



PHYTOCHEMICAL CHARACTERIZATION AND BIOACTIVITY OF HOP EXTRACTS: EMPHASIS ON PHENOLIC COMPOUNDS

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ABSTRACT

Objective: The objective of this study was to perform the phytochemical characterization of leaf extracts from three hop varieties (*Humulus lupulus* L.: Nugget, Comet, and Columbus) grown in Brazil, evaluating the influence of different solvents on the extraction of bioactive compounds.

Theoretical Framework: *Humulus lupulus* L. has emerged as a relevant source of bioactive metabolites due to its complex phytochemical profile. Plant-derived bioactive compounds, such as those found in hops, are promising due to their antioxidant and anti-inflammatory properties. However, a knowledge gap regarding the phytochemical profile of varieties grown in Brazil limits their pharmacological utilization and value addition.

Method: Lyophilized leaves of the three varieties were subjected to Microwave-Assisted Extraction (MAE) using water, ethanol, and hydroalcoholic solutions. Compound identification and quantification were performed by High-Performance Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS). Additionally, total phenolic content, flavonoids, and antioxidant potential were determined by the ABTS assay.

Results and Discussion: Thirteen phenolic compounds of pharmacological interest were identified. Hydroalcoholic solutions (50% and 70%) were more efficient in extracting total phenolics and flavonoids, highlighting rutin and chlorogenic acid. Notably, the aqueous extract of the Nugget variety showed the highest antioxidant potential, despite not having the highest total phenolic content, indicating the presence of molecules with high specific potency.

Research Implications: The results validate Brazilian hops as a rich source of raw material for the pharmaceutical and nutraceutical industries, providing a scientific basis for the development of herbal medicines aimed at neurological health.

Originality/Value: This study allowed for the detailed phytochemical characterization of three hop varieties cultivated in Paraná, Brazil. The discovery of the dissociation between compound content and bioactivity adds unique value, guiding future research towards the isolation of specific molecules with high therapeutic potential.

Keywords: *Humulus Lupulus*, Phenolic Acid, Flavonoids, ABTS, LC-MS/MS.

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CARACTERIZAÇÃO FITOQUÍMICA E BIOATIVIDADE DE EXTRATOS DE LÚPULO: ÊNFASE EM COMPOSTOS FENÓLICOS

RESUMO

Objetivo: O objetivo deste estudo foi realizar a caracterização fitoquímica de folhas de três variedades de lúpulo (*Humulus lupulus* L.: Nugget, Comet e Columbus) cultivadas no Brasil, avaliando a influência de diferentes solventes na extração de compostos bioativos com reconhecido potencial neuroprotetor.

Referencial Teórico: *Humulus lupulus* L. tem emergido como uma fonte relevante de metabólitos bioativos devido ao seu perfil fitoquímico complexo. Compostos bioativos de origem vegetal, como os encontrados no lúpulo, são promissores devido às suas propriedades antioxidantes e anti-inflamatórias. Contudo, uma lacuna de conhecimento sobre o perfil fitoquímico das variedades cultivadas no Brasil limita seu aproveitamento farmacológico e agregação de valor.

Método: Folhas liofilizadas das três variedades foram submetidas à Extração Assistida por Micro-ondas (MAE) utilizando água, etanol e soluções hidroalcoólicas. A identificação e quantificação dos compostos foi realizada por Cromatografia Líquida de Alta Eficiência acoplada à Espectrometria de Massas em Tandem (LC-MS/MS). Adicionalmente, foram determinados os teores de fenólicos totais, flavonoides e o potencial antioxidante pelo ensaio ABTS.

Resultados e Discussão: Foram identificados 13 compostos fenólicos de interesse farmacológico. As soluções hidroalcoólicas (50% e 70%) foram mais eficientes na extração de fenólicos e flavonoides totais, com destaque para a rutina e o ácido clorogênico. Notavelmente, o extrato aquoso da variedade Nugget apresentou o maior potencial antioxidante, apesar de não possuir os maiores teores de compostos fenólicos totais, indicando a presença de moléculas de alta potência específica.

Implicações da Pesquisa: Os resultados validam o lúpulo brasileiro como uma rica fonte de matéria-prima para as indústrias farmacêuticas e de nutracêuticos, fornecendo uma base científica para o desenvolvimento de produtos fitoterápicos voltados à saúde neurológica.

Originalidade/Valor: Este estudo permitiu a caracterização fitoquímica detalhada de três variedades de lúpulo cultivadas no Paraná, Brasil. A descoberta da dissociação entre o teor de compostos e a bioatividade agrega valor singular, orientando futuras pesquisas para o isolamento de moléculas específicas de alto potencial terapêutico.

Palavras-chave: *Humulus Lupulus*, Ácidos Fenólicos, Flavonoides, ABTS, LC-MS/MS.

CARACTERIZACIÓN FITOQUÍMICA Y BIOACTIVIDAD DE EXTRACTOS DE LÚPULO: ÉNFASIS EN COMPUESTOS FENÓLICOS

RESUMEN

Objetivo: El objetivo de este estudio fue realizar la caracterización fitoquímica de hojas de tres variedades de lúpulo (*Humulus lupulus* L.: Nugget, Comet y Columbus) cultivadas en Brasil, evaluando la influencia de diferentes solventes en la extracción de compuestos bioactivos con reconocido potencial neuroprotector.

Marco Teórico: *Humulus lupulus* L. se ha consolidado como una fuente relevante de metabolitos bioactivos debido a su complejo perfil fitoquímico. Los compuestos bioactivos de origen vegetal, como los encontrados en el lúpulo, son prometedores debido a sus propiedades antioxidantes y antiinflamatorias. Sin embargo, una laguna de conocimiento sobre el perfil fitoquímico de las variedades cultivadas en Brasil limita su aprovechamiento farmacológico y la agregación de valor.

