UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

Colegio de Ciencias Biológicas y Ambientales

Bridging Tradition and Science: Chemical Profiling and Antioxidant Activity of *Brunfelsia chiricaspi* Plowman (Solanaceae), a Medicinal Plant Used by the Shuar in the Ecuadorian Amazon.

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RESUMEN

Brunfelsia chiricaspi (parapra) es una planta medicinal utilizada tradicionalmente por la cultura Shuar de la Amazonía ecuatoriana para curar fiebres, dolores reumáticos y tos. A pesar de la recopilación oral, estos usos medicinales carecen de validación científica. Este estudio analizó infusiones usadas tradicionalmente y extractos hidroalcohólicos para la extracción máxima de compuestos de diferentes partes de *B. chiricaspi* (hojas, flores, corteza y mezclas de flor y hoja; flor, hojas y corteza) y así, determinar su contenido de fenoles totales, flavonoides y aminoácidos libres, junto con su capacidad antioxidante medida mediante los ensayos DPPH y FRAP. El individuo de *B. chiricaspi* fue colectado en Sevilla Don Bosco, Morona Santiago, Ecuador. Los resultados demostraron que los extractos hidroalcohólicos proporcionaron mayores concentraciones de compuestos bioactivos y actividades antioxidantes en comparación con las infusiones tradicionales, aunque las infusiones conservaron un nivel funcional significativo. Los análisis estadísticos confirmaron efectos significativos de la parte de la planta, el método de extracción y su interacción en todos los parámetros evaluados. Las hojas y las mezclas de hojas y flores mostraron las concentraciones más altas de compuestos fenólicos y flavonoides, mientras que la prolina fue el aminoácido libre más abundante, especialmente en las flores. Estos hallazgos respaldan el uso tradicional de *B. chiricaspi*, validan sus propiedades antioxidantes y destacan la influencia del método de preparación en la recuperación fitoquímica. Este trabajo constituye la primera caracterización química integral de *B. chiricaspi* y sienta las bases para futuras investigaciones orientadas al

aislamiento de bioactivos, validación farmacológica y desarrollo de productos fitoterapéuticos estandarizados.

Palabras clave: actividad antioxidante, compuestos fenólicos, flavonoides, medicina tradicional amazónica, Shuar, extractos hidroalcohólicos, infusiones, validación científica.

ABSTRACT

Brunfelsia chiricaspi (parapra) is a medicinal plant traditionally used by the Shuar culture of the Ecuadorian Amazon to treat fevers, rheumatic pain, and coughs. Despite oral recollection, these medicinal uses lack scientific validation. This study analyzed traditionally used infusions and hydroalcoholic extracts for the maximum extraction of compounds from different parts of *B. chiricaspi* (leaves, flowers, bark, and flower-leaf mixtures; flower, leaves, and bark) and thus, determined their total phenol, flavonoid, and free amino acid content, along with their antioxidant capacity measured by DPPH and FRAP assays. The *B. chiricaspi* individual was collected in Sevilla Don Bosco, Morona Santiago, Ecuador. The results demonstrated that the hydroalcoholic extracts provided higher concentrations of bioactive compounds and antioxidant activities compared to traditional infusions, although the infusions retained a significant functional level. Statistical analyses confirmed significant effects of plant part, extraction method, and their interaction on all parameters evaluated. Leaves and leaf-flower mixtures showed the highest concentrations of phenolic compounds and flavonoids, while proline was the most abundant free amino acid, especially in the flowers. These findings support the traditional use of *B. chiricaspi*, validate its antioxidant properties, and highlight the influence of the preparation method on phytochemical recovery. This work constitutes the first comprehensive chemical characterization of *B. chiricaspi* and lays the groundwork for future research aimed at isolating bioactive ingredients, pharmacological validation, and developing standardized phytotherapeutic products.

Keywords: antioxidant activity, phenolic compounds, flavonoids, Amazonian traditional medicine, Shuar, hydroalcoholic extracts, infusions, scientific validation.

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INTRODUCTION

Since ancient times, plants have played a fundamental role in human societies, providing essential resources such as food, medicine, and materials for daily life (Davis & Choisy, 2024). The relationship between humans and plants has been deeply intertwined, influencing cultural practices, healthcare systems, and economic structures. Civilizations across the world have developed extensive knowledge of plant properties, using them for nutrition, healing, and spiritual purposes (Nolan & Turner, 2011). Among these, Indigenous communities have preserved a rich body of ethnobotanical knowledge, typically transmitted orally across generations. Particularly in biodiverse regions like the Amazon, traditional medicine based on plant use remains central to health practices. These medical systems are grounded in empirical knowledge accumulated over centuries, applying botanical resources to treat infections, fevers, chronic diseases, and spiritual disorders (Alum, 2024).

However, this knowledge is increasingly endangered due to anthropogenic pressures such as deforestation, agricultural expansion, colonization, and the encroachment of modern society (Kennedy et al., 2023). Habitat destruction not only threatens plant biodiversity but also restricts access to medicinal species for Indigenous communities. Additionally, cultural erosion driven by globalization and displacement contributes to the loss of traditional knowledge, as younger generations adopt modern lifestyles and shift toward pharmaceutical-based healthcare (Ramirez, 2007).

In response, ethnobotany and bioprospecting have emerged as crucial scientific approaches to document, preserve, and validate traditional plant use. These interdisciplinary efforts involve close collaboration between scientists and Indigenous communities to record ethnomedicinal uses, study phytochemical profiles, and explore pharmacological potential. Ethnobotanical research bridges traditional knowledge and modern science, offering valuable insights into

novel bioactive compounds while supporting conservation of plant species and the protection of Indigenous intellectual property.

Despite the recognized importance of Amazonian medicinal plants, many species remain understudied. The *Solanaceae* Juss. family is among the most diverse and widely used in traditional medicine, particularly within Amazonian cultures. With 96 genera and approximately 2,800 species distributed across tropical and temperate regions (Souza et al., 2023), *Solanaceae* includes herbaceous plants, trees, shrubs, and climbers. Morphologically, these species are characterized by simple, alternate or opposite leaves, mostly radially symmetrical (actinomorphic) flowers, and fruits in the form of berries or capsules, containing numerous small seeds (Cornejo, 2006). The highest species diversity occurs in South America, which hosts many endemics (Martins & Barkman, 2005; Souza et al., 2023). Chemically, the family is rich in secondary metabolites, notably alkaloids, phenolic compounds, saponins, terpenes, and lipophilic metabolites, many of which have recognized pharmacological activities (Giacomin et al., 2023).

