characteristic	Characteristic
Foreign matter	None	/	None	None
Color	Characteristic	/	Dark green	Dark green
Form	Characteristic	/	Pellets	Pellets

Table 2. Content of bioactive fractions in hop extracts in *Herkules* vs. *Aurora* variety.

Parameter	Acceptable limits[1]	Unit	Herkules	Aurora
Total resins	11–40	% d.m.	16.41	26.41
Soft resins	9–25	% d.m.	12.83	22.01
Hard resins	2–12	% d.m.	3.85	4.80
α-acids	6–16	% d.m.	13.21	7.76
β-acids	5–20	% d.m.	-	-

[1] Reference values in % d.m given by the manufacturer of hop pellets.

Phytochemical composition

The results indicate significant differences in the phytochemical composition between the analyzed hop varieties. The variety *Aurora* exhibited higher values of total resins (26.42%) and soft resins (22.02%) compared to Herkules, which showed lower values of 16.41% and 12.84%, respectively.

On the other hand, Herkules demonstrated a notably higher content of α-acids (13.22%) compared to Aurora (7.76%), indicating a different distribution of bioactive components between the two varieties.

Resin fractions

Total resins represent the sum of all resinous bioactive compounds present in hop and are considered important indicators of biological activity. The results show that *Aurora* contains approximately 10% higher total resin content compared to *Herkules,* suggesting a greater concentration of resin-based bioactive substances.

Similarly, soft resins, which include α- and β-acids and are responsible for the majority of biological effects, were significantly higher in Aurora. The content of soft resins in *Aurora* (22.02%) was notably greater than in *Herkules* (12.84%), indicating a potentially higher overall biological/pharmacological activity.

In contrast, hard resins showed only minor differences between the two varieties, with slightly higher values observed in *Aurora* (4.80%) compared to Herkules (3.85%). These components are associated with oxidation processes and contribute more to structural stability than to biological activity.

The observed differences in bioactive component content between the two varieties can be attributed to genetic variability, as well as differences in cultivation conditions and processing methods. Our samples are commercially available for brewery industrial purposes, cultivated under standardized cultivation conditions, so we can assume that content differences can be mainly attributed to their genetic variability.

Aurora, characterized by higher total and soft resin content, may be more suitable for brewing applications where a broader spectrum of aromatic and resinous compounds is desired. Soft resins, which include α- and β-acids, contribute significantly to beer bitterness, aroma stability, and flavor complexity. In addition, Aurora contains notable amounts of essential oil components such as myrcene, humulene, caryophyllene, and farnesene, which are responsible for floral, citrus, herbal, spicy, and pleasant hoppy aroma characteristics in beer (Rutnik et al., 2021).

In contrast, *Herkules* exhibited a considerably higher α-acid content, indicating its suitability as a high-bittering hop variety in brewing applications. α-acids are primarily responsible for beer bitterness and contribute to microbiological stability after isomerization during wort boiling. Furthermore, *Herkules* is recognized for its excellent storage stability and high brewing efficiency, which additionally increases its industrial importance.

α-acids content

The content of α-acids, as one of the most important bioactive components of hop, showed a reverse trend compared to resin fractions. *Herkules* exhibited a significantly higher concentration of α-acids (13.22%) compared to *Aurora* (7.76%).

This difference is particularly important, as α-acids are directly associated with the pharmacological activity of hop, including sedative, anti-inflammatory, and spasmolytic effects. The higher concentration observed in *Herkules* suggests a greater potential for therapeutic applications where α-acids are the primary active constituents.

Among the investigated hop varieties, Herkules may also be considered more suitable for pharmaceutical applications targeting bitter-acid-mediated biological effects due to its higher α-acid content. Since α-acids, and their isomeric analogues iso α-acids have been associated with strengthening gut microbiota, cardioprotective, neuroprotective, metabolic, estrogen-like, anticancer, and immunomodulatory effects (Kogut et al., 2026), *Herkules* represents a promising source of bioactive compounds for the development of medicinal and nutraceutical preparations. Isomeric forms of α-acids can act synergistically to the inhibitory effects of α-acids on COX-2 driven PGE2 production, transcriptional activity of NF-κB, AP-1, CREP, and kinases involved in inflammatory signaling (Van Cleemput et al., 2009). However, scientific evidence suggests that the pharmacological activity of hops cannot be attributed only to α-acids. Other bioactive constituents, including β-acids, prenylated flavonoids such as xanthohumol and isoxanthohumol, as well as essential oil terpenes, may act synergistically with α-acids to enhance the overall biological response (Leto et al., 2025). Xanthohumol, for example, has demonstrated strong antioxidant, anti-inflammatory, chemopreventive, and neuroprotective properties, while isoxanthohumol serves as a precursor of the potent phytoestrogen 8-prenylnaringenin (Dzienisik et al., 2026). Similarly, hop terpenes such as humulene and caryophyllene have been shown to modulate inflammatory pathways and oxidative stress responses (Gullì et al., 2022). Therefore, the therapeutic potential of hop extracts is likely determined not only by the concentration of α-acids but also by the complex interactions among multiple classes of phytochemicals. Because of this, *Aurora* may also possess pharmaceutical value owing to its diverse phytochemical composition, highlighting the importance of considering the entire metabolite profile rather than α-acid content alone.