Método: Hojas liofilizadas de las tres variedades fueron sometidas a Extracción Asistida por Microondas (MAE) utilizando agua, etanol y soluciones hidroalcohólicas. La identificación y cuantificación de los compuestos se realizó mediante Cromatografía Líquida de Alta Eficacia acoplada a Espectrometría de Masas en Tándem (LC-MS/MS). Adicionalmente, se determinaron los contenidos de fenólicos totales, flavonoides y el potencial antioxidante mediante el ensayo ABTS.



Resultados y Discusión: Se identificaron 13 compuestos fenólicos de interés farmacológico. Las soluciones hidroalcohólicas (50% y 70%) fueron más eficientes en la extracción de fenólicos y flavonoides totales, destacando la rutina y el ácido clorogénico. Notablemente, el extracto acuoso de la variedad Nugget presentó el mayor potencial antioxidante, a pesar de no poseer los mayores contenidos de compuestos fenólicos totales, indicando la presencia de moléculas de alta potencia específica.

Implicaciones de la investigación: Los resultados validan el lúpulo brasileño como una rica fuente de materia prima para las industrias farmacéutica y de nutraceuticos, proporcionando una base científica para el desarrollo de productos fitoterápicos dirigidos a la salud neurológica.

Originalidad/Valor: Este estudio permitió la caracterización fitoquímica detallada de tres variedades de lúpulo cultivadas en Paraná, Brasil. El descubrimiento de la disociación entre el contenido de compuestos y la bioactividad agrega un valor singular, orientando futuras investigaciones hacia el aislamiento de moléculas específicas de alto potencial terapéutico.

Palabras clave: *Humulus Lupulus*, Ácido Fenólico, Flavonoides, ABTS, LC-MS/MS.

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1 INTRODUCTION

Bioactive compounds derived from plants, particularly flavonoids and phenolic acids, have emerged as promising alternatives due to their neuroprotective potential. Recent studies have demonstrated that these phytochemicals possess potent antioxidant and anti-inflammatory properties, capable of modulating key cellular pathways involved in the progression of neurological disorders (Xu & Wang, 2025; Nájera-Maldonado, Sánchez-Martínez, & Álvarez-Sánchez, 2024; Minocha, Liu, & Ning, 2022).

Humulus lupulus L. (hop), traditionally associated with the brewing industry, has gained increasing attention as a valuable source of these bioactive metabolites. Beyond its conventional use, the species has been extensively investigated for its pharmacological potential, supported by a complex and diversified phytochemical profile. The presence of flavonoids and phenolic acids in its leaves and cones positions hop as a promising candidate for the development of novel health-related applications (Piekara et al., 2025; Silva et al., 2025).

Despite the well-documented pharmacological potential of hop in the international literature, a significant knowledge gap remains regarding the phytochemical characterization of plants cultivated in emerging regions such as Brazil. Although hop cultivation is expanding in the country, studies addressing varieties adapted to local environmental conditions are still incipient. This lack of information limits the strategic utilization of Brazilian-grown hop as a valuable raw material for pharmaceutical and nutraceutical industries (Visin, Costa de Souza & Batista da Costa, 2025; Almeida & Conto, 2024; Jastrombek et al., 2022).



Chemical characterization is a fundamental step to verify whether hop varieties cultivated in Brazil retain the bioactive potential previously reported for those grown in temperate regions. Therefore, the lack of data on the flavonoid and phenolic acid composition of Brazilian hop represents a significant limitation for validating its therapeutic use and adding economic value to this emerging crop (Sabino et al., 2025; Pagliaro, Fabiano-Tixier, & Ciriminna, 2023).

In this context, the objective of the present study was to characterize the phytochemical profile of leaf extracts from three *Humulus lupulus* varieties (Nugget, Comet, and Columbus) cultivated under Brazilian conditions. Different extraction solvents were employed, and the total flavonoid and phenolic acid contents were quantified to assess the potential of these plants as sources of bioactive compounds with functional and pharmacological relevance.

2 THEORETICAL FRAMEWORKS

2.1 PHENOLIC COMPOUNDS

Flavonoids are a class of secondary metabolites belonging to the phenolic compound family, widely distributed throughout the plant kingdom and occurring in various organs, including leaves, flowers, bark, and seeds. These compounds possess a structure consisting of two aromatic rings linked by a heterocyclic bridge, into several subclasses, such as flavones, flavonols, flavanones, isoflavones, catechins, and anthocyanidins (Roy et al., 2022). Beyond their essential physiological roles in plants, such as protection against ultraviolet radiation, pathogens, and abiotic stress, these compounds exhibit significant antioxidant, anti-inflammatory, and antimicrobial activities (Chen et al., 2023; Yuan et al., 2023).

From a pharmacological perspective, flavonoids are increasingly investigated as promising therapeutic agents, such as cancer, cardiovascular disorders, and neurodegenerative pathologies. Their capacity to interact with multiple molecular targets and to modulate cellular processes related to oxidative stress and systemic inflammation underscores their relevance as multifunctional compounds for human health promotion (Yuan et al., 2023).

Phenolic acids, belonging to the class of phenolic compounds, are widely distributed across the plant kingdom and are found in leaves, fruits, seeds, and bark. These molecules consist of benzene rings substituted with one or more hydroxyl groups (Arruda et al., 2021). Alongside flavonoids, phenolic acids are recognized for their potent antioxidant, antimicrobial, anti-inflammatory, and antitumor properties (Yuan et al., 2023).