Within this family, the genus *Brunfelsia* comprises about 42 species of shrubs and small trees, with around 20 species found in the tropical forests of South America. In Ecuador, four species have been reported: *Brunfelsia chiricaspi* Plowman, *B. grandiflora* D. Don, *B. macrocarpa* Plowman, and *B. undulata* Sw. Of these, *B. chiricaspi* and *B. grandiflora* occur in the Ecuadorian Amazon (Jorgensen & Leon-Yanez, 1999). While *B. grandiflora* has received some scientific attention due to its bioactive properties, comprehensive phytochemical studies on *B. chiricaspi* are lacking.

Brunfelsia grandiflora, known locally as “chiricaspi,” is traditionally used by the Shuar people for its analgesic, anti-inflammatory, energizing, and aphrodisiac effects (Luzuriaga-Quichimbo et al., 2018). It is employed to treat rheumatism, arthritis, injuries, and muscle pain, as well as to enhance endurance, circulation, and libido. Its use extends to spiritual healing and

ritual cleansing. Despite its broad ethnomedical application, scientific knowledge about its chemical composition remains minimal. Concerns regarding its safety also exist due to the presence of potentially toxic alkaloids, highlighting the need for expert supervision during use.

On the other hand, *B. chiricaspi*, known as "parapra", has been little studied, with only taxonomic studies of this species suggesting a hallucinogenic effect, and in Doyle et al., its ethnomedicinal potential is highlighted (Doyle et al., 2019).

To date, no comprehensive phytochemical characterization of *B. chiricaspi* has been conducted. Understanding its chemical profile is essential to validate traditional uses, identify therapeutic compounds, and support conservation efforts for this underexplored species. Moreover, scientific validation can contribute to the preservation of Indigenous knowledge systems and promote their integration into broader health frameworks.

This study hypothesizes that *B. chiricaspi* contains bioactive compounds—particularly phenolic compounds—with significant antioxidant and therapeutic properties, and that its chemical composition and activity are influenced by the extraction method used

Phytochemical analysis is expected to reveal key secondary metabolites responsible for the plant's medicinal effects.

General Objective

To characterize the polyphenolic, flavonoid, and free amino acid composition of *Brunfelsia chiricaspi* extracts, evaluate their antioxidant potential, and assess how the extraction method influences both chemical profile and bioactivity.

Specific Objectives

To quantify total phenolic, flavonoid, and free amino acid content in different organs of *B. chiricaspi* using spectrophotometric assays.

To evaluate the antioxidant activity of traditional infusions and hydroalcoholic extracts using DPPH and FRAP assays.

To compare the chemical and functional profiles of extracts obtained through different extraction methods and assess the efficiency of traditional infusions relative to hydroalcoholic extraction.

Methodological Overview

Plant material will be collected following ethical guidelines and national regulations for ethnobotanical research. Both traditional infusions and hydroalcoholic extracts will be prepared from different plant parts. Spectrophotometric methods will be used for qualitative and quantitative analysis of bioactive compound families and antioxidant activity. Comparative analyses will be conducted to assess how extraction methods affect the chemical composition and functional properties of the plant, providing a foundation for further pharmacological studies.

METHODS

Reagents and Solvents

All reagents and solvents used in this study were of analytical grade and were purchased from Sigma-Aldrich (St. Louis, USA).

Sample Collection and Preparation

The plant samples (*B. chiricaspi*) which come from one individual were collected with the respective MAATE authorization MAATE-ARSFC-2023-0077 guide n° 02396 from a Evergreen piedmont forest of the north-central eastern Andes mountain range (Aguirre et al., 2013) in August 2024 from the "TSAPAU" farm, which belongs to the Shuar community of San Luis de Inimkis, coordinates 2°23'10.3"S 78°06'34.2"W UTM, located in the Sevilla Don Bosco canton, Morona Santiago province, Ecuador. This province is part of the southern Amazon region of Ecuador and is situated at an altitude of 874 meters above sea level (Figure 1).

A total of 1 kg of plant material—leaves, and stems, except flowers (0.5 kg), was collected from mature specimens growing in their natural environment. The taxonomic identification of the specimens was conducted at the Herbario de Botánica Económica del Ecuador of the Universidad San Francisco de Quito (USFQ) by the taxonomic curator, using specialized botanical literature, digital resources (tropicos.org, taxonomy paper and Ecuador vascular plants catalogue) and vouchers from another *Brunfelsia* species. Focusing on the biggest differences between other *Brunfelsia* species, we highlight the oblong shape of the leaves and their prominent veins, also the color and grouping of the flowers that are different in each *Brunfelsia* species (Plowman, 1973). A voucher specimen was deposited in the herbarium under reference number 35007 for future verification and research purposes (Figure 2).