CONCLUDING REMARKS

The present study provided a comparative analysis of bioactive component fractions in two *Humulus lupulus L.* varieties, *Herkules* and *Aurora*, using a spectrophotometric method. The results obtained here have demonstrated significant differences in their phytochemical composition.

Aurora was characterized by a higher content of total resins and soft resins, indicating a greater overall concentration of resin-based bioactive compounds. In contrast, *Herkules* exhibited a significantly higher level of α-acids, which are directly associated with the pharmacological activity of hop, particularly its sedative, anti-inflammatory, and spasmolytic effects.

These findings highlight the importance of varietal selection in determining the chemical profile and biological potential of hop extracts. Depending on the intended application, different varieties may offer specific advantages, with *Aurora* being more suitable for broader industrial applications, while *Herkules* may be preferable for targeted therapeutic effects related to α-acids. Detailed profiling of humulone analogues and their oxidation- and isomerization-derived metabolites is required, as these constituents may significantly contribute to the therapeutic potential of the studied hop varieties. Future research should focus on comprehensive phytochemical characterization using advanced analytical techniques, including HPLC-MS/MS and metabolomic approaches, to identify and quantify individual bitter acids, prenylated flavonoids, polyphenols, terpenoids, and their transformation products. Determination of these compounds is essential as the biological activity of hop extracts is likely mediated by the combined action of multiple phytochemical classes rather than by individual constituents alone.

However, this study contributes to a better understanding of the phytochemical variability of two *Humulus lupulus L.* varieties, *Herkules* vs. *Aurora*, confirming that the selection of hop variety plays a crucial role in determining the phytochemical profile and biological potential of hop-derived products, supporting its potential application in phytotherapy, nutraceutical development, and pharmaceutical formulations.

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КОМПАРАТИВНА АНАЛИЗА НА СОДРЖИНА НА БИОАКТИВНИ КОМПОНЕНТИ ВО ДВА ВАРИЕТЕТИ НА *Humulus lupulus L.*

Матеа Кузманоска[1], Татјана Ристеска[2], Викторија Максимова[1*]
*[1]Факултет за медицински науки, Универзитет „Гоце Делчев", Штип, „Крсте Мисирков"
10-А, 2000 Штип, Република Северна Македонија*
[2]Прилепска пиварница АД Прилеп, Република Северна Македонија

**Контакт автор:* viktorija.maksimova@ugd.edu.mk

Резиме

Humulus lupulus L. претставува растителен вид со значаен фитотерапевтски потенцијал, поради присуството на биоактивни компоненти, особено горчливите киселини. Овие соединенија се познати по своите антиоксидативни, антиинфламаторни и невропротективни својства. Целта на ова истражување беше да се изврши компаративна анализа на содржината на биоактивни компоненти кај два вариетети на *Humulus lupulus L.*, односно *Herkules* и *Aurora*.

Експерименталната анализа беше спроведена со примена на спектрофотометриска метода (UV/Vis spectrophotometer Cary 60 UV-Vis, Agilent Technologies, USA) како стандардизирана аналитичка техника. Подготовката на примероците опфати екстракција со метанол и диетил-етер, проследена со мерење на апсорбанцијата при бранови должини од 355 nm, 325 nm и 275 nm. Беше определена содржината на вкупни смоли, меки смоли, тврди смоли и α-киселини.

Добиените резултати покажаа разлики во содржината на биоактивни компоненти помеѓу двата анализирани вариетети, што укажува на варијабилност во нивниот фитохемиски состав. Еден од вариететите покажа повисока концентрација на α-киселини, што укажува на потенцијално поголема биолошка активност.

Во заклучок, добиените резултати ја нагласуваат важноста на вариететските разлики кај *Humulus lupulus* и нивното влијание врз фитотерапевтската примена. Наодите придонесуваат за подобро разбирање на хемискиот состав и потенцијалната употреба на екстрактите од хмељ во современата фитотерапија.

Клучни зборови: *горчливи киселини, ектракт од хмељ, фитотерапија, спектрофотометриска анализа.*

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