These biological effects are primarily associated with their ability to modulate key cellular processes, including inflammatory signaling pathways and oxidative stress responses (Chen et al., 2023). Phenolic acids have gained increasing attention from both the food and pharmaceutical industries, where they are being explored as bioactive ingredients for the development of functional foods, nutraceuticals, and phytotherapeutic formulations (Arruda et al., 2021).

The bioactivity of plant extracts is intrinsically linked to the presence of phenolic compounds such as flavonoids and phenolic acids (Yuan et al., 2023). Accumulating evidence indicates that these compounds can reduce the generation of reactive oxygen species (ROS), enhance cellular viability, and protect neuronal cells from oxidative and inflammatory damage. Their ability to regulate molecular pathways involved in apoptosis and inflammation has broadened their therapeutic relevance, particularly in the prevention and treatment of neurodegenerative disorders (Fátima et al., 2022; Rad et al., 2022).

Phenolic acids have been associated with a lower risk of cardiovascular diseases and with the modulation of immune system function. Moreover, dietary intake of foods rich in these compounds has demonstrated preventive effects against certain types of cancer, attributed to their capacity to inhibit cellular processes that drive tumor growth and progression (Nguyen et al., 2022).

The phytochemical profile of hop cones (*Humulus lupulus* L.) is particularly remarkable for its abundance of prenylated flavonoids—a rare class of compounds in the plant kingdom. Among them, xanthohumol is the most abundant and extensively studied molecule, recognized for its potent antioxidant and chemopreventive activities, along with its derivative, isoxanthohumol. Of particular importance for neuroprotective potential, these compounds have been identified as positive modulators of γ -aminobutyric acid (GABA) receptors, thereby supporting their potential application in the management of neurological disorders (Lyu et al., 2022).

The identification and quantification of phenolic compounds in plant extracts requires the use of high-performance analytical techniques. LC-MS/MS is regarded as the gold-standard method for this purpose. The LC step enables the separation of the various constituents within the extract, while MS provides compound identification based on their molecular masses. The use of tandem mass spectrometry (MS/MS) adds a level of selectivity, ensuring unequivocal identification and exceptional analytical sensitivity (Silva et al., 2025).



3 METHODOLOGY

3.1 PLANT MATERIAL AND SAMPLE PREPARATION

The leaves of *Humulus lupulus* L. varieties Nugget, Comet, and Columbus were obtained from plants cultivated at the Experimental Farm of Universidade Paranaense, Umuarama, Paraná, Brazil (23°45'51"S, 53°16'14"W). After manual harvesting, the samples were transported to the Interdisciplinary Laboratory of Biological and Chemical Analyses (LIABQ) of the Cesumar Institute of Science, Technology and Innovation (ICETI/UniCesumar). The plant material was freeze-dried at −86 °C (JJ Científica, model LJJ), ground using a knife mill (Quimis Q298A21), and stored at −26 °C until extraction.

3.2 MICROWAVE-ASSISTED EXTRACTION (MAE)

Microwave-assisted extraction (MAE) was performed using solid-to-solvent ratios of 1:10 and 1:20 (w/v), as described in Table 1. The extraction solvents tested included distilled water (H₂O), hydroalcoholic solutions (50% and 70% v/v ethanol), and absolute ethanol (99%).

Table 1

Extraction conditions and yield of hop leaf extracts obtained by microwave-assisted extraction.

Extract	Hop Variety	Solvent	Concentration (g mL ⁻¹)	Yield %
E1	Columbus	H ₂ O	0.1	10.0
E2	Columbus	EtOH 50 %	0.1	24.5
E3	Columbus	EtOH 70 %	0.1	18.8
E4	Columbus	EtOH 99 %	0.1	8.5
E5	Comet	H ₂ O	0.1	1.2
E6	Comet	EtOH 70 %	0.1	15.1
E7	Comet	EtOH 99 %	0.05	8.2
E8	Nugget	H ₂ O	0.1	8.8
E9	Nugget	EtOH 70 %	0.1	16.0
E10	Nugget	EtOH 99 %	0.05	8.0

Source: Elaborated by the authors.

The MAE was carried out using a rotary-base microwave oven (Provecto Analítica, DGT 100-Plus). The extraction was performed with 1 g or 2 g of dried hop leaf and 10 mL or 20 mL of solvent, respectively. The irradiation cycle consisted of sequential steps of 200 W for 1 min, 400 W for 5 min, and 600 W for 1 min, followed by a 5 min cooling period (0 W),



totaling 12 min of processing time. After extraction, the mixture was filtered, and the filtrate was transferred to an amber glass vial and stored at -26°C until further analyses.

The extract yield and its compounds were evaluated to determine the best extraction condition. Yield was calculated using the extract mass and the initial residue weight. All determinations were performed in triplicate.

3.3 DETERMINATION OF TOTAL PHENOLIC COMPOUNDS (TPC)

The TPC was measured using the colorimetric Folin–Ciocalteu method (Dala-Paula et al., 2019; Untea et al., 2018), with modification. In test tubes, 50 μL of each extract was mixed with 250 μL of Folin–Ciocalteu reagent and 2.5 mL of distilled water. The mixture was homogenized for 1 min, followed by the addition of 1.0 mL of Na_2CO_3 , 7% w/v solution. Incubated for 1 h at room temperature, protected from light. The absorbance was measured at 750 nm using a UV–Vis spectrophotometer. The TPC was calculated from a calibration curve constructed with gallic acid as the standard, and the results were expressed $\mu\text{g GAE/mL}$.