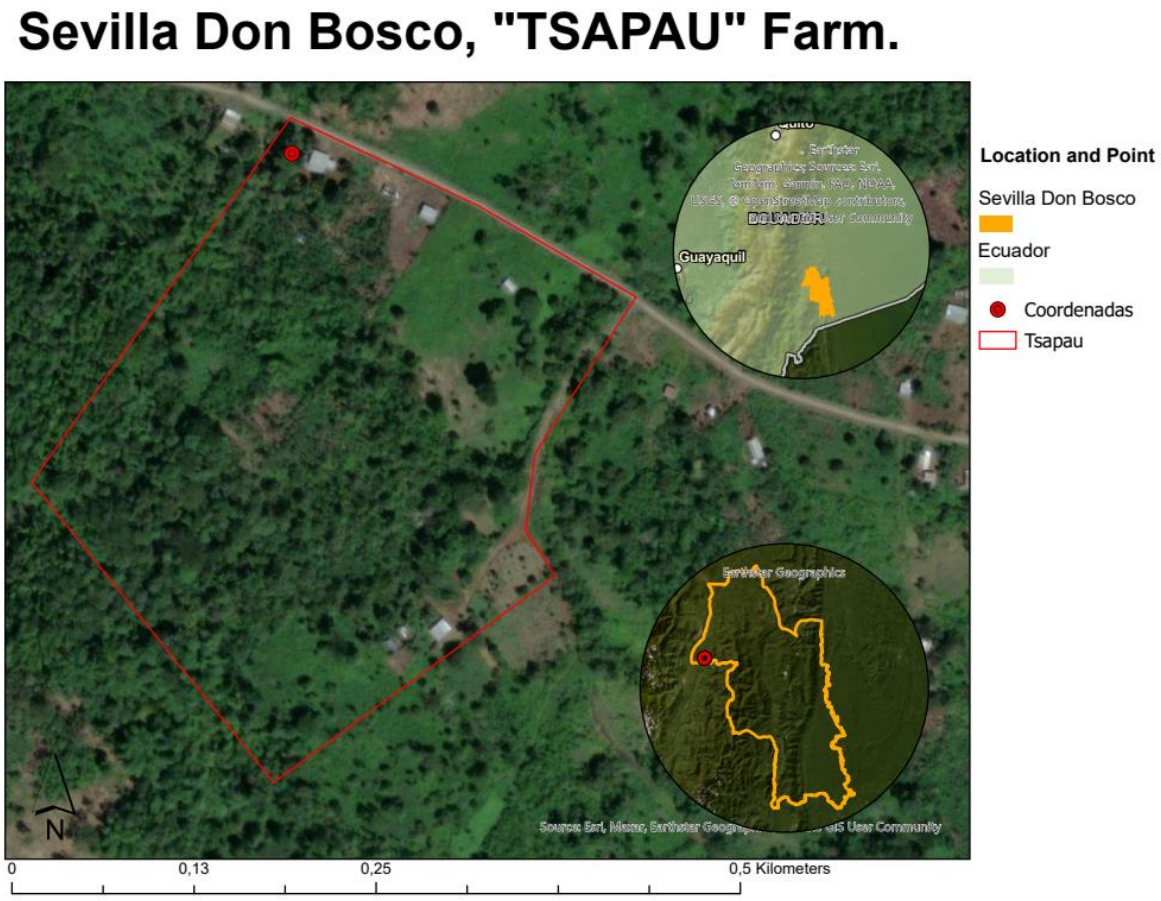

Figure 1. Geographic location of *Brunfelsia chiricaspi* individual collection site in Sevilla Don Bosco Finca "TSAPAU".

Figure 2. Plant individual voucher collected in Sevilla Don Bosco, "TSAPAU" farm.

After collection, the plant material was thoroughly cleaned to remove any impurities, carefully cut into small pieces, and the stem was scraped to obtain the bark. Then stored at -20°C to preserve its integrity, and for a minimum time of 24 hours to ensure proper dehydration in next process. The samples were then freeze-dried using a BIOBASE freeze-dryer (BIOBASE, Shandong, CN) for 72 hours. Once the process was complete, the dried samples were finely ground using an analytical mill (IKA A11 basic) to obtain a homogeneous powder (Figure 3). The samples were stored at - 20°C until the time of analysis.

Figure 3. Milled sample powders (bark, leaves, flowers and mixes flower and leaves, and flower, leaves and bark) from the individual of *Brunfelsia chiricaspi* (Solanaceae).

Preparation of Infusions and Hydroalcoholic Extracts

For the preparation of extracts or infusions, three individual plant parts (flowers, leaves, and stem) were used, along with two mixed combinations: (1) flowers and leaves, and (2) flowers, leaves, and bark.

Infusions.

The infusions were prepared following the traditional method of the Shuar indigenous nationality. For this process, 10 mL of distilled water was brought to boil. One gram of plant powder from each plant part (leaf, flower, and stem) was accurately weighed separately. In the case of mixtures, equal amounts of the finely ground powder from each plant part were weighed independently and then combined to form a homogeneous mixture equivalent to 1 g. Once the water reached boiling point, the plant powder was added, thoroughly mixed with the water, and left to boil for 10 minutes. The samples were then allowed to cool to room temperature. The infusions were sequentially filtered, first through paper filters and then through 0.45 µm PTFE syringe filter to ensure the removal of solid residues. Finally, the filtered infusions were stored at -20°C for later analysis.

Hydroalcoholic Extracts.

The hydroalcoholic extracts were prepared using a mixture of methanol analytical grade and distilled water in an 80:20 *v/v* ratio, following the procedure previously reported and employed in the Bio-exploration Laboratory at Universidad San Francisco de Quito USFQ (Guevara et al., 2019). Each extract was prepared with a total volume of 10 mL, to which 1 g of plant powder was added and thoroughly mixed. The mixture was then homogenized and then placed in an orbital shaker to macerate for 24 hours in a dark room to enhance compound extraction. After the maceration process, the extracts were centrifuged twice for 10 minutes at 1500 g using a HERMLE Z206A centrifuge (HERMLE Labortechnik GmbH, Wehingen, GER) to separate any suspended particles. The resulting supernatant was subsequently filtered twice, first using filter paper and then 0.45 µm Minisart syringe filters, to remove any remaining plant residues. The final hydroalcoholic extracts were stored at -20°C for further analysis.

Determination of Total Phenolic Content – Folin-Ciocalteu Method

The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu method (Singleton et al., 1999). First, the Folin-Ciocalteu reagent was prepared by diluting 1 mL of FC reagent in 10 mL of distilled water, ensuring minimal exposure to light. The sodium carbonate solution was then prepared by dissolving 3.75 g of sodium carbonate in 50 mL of distilled water. Next, 100 µL of the hydroalcoholic extract or infusion sample was mixed with 500 µL of the Folin-Ciocalteu reagent. The reaction mixture was incubated for 5 minutes at room temperature in the dark, followed by the addition of 400 µL of the sodium carbonate solution. The mixture was then incubated at room temperature for 2 hours in the dark to allow for the reaction to proceed. After incubation, the samples were centrifuged for 10 minutes at 1500 g using a HERMLE Z206A centrifuge (HERMLE Labortechnik GmbH, Wehingen, Germany) to remove any precipitate. The absorbance of the samples was measured spectrophotometrically at 760 nm using an i3 UV–Vis spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China) with a quartz cuvette. A blank sample, prepared with the same reaction mixture but replacing the extract with a hydroalcoholic methanol/water solution (80:20 *v/v*), was used as a reference. To determine the total phenolic content, a gallic acid standard curve was generated (0.031– 0.6 mM, $y = 1.6509x - 0.007$, $R^2 = 0.9983$) per 1 g of fresh weight of plant, and the results were expressed as mg of gallic acid equivalents (GAEq) per-mL of extract.