3.4 DETERMINATION OF TOTAL FLAVONOID CONTENT (TFC)

The TFC was performed using the AlCl_3 colorimetric complexation method (Dala-Paula et al., 2019; Gómez-Mejía et al., 2019), with modification. In test tubes, 250 μL of each extract was diluted in 1.0 mL of distilled water, followed by the addition of 75 μL of sodium nitrite (NaNO_2 , 5% w/v). After incubation for 5 min at room temperature, 75 μL of aluminum chloride solution (AlCl_3 , 10% w/v) was added, homogenized and incubated for another 5 min. Subsequently, 0.5 mL of NaOH , 1 mol/L solution was added to complete the reaction. The absorbance was measured at 510 nm using a UV–Vis spectrophotometer. The TFC was determined from a six-point catechin calibration curve, and the results were expressed $\mu\text{g CE/mL}$.

3.5 EVALUATION OF ANTIOXIDANT POTENTIAL BY ABTS

The antioxidant activity of the extracts was determined using the ABTS radical cation decolorization assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) with modification (Dala-Paula et al., 2019; Untea et al., 2018). The antioxidant potential was



quantified based on a calibration curve constructed with Trolox. The results were expressed in $\mu\text{mol TE/mL}$.

3.6 PHYTOCHEMICAL ANALYSIS BY LC–MS/MS

After extraction, the samples were filtered through PTFE membrane filters (0.45 μm and 0.22 μm) and sent to the State University of Maringá (UEM), where the phytochemical analysis was performed in collaboration. The profile of active compounds was determined using a ultra-high-performance liquid chromatography (UPLC) system (ACQUITY UPLC® H-Class, Waters, Milford, CO, USA) equipped with an electrospray ionization source (Zspray™, Waters, Milford, CO, USA) and a triple-quadrupole mass spectrometer (Xevo TQDTM, Waters, Milford, CO, USA). Samples were diluted in methanol (1:9, v/v) and acidified with formic acid before analysis. Chromatographic separation was achieved with a C18 column at a 0.150 mL min^{-1} flow rate. The column temperature was maintained at 30 ± 1 °C, and the injection volume was set to 1.5 μL . The mobile phase consisted of Milli-Q® water with 0.1% formic acid (solvent A) and methanol (solvent B), utilizing a gradient elution program. The infusion into the mass spectrometer was carried out at a flow rate of 10 $\mu\text{L} \cdot \text{min}^{-1}$ under the following conditions: interface temperature of 350 °C, nebulizing gas (nitrogen) flow of 600 L h^{-1} , and mass scan range from 50 to 700 m/z . Data acquisition and processing were performed using the MassLynx software package (Waters Corp.), and compound identification was supported by comparison with spectral data available in the PubChem database.

4 RESULTS AND DISCUSSIONS

4.1 EXTRACTION PROCESS YIELD

The extraction yield represents a critical parameter for assessing the industrial feasibility of a process, as it reflects the amount of dry extract obtained from a defined mass of plant material. The yields for each hop variety and solvent system are summarized in Table 1.

The MAE proved to be an effective technique for recovering bioactive constituents from hop leaves, with yields ranging from 8.0% to 24.5%. The only exception was extract E5, which presented the lowest yield among all samples. The highest extraction efficiencies were obtained for extracts E2, E3, E6, and E9, highlighting the decisive influence of the solvent composition on extraction performance (Li et al., 2024; Jurinjak Tušek, Šamec & Šalić, 2022).



A consistent trend of higher yields was observed when hydroalcoholic solvents, particularly 70% ethanol, were used. This behavior can be attributed to the intermediate polarity of the ethanol–water mixture, which enhances the solubilization of a broad spectrum of hydrophilic compounds, including phenolics, flavonoids, sugars, proteins, and mineral salts. These compounds contribute substantially to the dry extract mass. The synergistic effect between ethanol and water likely promotes enhanced compound diffusion and facilitates cell wall disruption under microwave irradiation, resulting in greater extraction efficiency (Martins et al., 2023; Subra-Paternault et al., 2022).

4.2 TOTAL PHENOLIC COMPOUNDS, TOTAL FLAVONOIDS, AND ANTIOXIDANT POTENTIAL

To evaluate the overall bioactive profile of hop leaf extracts from the Nugget, Comet, and Columbus varieties obtained with different solvents (water, 50% ethanol, 70% ethanol, and absolute ethanol), the TPC, TFC, and antioxidant potential determined by the ABTS radical-scavenging assay were quantified (Table 2).

Table 2

Total phenolic and flavonoid contents, and antioxidant potential of hop leaf extracts obtained by microwave-assisted extraction.

Extract	Hop	Total phenolic ($\mu\text{g GAE/mL}$)	Total flavonoid ($\mu\text{g EC/mL}$)	ABTS ($\mu\text{mol TE/mL}$)
E1	Columbus	597.66 ± 7.62	161.44 ± 6.13	48.75 ± 4.70
E2	Columbus	615.88 ± 10.46	170.16 ± 7.25	51.90 ± 5.30
E3	Columbus	603.10 ± 8.21	169.91 ± 5.18	65.20 ± 2.15
E4	Columbus	504.28 ± 8.83	149.65 ± 4.72	49.85 ± 4.20
E5	Comet	483.01 ± 9.87	158.75 ± 5.54	55.12 ± 6.10
E6	Comet	454.32 ± 9.91	164.82 ± 7.01	73.10 ± 6.70
E7	Comet	491.43 ± 9.15	141.26 ± 3.91	75.56 ± 7.15
E8	Nugget	461.97 ± 8.44	143.90 ± 4.95	105.50 ± 10.60
E9	Nugget	527.07 ± 10.02	146.54 ± 5.27	59.40 ± 4.80
E10	Nugget	495.39 ± 9.86	146.28 ± 4.38	95.90 ± 9.51

EC: catechin equivalent; GAE: gallic acid equivalent; TE: Trolox equivalent.

Source: Elaborated by the authors.

Overall, extracts obtained with hydroalcoholic solvents—particularly those containing 50% and 70% ethanol—exhibited the highest total phenolic content (TPC) and total flavonoid content (TFC), suggesting that solvents of intermediate polarity were more effective in recovering bioactive constituents from the plant matrix. This trend was especially evident for



the Columbus variety, which showed superior extraction performance when 50% and 70% ethanol were employed.

In contrast, extracts produced with absolute ethanol showed the lowest overall levels of phenolics and flavonoids, reinforcing the limitation of low-polarity solvents for extracting predominantly hydrophilic metabolites. Aqueous extraction also revealed variable behavior among varieties; notably, the Nugget extract obtained with water displayed not only relevant concentrations of phenolic and flavonoid compounds but also the highest antioxidant potential among all tested samples.

This pattern aligns with the findings of Subra-Paternault et al. (2022), who demonstrated that solvents of intermediate polarity enhance the solubilization of phenolic compounds in various plant matrices. Similarly, El Mannoubi et al. (2023) reported that ethanol–water mixtures ranging from 50% to 80% maximize the extraction efficiency of hydrophilic and moderately polar metabolites, which explains the high extractive yields and bioactive compound recovery observed in the present study.

Despite trends in total phenolic content (TPC) and flavonoid content (TFC), the antioxidant activity exhibited a distinct pattern. The aqueous extract of the Nugget variety showed the highest ABTS value ($105.50 \pm 10.60 \mu\text{mol TE/mL}$), even though it was not among the extracts with the highest overall phenolic content. This finding suggests that the antioxidant bioactivity is more strongly associated with the presence of highly reactive specific molecules or synergistic effects among minor compounds rather than with the total phenolic concentration alone (George et al., 2022). Recent studies have also indicated that correlations between TPC and antioxidant assays such as ABTS, DPPH, or FRAP may vary depending on extract composition and assay sensitivity (Martins et al., 2023).

The high ABTS scavenging capacity observed in the aqueous Nugget extract indicates that hydrophilic compounds in this cultivar possess strong radical-quenching potential. Martins et al. (2024) emphasized that in plant extracts from species such as *Humulus lupulus*, specific compounds can contribute disproportionately to antioxidant performance even when the total phenolic content is moderate. Furthermore, Li et al. (2024) noted that antioxidant potency depends not only on the overall concentration of phenolics but also on their molecular structure and the extraction environment, which can enhance compound stability.

Genotypic variability must also be considered, as genetic differences can influence both the phytochemical profile and solvent–compound affinity. Afonso et al. (2022) demonstrated that hop cultivars exhibit pronounced variation in phenolic composition depending on genotype, crop year, and environmental conditions. Similarly, Kolenc et al. (2023) reported that



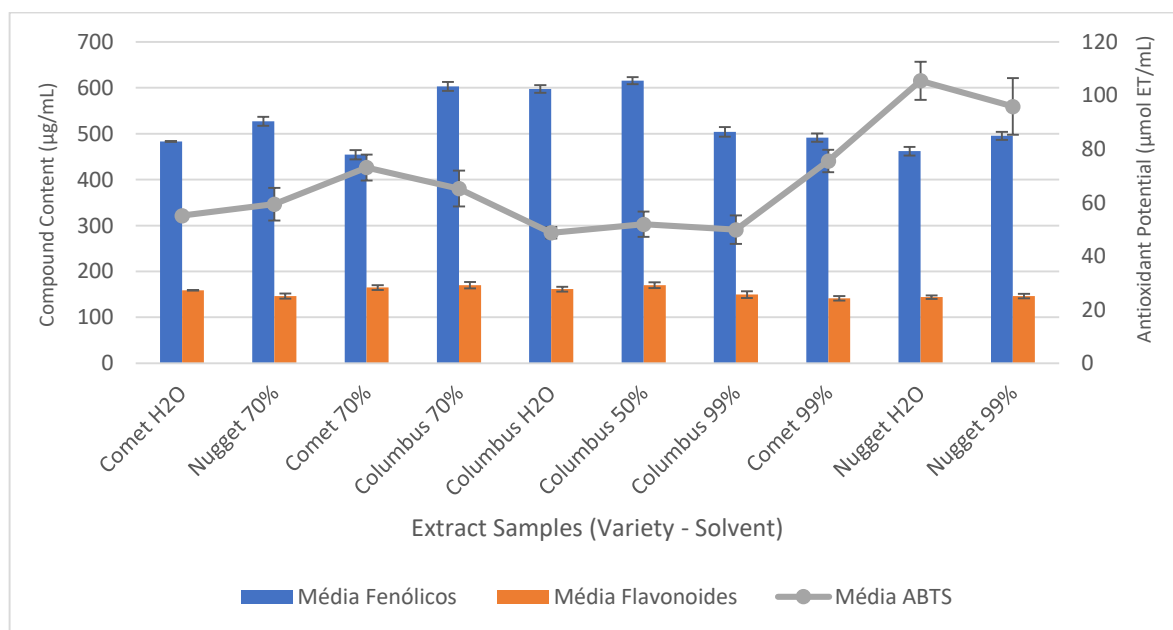
antioxidant capacity can vary substantially among genotypes even under identical extraction conditions, corroborating the differences observed among the Columbus, Nugget, and Comet varieties in the present study.

From a methodological standpoint, these findings indicate that solvent selection should be guided by the intended application of the extract. When maximizing overall phenolic and flavonoid recovery is desired, hydroalcoholic solvents such as 70% ethanol tend to be more effective. Conversely, when the goal is to optimize specific antioxidant capacity, a combined evaluation using multiple assays and compound-level identification by LC–MS/MS is recommended (Shi et al., 2022). This integrated approach facilitates the selection of molecules with greater biological relevance and supports the optimization of extraction processes for nutraceutical and pharmaceutical applications.