Determination of Total Flavonoid Content – Aluminum Chloride Method

The total flavonoid content was determined spectrophotometrically following the aluminum chloride colorimetric method (Shraim et al., 2021). First, all reagents were prepared and kept protected from light. A 10 % (w/v) aluminum chloride hexahydrate ($AlCl_3 \cdot 6H_2O$) solution was prepared by dissolving 2 g of $AlCl_3 \cdot 6H_2O$ in 20 mL of methanol. A 1 M sodium hydroxide

solution was prepared by dissolving 1 g of NaOH in 25 mL of distilled water. Finally, a 5 % (w/v) sodium nitrite ($NaNO_2$) solution was obtained by dissolving 0.5 g of $NaNO_2$ in 10 mL of distilled water. For each assay, 160 µL of the hydroalcoholic extract or infusion (previously diluted 1:2 v/v based on preliminary tests) was placed into an-Eppendorf tube and brought to 960 µL with distilled water. Then, 48 µL of the 5 % $NaNO_2$ solution was added; the mixture was vortexed and incubated for 6 minutes at room temperature in the dark. Subsequently, 96 µL of the 10 % $AlCl_3 \cdot 6H_2O$ solution was added, vortexed, and incubated for an additional 5 minutes under the same conditions. Finally, 320 µL of the 1 M NaOH solution and 176 µL of distilled water were added, the reaction mixture was vortexed once more, and allowed to develop for 10 minutes at room temperature in the dark. After incubation, absorbance was measured at 510 nm using an i3 UV–Vis spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China) with plastic cuvettes. A reagent blank—prepared by replacing the sample with distilled water—served as reference. Total flavonoid content was quantified against a catechin standard curve (0.06–500 µM; $y = 0.8951x - 0.0016$, $R^2 = 0.9992$) and expressed as miligram of catechin equivalents (mg Catq) per mL of extract.

Determination of Free Amino Acids – Ninhydrin–Cadmium Method

Free amino acids were quantified spectrophotometrically using the ninhydrin–cadmium assay (Doi et al., 1981). First, the working reagent was prepared by dissolving 1 g of $CdCl_2$ in 1 mL of distilled water and, in a separate flask, mixing 80 mL of 99.5 % ethanol with 10 mL of glacial acetic acid before dissolving 0.8 g of ninhydrin; the $CdCl_2$ solution was then added to the ninhydrin mix and gently homogenized. For each determination, 1 mL of the hydroalcoholic extract or infusion was combined with 2 mL of the ninhydrin–cadmium reagent in a test tube, heated at 80 °C for 5 minutes, and immediately cooled under running water to room temperature. The absorbance of the resulting chromophore was measured at 507 nm

using an i3 UV–Vis spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China) with a reagent blank prepared in distilled water. Free leucine and proline were quantified against their respective standard curves (leucine: 2.4 – 24 mg/L, $y = 0.0305\ x - 0.03$, $R^2 = 0.9942$; proline: 50 – 800 mg/L, $y = 0.0012\ x - 0.0345$, $R^2 = 0.9861$) and the results expressed as mg amino acid per mL of extract.

Determination of Total Antioxidant Capacity (TAC)

The total antioxidant capacity of the hydroalcoholic extracts and infusions was determined in parallel using Ferric Reducing Antioxidant Power (FRAP) and 2,2 – diphenyl – 1-picrylhydrazyl free radical method (DPPH) assays.

The Ferric Reducing Antioxidant Power (FRAP) assay was performed following the method previously described by Benzie and Strain (Benzie & Strain, 1996).

The FRAP working solution was prepared 15 minutes before beginning the experimental protocol by mixing: 10 parts sodium acetate buffers (300 mM, pH 3.6), 1 part 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM hydrochloric acid and 1 part 20 mM ferric chloride ($FeCl_3$) solution. Preliminary tests were performed to determine the appropriate dilution for each hydroalcoholic extract and infusion. For sample analysis, 100 µL of each diluted infusion or hydroalcoholic extract was mixed with 900 µL of freshly prepared FRAP reagent. The samples were then incubated for 15 minutes at room temperature in the dark to allow the reaction to develop. The antioxidant capacity was determined by measuring the absorbance of the samples spectrophotometrically at 539 nm using an i3 UV–Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China) with a quartz cuvette against a blank consisting of 900 µL of the FRAP reagent with 100 µL of methanol (for hydroalcoholic extracts) or distilled water (for infusions). A Trolox standard curve (12.5–

300 µM, y = 0.0028 + 0.0232, R^2 = 0.9938) was used to quantify antioxidant activity, and the results were expressed as micromoles of Trolox equivalents (TEq) per mg of fresh weight.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was performed following the method previously described by Prymont Przyminska et al. (Prymont-Przyminska et al., 2014).

The DPPH working solution was prepared by dissolving 2.5 mg of DPPH radical in 100 mL of methanol and adjusting its absorbance to 0.7 at 517 nm. Preliminary tests were performed to assess the need for dilution; however, this was not necessary, as the extracts and hydroalcoholic infusions were within the appropriate absorbance range. For each reaction, 10 µL of the hydroalcoholic extract or infusion was mixed with 790 µL of the prepared DPPH solution, and the reaction mixture was incubated in the dark for 15 minutes at room temperature. The absorbance was then measured spectrophotometrically at 517 nm against a blank containing the DPPH reagent and a methanol-water solution (80:20 v/v) instead of the sample. The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = (\text{Abs of control} - \text{Abs of sample} / \text{Abs of control})$$

Where Abs consists of the absorbance of the sample or blank determined spectrophotometrically at 517 nm using an i3 UV–Vis Spectrophotometer (Hanon Advanced Technology Group Co., Ltd., Jinan, China) with a quartz cuvette.

A Trolox standard curve (0.1–2 µM, y = -3.3164 + 02.2931, R^2 = 0.972) was used and the results were expressed as micromoles of Trolox equivalents (TEq) per mg of fresh weight.

Statistical analysis

The data from the five spectrophotometric assays were processed in RStudio as follows: first, descriptive statistics (mean ± standard deviation) were calculated for each floral part and extraction type (infusion or hydroalcoholic extract). Next, data normality was assessed using the Shapiro–Wilk test. Measurements that met the normality assumption were analyzed by two-way ANOVA, while non-normal data were transformed—using the bestNormalize function—to approximate a normal distribution before conducting two-way ANOVA. All parametric tests were performed at a significance level of $p < 0.001$. Finally, post hoc multiple comparisons were carried out with the emmeans package in R, applying Tukey's correction to identify significant differences between infusion and hydroalcoholic extract treatments within each floral part.