These results, illustrated in **Figure 1**, clearly demonstrate the combined influence of plant variety and solvent system on the chemical composition and functional properties of the hop leaf extracts. The data further highlight the effectiveness of hydroalcoholic extraction systems as an efficient strategy for recovering phenolic and flavonoid compounds with marked antioxidant potential.

Figure 1

*Total phenolic content, total flavonoid content, and antioxidant potential (ABTS assay) of *Humulus lupulus* leaf extracts.*



Source: Elaborated by the authors.



4.3 INDIVIDUAL PHENOLIC PROFILE BY LC–MS/MS

The LC–MS/MS analysis enabled the identification and quantification of six phenolic acids of interest in the leaf extracts of *Humulus lupulus* L. from the Columbus, Comet, and Nugget varieties (Table 3). The concentrations varied significantly according to both solvent type and plant variety, indicating a strong interaction between genetic factors and extraction conditions.

Among the identified compounds, chlorogenic acid exhibited the highest concentrations, particularly in the Nugget 70% ethanol extract ($1.1460 \pm 0.0199 \mu\text{g g}^{-1}$), followed by the Comet water extract ($0.8601 \pm 0.0222 \mu\text{g g}^{-1}$). This behavior confirms chlorogenic acid as a predominant metabolite in hop leaves and cones, consistent with the findings of Lyu et al. (2022) and Silva et al. (2025), who reported comparable levels in hydroalcoholic extracts analyzed by UHPLC–MS/MS. Such predominance is attributed to its central role in the biosynthetic pathways of hydroxycinnamic acids and its chemical stability under hydroalcoholic extraction conditions. Chlorogenic acid is widely recognized for its potent neuroprotective, antioxidant, and anti-inflammatory properties in central nervous system models (Nguyen et al., 2024).

Table 3

*Composition of phenolic acids ($\mu\text{g/g}$) in crude leaf extracts of *Humulus lupulus* L. (Columbus, Comet, and Nugget varieties).*

Samples	Gallic acid	Caffeic acid	Chlorogenic acid	p-Coumaric acid	Ferulic acid	Protocatechuic acid
Columbus H ₂ O	ND	0.1333 ± 0.0001	0.3914 ± 0.0015	0.4143 ± 0.0251	0.0338 ± 0.0005	0.0710 ± 0.0005
Columbus EtOH 50%	ND	$0.1343 \pm 1.2\text{E}^{-05}$	0.4280 ± 0.0060	0.3822 ± 0.0226	0.0969 ± 0.0033	0.1107 ± 0.0157
Columbus EtOH 70%	ND	0.1374 ± 0.0004	0.4650 ± 0.0039	0.3921 ± 0.0204	0.0808 ± 0.0089	0.1082 ± 0.0040
Columbus EtOH 99%	ND	0.1368 ± 0.0002	0.3959 ± 0.0078	0.3238 ± 0.0020	0.0329 ± 0.0024	0.0710 ± 0.0003
Comet H ₂ O	0.1035 ± 0.0028	0.2385 ± 0.0006	0.8601 ± 0.0222	0.9173 ± 0.0404	0.2648 ± 0.0039	0.4035 ± 0.0178
Comet EtOH 70%	0.0582 ± 0.0050	$0.1458 \pm 7.2\text{E}^{-05}$	0.5612 ± 0.0130	0.4242 ± 0.0152	0.0495 ± 0.0019	0.1698 ± 0.0026
Comet EtOH 99%	ND	0.1407 ± 0.0003	0.4080 ± 0.0020	ND	0.0365 ± 0.0027	0.0846 ± 0.0024
Nugget H ₂ O	ND	0.1396 ± 0.0005	0.5312 ± 0.0164	0.3705 ± 0.0119	0.0775 ± 0.0033	0.1610 ± 0.0018
Nugget EtOH 70%	0.0691 ± 0.0020	0.1745 ± 0.0018	1.1460 ± 0.0199	0.5208 ± 0.0332	0.0830 ± 0.0003	0.1865 ± 0.0129
Nugget EtOH 99%	ND	0.1412 ± 0.0015	0.4213 ± 0.0019	0.3634 ± 0.0132	0.0305 ± 0.0012	0.0774 ± 0.0006

ND: Not Detected

Source: Elaborated by the authors.



The aqueous extract of the Comet variety displayed the most diversified phenolic acid profile, featuring the highest contents of *p*-coumaric acid ($0.9173 \pm 0.0404 \mu\text{g g}^{-1}$), protocatechuic acid ($0.4035 \pm 0.0178 \mu\text{g g}^{-1}$), and caffeic acid ($0.2385 \pm 0.0006 \mu\text{g g}^{-1}$). These results corroborate the findings of Carbone and Gervasi (2022), who observed a similar distribution in aqueous extracts of *H. lupulus*, emphasizing the efficiency of polar solvents in releasing hydrophilic compounds. Both *p*-coumaric and protocatechuic acids are key metabolites involved in modulating antioxidant and anti-inflammatory responses, which may explain the high antioxidant capacity recorded for this variety (El Mannoubi et al., 2023; Nguyen et al., 2022). This evidence suggests that, for the Comet variety, aqueous extraction is particularly effective in obtaining a broad spectrum of phenolic acids with potential bioactive relevance.

Conversely, gallic acid was detected only in a few samples (Comet H₂O, Comet EtOH 70%, and Nugget EtOH 70%) at concentrations below $0.10 \mu\text{g g}^{-1}$. Its absence in most samples may be associated with thermal degradation during microwave-assisted extraction or with its low affinity for less polar solvents, a phenomenon also reported by Kolenc et al. (2023).