RESULTS

According to the results, total phenolic content in infusions (Figure *5*) ranged from 0.31 ± 0.04 mg GAE/mL in stem to 0.54 ± 0.06 mg GAE/mL in the leaf–flower mixture, whereas in hydroalcoholic extracts (Figure *4*) it varied from 0.23 ± 0.05 mg GAE/mL in stem to 0.61 ± 0.12 mg GAE/mL in the same mixture. Two-way ANOVA revealed a significant effect of plant tissue ($F(4, 80) = 47.64$, $p < 2 \times 10^{-16}$) and a tissue × method interaction ($F(4, 80) = 10.17$, $p = 1.04 \times 10^{-6}$), but no main effect of preparation type ($F(1, 80) = 0.91$, $p = 0.344$). Post hoc comparisons showed that extracts contained significantly more phenolics than infusions in flowers (estimate = 1.31, *$p < 0.0001$*), while infusions exceeded extracts in bark (estimate = –0.61, $p = 0.0184$) and in the leaf–flower–bark mixture (estimate = –0.61, $p = 0.0183$), with no differences in leaves ($p = 0.971$) or in the leaf–flower mix ($p = 0.0928$). Total flavonoid content in infusions (Figure *5*) ranged from 0.07 ± 0.01 mg Catq/mL in the leaf–flower–stem mix to 0.09 ± 0.01 mg Catq/mL in leaves, while in extracts (Figure *4*) it

spanned 0.05 ± 0.01 mg Catq/mL in bark to 0.42 ± 0.06 mg Catq/mL in the leaf–flower mix. Two-way ANOVA indicated highly significant effects of tissue ($F(4, 80) = 94.06$, $p < 2 \times 10^{-16}$), preparation ($F(1, 80) = 311.93$, $p < 2 \times 10^{-16}$) and their interaction ($F(4, 80) = 14.20$, $p = 8.46 \times 10^{-9}$); post hoc tests confirmed that extracts had significantly higher flavonoid levels than infusions across all tissues (bark: estimate = 0.88; flowers: 1.75; leaves: 0.67; leaves–flowers–bark: 0.86; leaves–flowers: 1.94; all $p < 0.0001$).

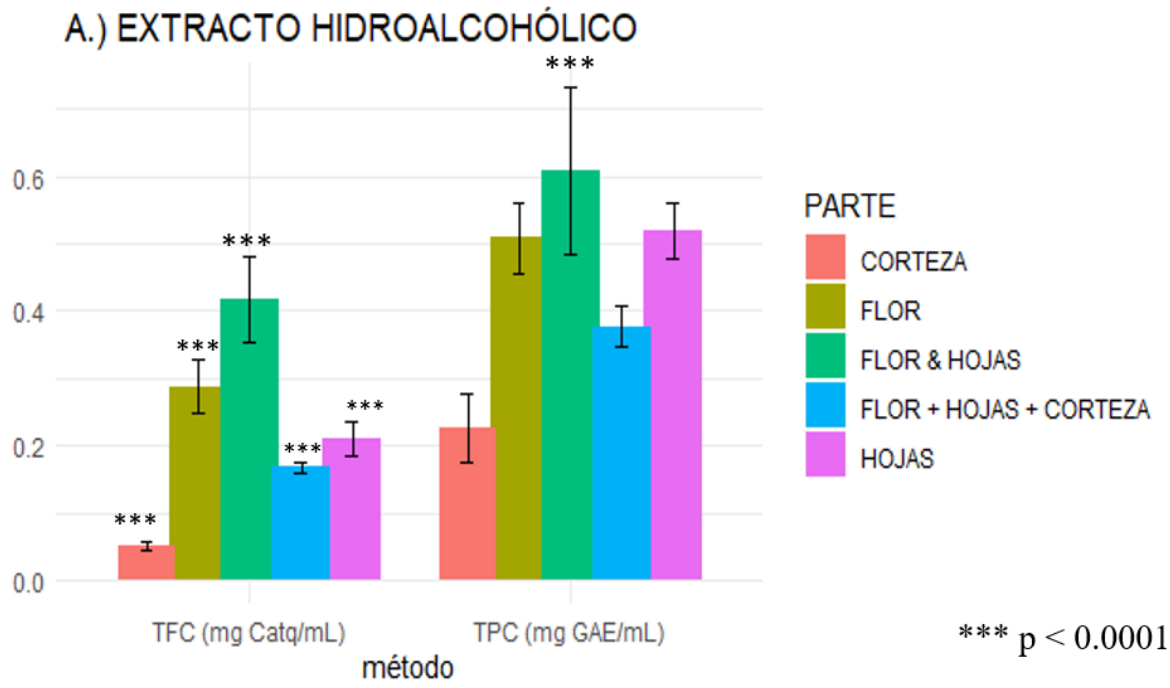

Figure 4. Total phenol content (TPC) expressed in milligrams of gallic acid equivalent per milliliter of extract and total flavonoid content (TFC) expressed in milligrams of catechin equivalent per milliliter of extract (right to left).

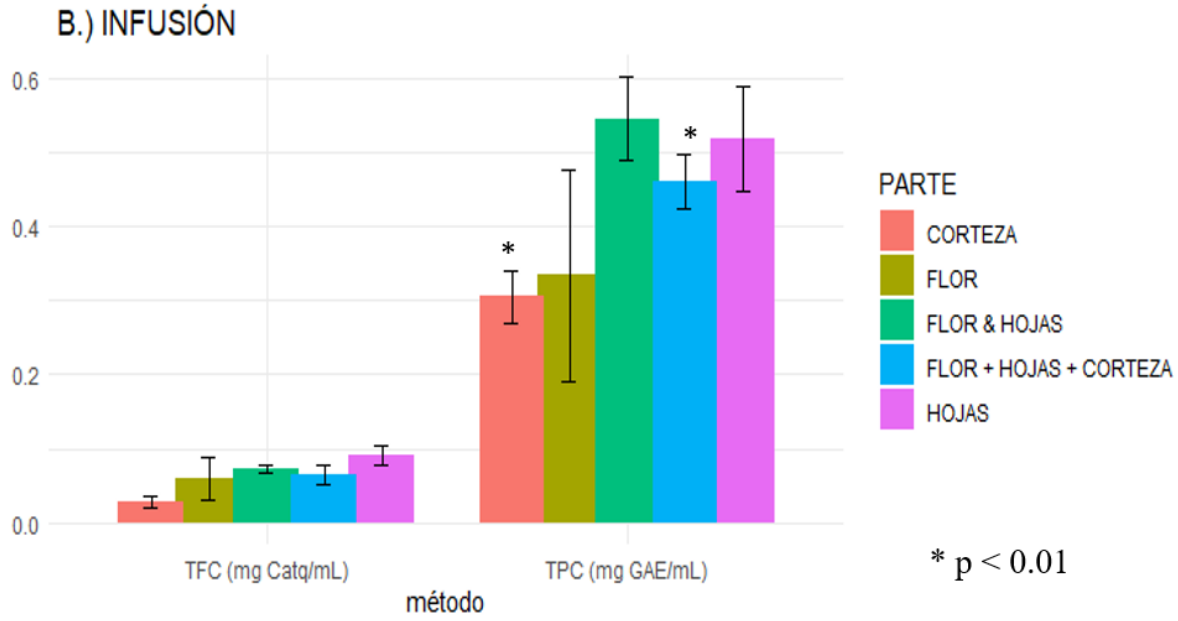

Figure 5. Total phenol content (TPC) expressed in milligrams of gallic acid equivalent per milliliters of infusion extract and total flavonoid content (TFC) expressed in milligrams of catechin equivalent per milliliter of infusion extract (right to left).

Free amino acid analysis showed that infusion (Figure *7*) proline ranged from 0.37 ± 0.13 mg/mL in bark to 1.12 ± 0.30 mg/mL in the leaf–flower mix, and infusion leucine (Figure *7*) peaked at 0.06 ± 0.02 mg/mL in flowers; in extracts (*Figure 6*) both amino acids increased by 40–60 %, with proline highest in leaves (~1.30 mg/mL) and leucine highest in leaves (0.34 ± 0.09 mg/mL). Two-way ANOVAs revealed significant effects of tissue (proline: $F(4, 75) = 3.71$, $p = 0.0083$; leucine: $F(4, 79) = 5.86$, $p = 0.00035$), preparation (proline: $F(1, 75) = 176.02$, $p < 2 \times 10^{-16}$; leucine: $F(1, 79) = 308.03$, $p < 2 \times 10^{-16}$), and their interaction (proline: $F(4, 75) = 8.46$, $p = 1.09 \times 10^{-5}$; leucine: $F(4, 79) = 16.91$, $p = 4.7 \times 10^{-10}$). Post hoc analyses showed that extracts contained significantly more proline (bark: 2.33; flowers: 0.71; leaves: 2.18; leaves–flowers–bark: 1.38; leaves–flowers: 0.84) and

leucine (bark: 2.54; flowers: 0.76; leaves: 2.12; leaves–flowers–bark: 1.84; leaves–flowers: 0.69) than infusions in every tissue (all $p < 0.001$).

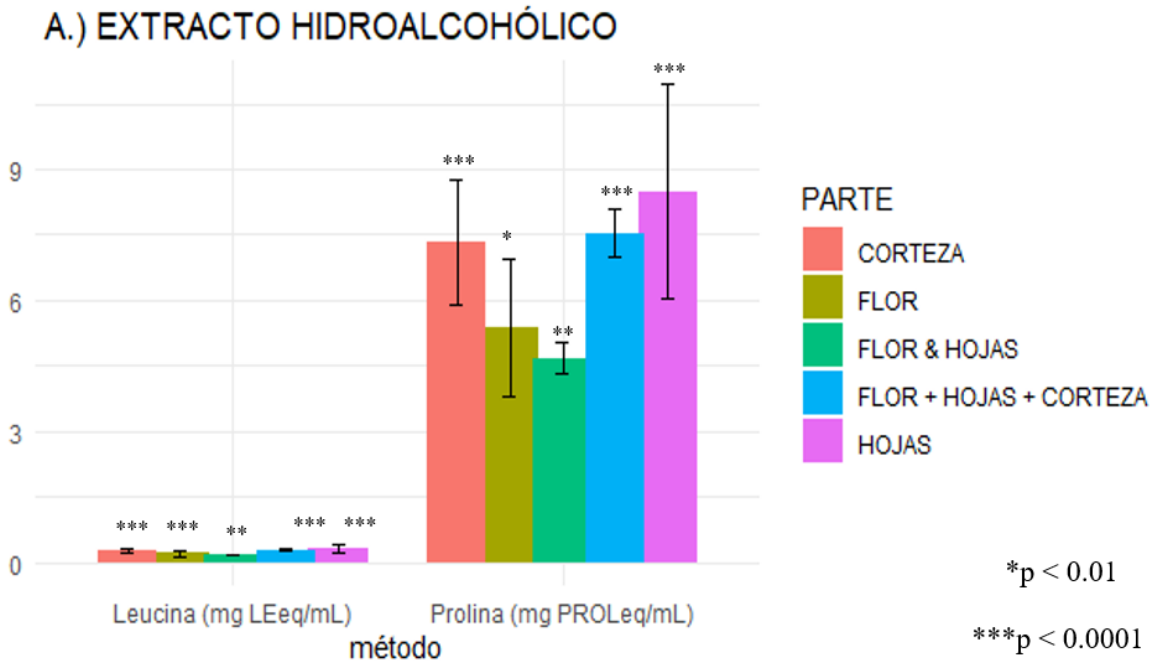

Figure 6. Total Proline content expressed as milligram proline equivalent (PROLeq) per milliliter of extract and total Leucine content expressed as milligram (mg) leucine equivalent (LEeq) per milliliter (mL) of extract (right to left).

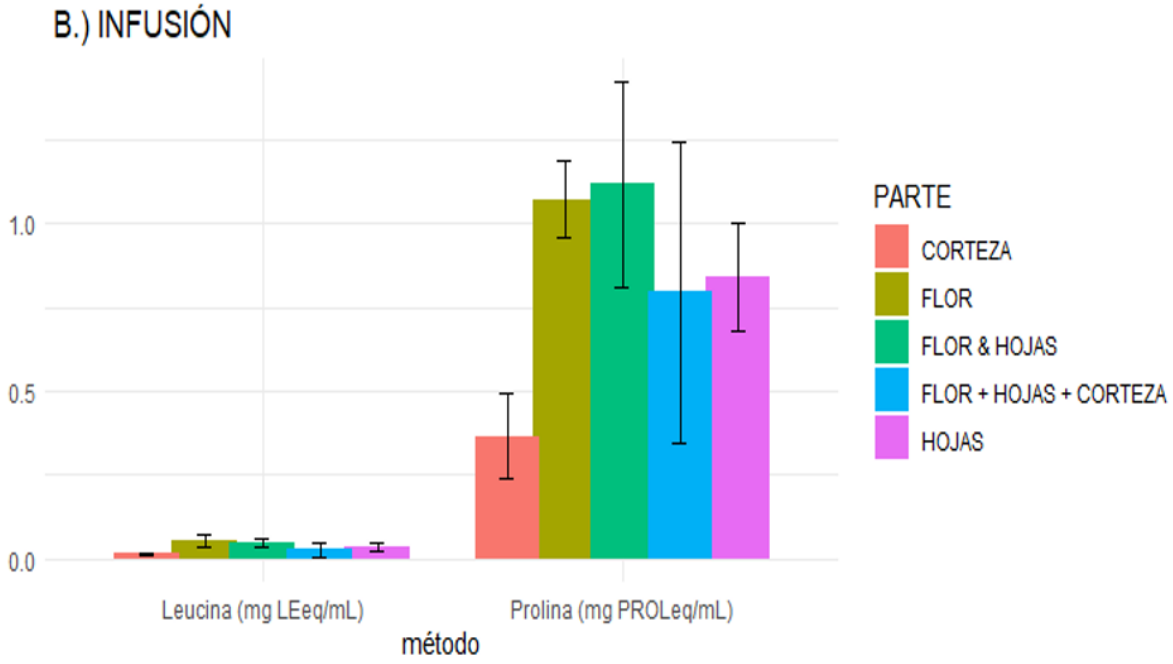

Figure 7. Total Proline content expressed as milligram proline equivalent (PROLeq) per milliliter of infusion extract and total Leucine content expressed as milligram (mg) leucine equivalent (LEeq) per milliliter (mL) of infusion extract (right to left).