Ferulic acid exhibited moderate concentrations ($0.03\text{--}0.26 \mu\text{g g}^{-1}$), with the highest value again observed in Comet H₂O. The predominance of this variety in aqueous medium agrees with the findings of Sultana et al. (2009) and Bilska et al. (2024), who reported enhanced recovery of hydroxycinnamic acids in highly polar solvent systems. In addition, ferulic acid is well recognized for its strong antioxidant and neuroprotective properties, as demonstrated by Xu and Wang (2025) in cellular oxidative stress models.

The comparative solvent analysis revealed that 70% ethanol provided more balanced extractions, yielding higher levels of compounds with intermediate polarity, thereby confirming the efficiency of hydroalcoholic systems. This trend was also reported by Subra-Paternault et al. (2022) and Martins et al. (2023), who observed a synergistic effect of ethanol–water mixtures on cell wall disruption and phenolic compound diffusion.

From a metabolic standpoint, the predominance of cinnamic acid derivatives (chlorogenic, *p*-coumaric, ferulic, and caffeic acids) reflects the phenylalanine ammonia-lyase (PAL) pathway, which plays a key role in the biosynthesis of plant defense metabolites. The variability among cultivars (Columbus, Comet, and Nugget) corroborates the observations of Afonso et al. (2022) regarding the genetic and edaphoclimatic influences on the phenolic expression of *Humulus lupulus*.

In functional terms, chlorogenic and protocatechuic acids are noteworthy for their ability to cross the blood–brain barrier and their neuroprotective potential (Nguyen et al., 2024).



These properties explain the growing interest in hop extracts as sources of bioactive molecules with potential applications in neurological health, supporting the findings of the present study and reinforcing the pharmacological value of Brazilian hop cultivars.

Overall, the individual phenolic acid profile determined by LC–MS/MS not only reveals significant differences among cultivars and solvents but also confirms the high extraction efficiency of 70% ethanol for compounds of therapeutic relevance. These findings consolidate the potential of *H. lupulus* leaves cultivated in Brazil as a sustainable source of phenolic compounds with antioxidant and neuroprotective properties.

4.4 INDIVIDUAL PROFILE OF FLAVONOIDS AND VANILLIN BY LC–MS/MS

The LC–MS/MS analysis enabled the identification and quantification of four flavonoids (catechin, quercetin, rutin, and myricetin) as well as the aromatic phenolic compound vanillin in the leaves of *Humulus lupulus* L. cultivars Columbus, Comet, and Nugget (Table 4). The concentrations of these compounds varied widely among solvents and cultivars, reflecting both differences in compound solubility and genetic particularities inherent to each variety.

Table 4

Composition of flavonoids in crude leaf extracts of Humulus lupulus L. cultivars Columbus, Comet, and Nugget ($\mu\text{g g}^{-1}$).

Samples	Quercetin	Rutin	Catechin	Myricetin	Vanillin
Columbus H ₂ O	ND	ND	ND	0.1359 $\pm$ 0.0058	0.6874 $\pm$ 0.0321
Columbus EtOH 50%	ND	0.4438 $\pm$ 0.0108	ND	0.1258 $\pm$ 0.0088	0.7963 $\pm$ 0.0717
Columbus EtOH 70%	ND	0.4678 $\pm$ 0.0018	0.6508 $\pm$ 0.0031	0.0548 $\pm$ 0.0020	0.7781 $\pm$ 0.0569
Columbus EtOH 99%	0.0505 $\pm$ 0.0035	0.4395 $\pm$ 0.0055	ND	0.2393 $\pm$ 0.0144	0.5117 $\pm$ 0.0082
Comet H ₂ O	0.0389 $\pm$ 0.0016	2.6374 $\pm$ 0.0590	0.4710 $\pm$ 0.0010	0.1475 $\pm$ 0.0060	1.1057 $\pm$ 0.1518
Comet EtOH 70%	0.0476 $\pm$ 0.0024	4.7965 $\pm$ 0.0545	2.2354 $\pm$ 0.2316	0.2064 $\pm$ 0.0534	0.5579 $\pm$ 0.0775
Comet EtOH 99%	0.0481 $\pm$ 0.0004	1.0852 $\pm$ 0.0099	1.5552 $\pm$ 0.3554	0.1828 $\pm$ 0.0603	0.6948 $\pm$ 0.0231
Nugget H ₂ O	0.0291 $\pm$ 0.0008	0.8266 $\pm$ 0.0077	0.5112 $\pm$ 0.0052	0.1948 $\pm$ 0.0098	0.5975 $\pm$ 0.0313
Nugget EtOH 70%	0.0344 $\pm$ 0.0010	2.9878 $\pm$ 0.0611	2.8299 $\pm$ 0.0602	0.2126 $\pm$ 0.0453	0.6651 $\pm$ 0.0825
Nugget EtOH 99%	0.0382 $\pm$ 0.0039	0.8897 $\pm$ 0.0042	2.4491 $\pm$ 0.0285	0.2247 $\pm$ 0.0004	0.5108 $\pm$ 0.0173

ND: Not Detected

Source: Elaborated by the authors.



Among the flavonoids identified, rutin was the predominant compound, reaching its highest concentration in the Comet EtOH 70% extract ($4.7965 \pm 0.0545 \mu\text{g g}^{-1}$), followed by Nugget EtOH 70% ($2.9878 \pm 0.0611 \mu\text{g g}^{-1}$). These results confirm that hydroalcoholic solvents of intermediate polarity favor the extraction of glycosylated flavonoids due to their mixed solubility in both water and ethanol (Jurinjak Tušek et al., 2022; Martins et al., 2023). Similar findings were reported by Gullón et al. (2017) and Kolenc et al. (2023), who observed high rutin contents in hop extracts obtained through hydroalcoholic extraction, corroborating the data obtained in this study. Rutin is widely recognized for its antioxidant, anti-inflammatory, and neuroprotective properties, modulating signaling pathways related to apoptosis and oxidative stress, thus reinforcing the pharmacological potential of *H. lupulus* leaves cultivated in Brazil (Gullón et al., 2017).

Catechin was the second most abundant flavonoid, with the highest levels observed in Nugget EtOH 70% ($2.8299 \pm 0.0602 \mu\text{g g}^{-1}$) and Nugget EtOH 99% ($2.4491 \pm 0.0285 \mu\text{g g}^{-1}$). The enhanced extraction in less polar solvents is consistent with the semi-crystalline nature and lower polarity of catechin (Li et al., 2024). Comparable results were reported by Piekara et al. (2025), who demonstrated that *H. lupulus* cultivars accumulate higher concentrations of catechin and related flavonols in the leaves, particularly under ethanolic extraction. In addition to its antioxidant potential, catechin has been associated with improved mitochondrial function and neuronal protection against oxidative damage (Nguyen et al., 2024).

Although quercetin was present in lower amounts (maximum $0.0505 \pm 0.0035 \mu\text{g g}^{-1}$ in Columbus EtOH 99%), it was detected in all cultivars, except for some aqueous and diluted ethanolic extractions. The low concentration of this flavonol may be related to partial degradation during microwave-assisted extraction, since thermolabile compounds are prone to oxidation at elevated temperatures (Silva et al., 2025). Lyu et al. (2022) also reported relatively low levels of quercetin in hop extracts, reinforcing its limited thermal stability in polar solvent systems.

Although myricetin was present at relatively low concentrations ($0.0548\text{--}0.2393 \mu\text{g g}^{-1}$), it exhibited an interesting extraction behavior: the highest content was observed in Columbus EtOH 99% ($0.2393 \pm 0.0144 \mu\text{g g}^{-1}$), indicating a greater affinity for less polar solvents. This trend is consistent with the findings of Afonso et al. (2022) and Bilska et al. (2024), who reported that myricetin shows higher solubility in concentrated ethanolic media and plays an important role in antioxidant defense and the modulation of pro-inflammatory enzymes.

Among the non-flavonoid phenolic compounds, vanillin was detected in all extracts, with the highest concentration observed in the Comet H₂O extract ($1.1057 \pm 0.1518 \mu\text{g g}^{-1}$).



This result highlights the high solubility of vanillin in polar media and suggests that a significant portion of the antioxidant bioactivity observed in aqueous extracts (Table 2) may be attributed to this compound. Vanillin is well known for its strong antioxidant capacity and neuroprotective effects, acting through the suppression of inflammatory mediators (Nguyen et al., 2024). A recent study by Piekara et al. (2025) also confirmed the presence of aromatic derivatives such as vanillin in hop tissues, reinforcing the multifunctional biological relevance of this molecule.

Overall, 70% ethanol extraction proved to be the most efficient condition for obtaining a balanced flavonoid profile, characterized by high levels of rutin and catechin along with satisfactory recovery of quercetin, myricetin, and vanillin. This behavior can be attributed to the synergistic interaction between the polar properties of water and the organic characteristics of ethanol, which enhances solvent penetration into the plant matrix and facilitates cell wall disruption (Subra-Paternault et al., 2022; Martins et al., 2023).

The variability observed among the Columbus, Comet, and Nugget cultivars confirms the pivotal role of genetic factors in the biosynthesis of flavonoids, as demonstrated by Afonso et al. (2022) and Kolenc et al. (2023). The predominance of rutin and catechin in the extracts of Comet and Nugget highlights the potential of these varieties as promising sources of neuroprotective compounds, adding significant pharmacological value to hops cultivated under Brazilian conditions.

Taken together, the results of this study unequivocally demonstrate the potential of hop leaves cultivated in Brazil as a rich and diversified source of bioactive compounds with direct relevance to neurological health. It became evident that both cultivar selection and extraction solvent are critical factors that selectively modulate the phytochemical profile, allowing for the optimized recovery of high-value compounds such as rutin and chlorogenic acid.

In addition, the remarkable dissociation between the high total phenolic content and the peak antioxidant activity observed in the Nugget aqueous extract suggests the presence of molecules with high specific potency, opening a promising avenue for future investigations. Therefore, these findings not only fill a critical gap in the chemical characterization of Brazilian hop varieties but also provide a solid foundation for the conclusions of this study and for the development of future therapeutic applications.

5 CONCLUSION

This study characterized the phytochemical profile of leaves from three *Humulus lupulus* L. cultivars grown in Brazil, demonstrating that both cultivar and solvent selection



significantly influence the extraction efficiency of bioactive compounds. Hydroalcoholic solutions, particularly 50% and 70% ethanol, yielded higher levels of phenolics and flavonoids, including pharmacologically relevant molecules such as rutin and catechin. These findings confirm that Brazilian-grown hops represent a diverse and promising source of antioxidant and neuroprotective compounds, addressing an important knowledge gap regarding hops cultivated in subtropical regions.

An important result was the dissociation between total phenolic content and antioxidant activity, as observed in the Nugget aqueous extract, indicating that biological efficacy is driven not only by concentration but also by molecular structure, polarity and synergistic interactions among compounds. Overall, the study reinforces the pharmacological and nutraceutical potential of hop leaves as sustainable sources of bioactive molecules and provides a solid foundation for future investigations exploring their technological applications, particularly in products aimed at neurological health and healthy aging.

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