In antioxidant assays, infusion DPPH (Figure 9) scavenging ranged from 25.42 ± 0.46 µmol/g in flowers to 28.24 ± 0.18 µmol/g in bark, while extracts(*Figure 8*) reached 30.51 ± 1.35 µmol/g in flowers and 30.22 ± 0.93 µmol/g in leaves; infusion FRAP values were 13.53 ± 2.02 µmol/g in flowers, 35.48 ± 3.67 µmol/g in bark and 68.11 ± 10.21 µmol/g in leaves, versus 90.69 ± 9.11 µmol/g in flowers, 10.16 ± 2.30 µmol/g in bark and 113.12 ± 16.86 µmol/g in leaves for extracts. Two-way ANOVAs confirmed significant effects of tissue (DPPH: $F(4, 80) = 164.53$; FRAP: $F(4, 79) = 107.69$; both $p < 2 \times 10^{-16}$), preparation (DPPH: $F(1, 80) = 130.27$; FRAP: $F(1, 79) = 125.69$; both $p < 2 \times 10^{-16}$), and interactions (DPPH: $F(4, 80) = 16.88$, $p = 4.53 \times 10^{-10}$; FRAP: $F(4, 79) = 60.77$, $p < 2 \times 10^{-16}$). Post hoc analyses showed that extracts exhibited significantly higher DPPH activity in flowers (estimate = 1.564, $p < 0.0001$) and leaves (1.165, $p < 0.0001$) but not in the leaf–flower mix ($p = 0.2444$), and higher FRAP in leaves (1.04), flowers (1.52), leaves–flowers–bark (1.05) and leaves–flowers (1.44; all $p < 0.0001$), whereas bark infusions outperformed extracts (–1.29; $p < 0.0001$). These results demonstrate that hydroalcoholic

extraction maximizes the recovery of bioactive compounds and antioxidant capacity, while traditional infusions still provide appreciable levels of functional metabolites.

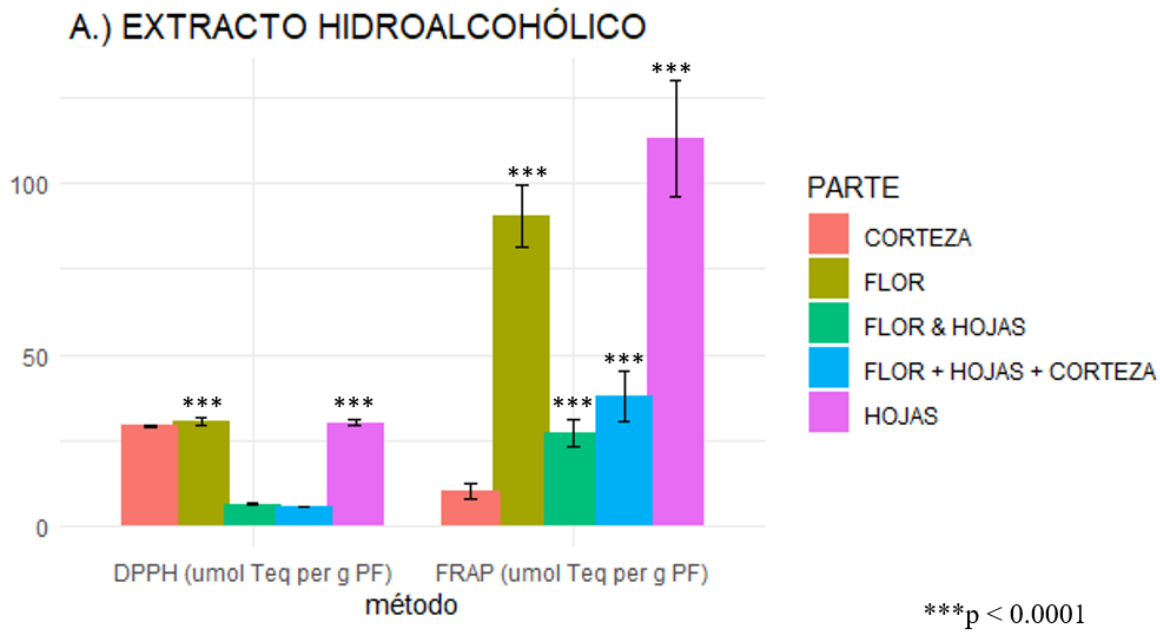

Figure 8. FRAP and DPPH results are expressed in micromoles (umol) of trolox equivalent (Teq) per gram (g) of fresh weight.

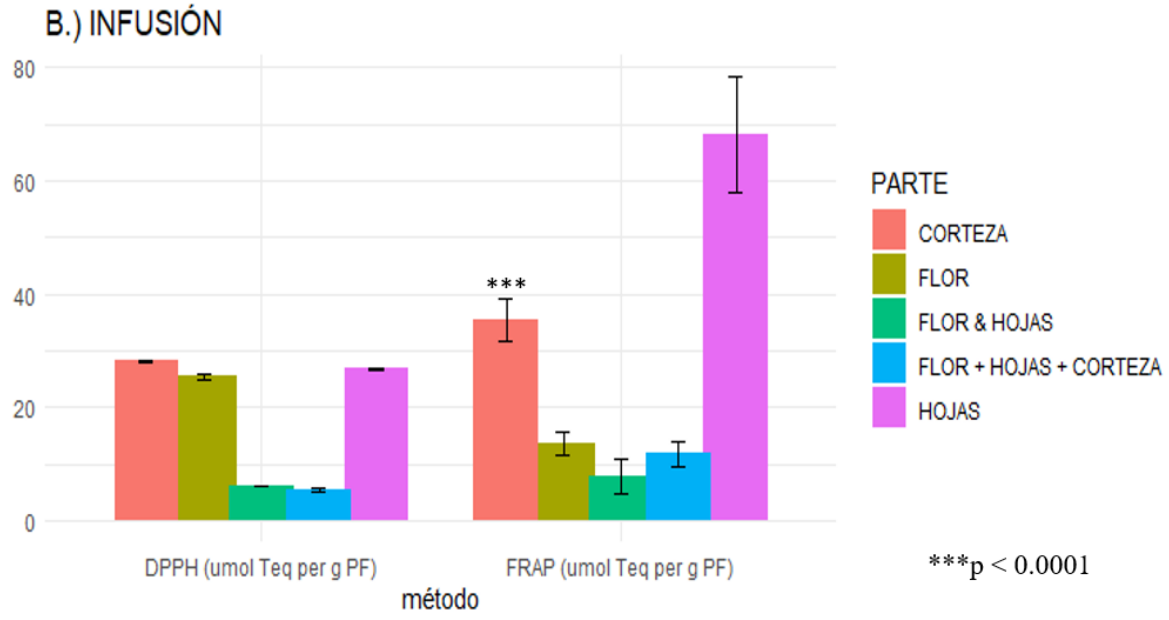

Figure 9. FRAP and DPPH results are expressed in micromoles (umol) of trolox equivalent (Teq) per gram (g) of fresh weight.

DISCUSION

Hydroalcoholic extraction was employed to establish the maximal yield of bioactive constituents, providing a benchmark against which infusion recoveries—and thus likely intake via traditional consumption—could be assessed. Total phenolic content determined by the Folin–Ciocalteu method was consistently higher in hydroalcoholic extracts than in infusions, with leaves and the leaf–flower mixture yielding the greatest concentrations (Figure *4*). Infusions recovered approximately 60–70 % of the extractable phenolic pool, underscoring that a single cup of infusion delivers a substantial, but partial, share of the plant's antioxidant potential (Rojas Canessa, 2019). Ethanol–water mixtures enhance cell-wall disruption and solubilize a broader spectrum of phenolic subclasses, whereas hot water alone primarily extracts low-molecular-weight phenolics. The pronounced phenolic richness of the leaf–flower blend suggests synergistic co-extraction of complementary metabolites, a "phyto-complex" effect worthy of targeted LC–MS/MS profiling to isolate the active constituents (Plaskova & Mlcek, 2023a) Flavonoid quantification mirrored these trends: hydroalcoholic extracts exhibited markedly higher total flavonoids across all tissues—particularly in the leaf–flower mixture and flowers—while infusions achieved roughly half those levels (Figure *4*). This differential recovery highlights how solvent polarity dictates the liberation of flavonoid glycosides and aglycones, yet even the infusion's lower flavonoid content remains physiologically relevant given micromolar bioactivity thresholds (Plaskova & Mlcek, 2023b). By comparing infusion yields to maximal extractable amounts, we can estimate the actual flavonoid intake per serving, informing dosage considerations for nutraceutical development. Free amino acid profiling revealed that proline accumulates preferentially in reproductive tissues: hydroalcoholic extracts of flowers and leaf–flower mixtures contained the highest proline levels, whereas infusions retained approximately 40–50 % of those concentrations

(*Figure 6* and Figure *7*). This pattern aligns with proline's role as an osmoprotectant during flower development (Mattioli et al., 2009). Leucine remained uniformly low across all preparations, suggesting minimal stress-induced biosynthesis under the sampled conditions (Sun et al., 2022). Understanding the infusion's contribution to amino acid intake may have implications for flavor profile and nitrogen nutrition.

Antioxidant assays using DPPH and FRAP confirmed that hydroalcoholic extracts achieve 1.3–1.5-fold greater radical-scavenging and reducing power than infusions of the same tissues (*Figure 8* and Figure 9). Strong correlations between total phenolics and DPPH activity ($r = 0.87$) and between total flavonoids and FRAP values ($r = 0.91$) validate that these classes drive antioxidant potential. Yet infusions still delivered antioxidant capacities within effective nutraceutical ranges, emphasizing their value in traditional contexts (Guevara et al., 2019). By relating infusion activity to the maximal extraction benchmark, we can quantify the degree to which a customary preparation contributes to daily antioxidant intake and adjust preparation parameters (e.g., temperature, time, plant-to-water ratio) to optimize efficacy.

In sum, hydroalcoholic extraction provides a reference for maximal recovery of phenolics, flavonoids, free amino acids, and antioxidant activity, while traditional infusions offer a practical, lower-toxicity method that still captures a meaningful fraction of these metabolites. Future work should integrate bioactivity-guided fractionation, advanced chromatographic and spectrometric analyses, and both in vitro and in vivo validation to isolate key compounds, elucidate synergistic "phyto-complex" effects, and refine infusion protocols—thereby bridging ancestral knowledge with evidence-based phytopharmaceutical development.

The results obtained in this study support the initial hypothesis that *Brunfelsia chiricaspi* contains bioactive compounds with significant antioxidant properties, whose composition and functional activity are influenced by the extraction method. This observation reinforces the

importance of considering both traditional and modern preparation methods when studying medicinal species used in ancient times.

CONCLUSIONS

The present study demonstrates that *Brunfelsia chiricaspi* harbors a rich spectrum of bioactive metabolites whose yields and functional properties are strongly modulated by extraction medium. Hydroalcoholic extraction consistently achieved maximal recovery of total phenolics, total flavonoids, free amino acids, and antioxidant activity, whereas parallel infusions captured roughly 60–70 % of phenolics, 40–50 % of flavonoids, and 40–60 % of amino acids, while still delivering substantial radical-scavenging and reducing power. The results also confirmed that both plant tissue and preparation type, and their interaction, significantly affect all measured parameters. Strong correlations between phenolic content and DPPH, and between flavonoids and FRAP, validate these compounds as primary drivers of antioxidant potential. Collectively, these findings validate traditional infusions as accessible, safe sources of functional metabolites and establish hydroalcoholic extracts as benchmarks for maximal bioactive recovery.

FUTURE PERSPECTIVE

To bridge ancestral knowledge with evidence-based applications, future work should:

Bioactivity-guided fractionation employing HPLC–MS/MS and NMR to isolate, identify, and quantify individual phenolic and flavonoid constituents—and to characterize potential synergistic "phyto-complex" adducts in leaf–flower blends.

In vitro and *in vivo* validation of isolated compounds and standardized extracts to assess pharmacological efficacy, bioavailability, and safety, including cellular antioxidant assays, anti-inflammatory models, and animal studies.

Optimization of infusion parameters (temperature, time, plant-to-water ratio) to enhance yield and reproducibility in home and industrial settings, and exploration of green solvent systems that balance extraction efficiency with environmental sustainability.

Formulation development of functional beverages or nutraceutical prototypes, with dosage standardization based on infusion yields and body-weight–normalized intake, complemented by sensory evaluation and stability testing.

These integrated approaches will deepen mechanistic insights, validate the therapeutic potential of *B. chiricaspi*, and guide the rational design of novel phytopharmaceuticals and functional food products.

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