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ASSESSMENT OF CHEMICAL PROFILE AND ANTIOXIDANT POTENTIAL IN AQUEOUS EXTRACTS OF DIFFERENT ANATOMICAL PARTS OF *ARRABIDAEA CHICA* (BIGNONIACEAE) FROM TABATINGA, AM

AVALIAÇÃO DO PERFIL QUÍMICO E DO POTENCIAL ANTIOXIDANTE EM EXTRATOS AQUOSOS DE DIFERENTES PARTES ANATÔMICAS DE ARRABIDAEA CHICA (BIGNONIACEAE) DE TABATINGA, AM

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ABSTRACT

Arrabidaea chica, commonly known as "pariri," "cipó-pau," or "crajiru," boasts numerous medicinal properties, including the treatment of various ailments such as uterine and ovarian inflammation, syphilis, leukemia, conjunctivitis, diarrhea, and psoriasis, among others. Despite its extensive use in traditional medicine, its pharmacological potential remains underestimated, with fewer than 50 studies conducted in the last decade, and only four focused on the Amazon region. This study aimed to analyze the chemical profile and determine the antioxidant potential of aqueous extracts from different anatomical parts of A. chica collected in Tabatinga. Material collected underwent exsiccate preparation and extract extraction. Exsicata were deposited in the Didactic Herbarium UEA/CESTB, while extracts were analyzed for antioxidant activity and mass spectrometry. Stem, root, and leaf extracts showed antioxidant potential mainly in the leaves (88%). Mass spectrometry revealed six flavonoids: Vicenin II, Scutellarein-O-glucuronide, 6-Hydroxyluteolin-O-glucuronide, 6-Methoxyluteolin-O-glucuronide, Scutellarein-(6 "-O-cafeoyl)-glucopyranoside, and Carajurin. These findings underscore A. chica high pharmacological potential, especially its antioxidant activity, and the identification of bioactive compounds, primarily flavonoids, holds promise for pharmaceutical and cosmetic applications, positioning A. chica as a valuable resource for future research and biotechnological advancements.

Keywords: Arrabidaea chica, Crajiru, Antioxidant.

RESUMO

A Arrabidaea chica, conhecida popularmente como "pariri", "cipó-pau" ou "crajiru", possui diversas propriedades medicinais, incluindo o tratamento de várias

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enfermidades como inflamação uterina e ovariana, sífilis, leucemia, conjuntivite, diarreia e psoríase, entre outras. Apesar de seu amplo uso na medicina tradicional, seu potencial farmacológico ainda é subestimado, com menos de 50 estudos realizados na última década, sendo apenas quatro focados na região amazônica. Este estudo teve como objetivo analisar o perfil químico e determinar o potencial antioxidante de extratos aquosos de diferentes partes anatômicas de A. chica coletadas em Tabatinga. O material coletado passou por preparo de exsicatas e extração de extratos. As exsicatas foram depositadas no Herbário Didático UEA/CESTB, enquanto os extratos foram analisados quanto à atividade antioxidante e espectrometria de massa. Os extratos de caule, raiz e folha mostraram potencial antioxidante principalmente nas folhas (88%). A espectrometria de massa revelou seis flavonoides: Vicenin II, Escutelareina-O-glicuronídeo, 6-Hidroxiluteolina-O-glicuronídeo, 6-Metoxiluteolina-O-glicuronídeo, Escutelareina-(6 “-O-cafeoil)-glucopiranosídeo e Carajurina. Esses achados destacam o alto potencial farmacológico de A. chica, especialmente sua atividade antioxidante, e a identificação de compostos bioativos, principalmente flavonoides, promete aplicações farmacêuticas e cosméticas, posicionando A. chica como um recurso valioso para pesquisas futuras e avanços biotecnológicos.

Palavras-chave: Arrabidaea chica, Crajiru, Antioxidante.

1. INTRODUCTION

The North of Brazil is endowed with vast biodiversity, it is a paradise of unique life forms and ecosystems, maintaining a complex balance. Amid this diversity, medicinal plants stand out as an alternative treatment and cure for certain diseases. Knowledge about the use of these medicinal plants is transmitted between generations. The region has a wide biodiversity of fauna and flora. Of these, plants with medicinal properties have gained notoriety and have gained space in the world of research. These vegetables are used in the preparation of medicines both by pharmaceutical industries for the manufacture of industrialized medicines and by individuals in homemade therapeutic practices and are used in different ways, such as teas, baths, infusions, decoctions, macerations, ointments, syrups, ointments, juices, dyes, among others [1].

According to Cragg and Newman (2005) [2] they highlight that plants have been an important source of drugs over the centuries, including for the treatment of cancer. *A. chica*, known as Crajiru, which has varied medicinal properties, such as antineoplastic, antitumor and antimicrobial, in addition to being used in the treatment of various diseases. The species *A. chica* belongs to the Bignoniaceae family and is distributed in tropical areas of America and Africa. This plant is characterized as a climbing liana with cylindrical stems, its leaves are petiolate, composed of 2 or 3 leaflets with a simple intermediate and terminal cirrus [3-4].

Phytochemical studies verified the presence of compounds such as anthocyanins and anthocyanidins belonging to the flavonoid class, phytosterols belonging to the terpene class and tannins such as polyphenols in its composition. This plant also has great potential for future applications such as the presence of biologically active compounds with hypotensive activity

[5]. Anthocyanins and anthocyanidins are plant pigments responsible for the colors in fruits, vegetables and flowers. These compounds have been associated with several health benefits due to their antioxidant and anti-inflammatory properties and are studied for their potential in preventing chronic diseases [6].

Therefore, the present study aimed to determine the chemical profile and evaluate the antioxidant potential of aqueous extracts from the different anatomical parts of *A. chica*, a plant found in the Amazon rainforest and traditionally used in folk medicine.

2. MATERIALS AND METHODS

2.1 Description of the study area, collection of botanical material and preparation of exsicata

Located in the interior of the State of Amazonas, the municipality of Tabatinga is located in the triple border region of Brazil, Colombia and Peru. The city is 1,105 km from Manaus and is located on the left bank of the Solimões River. The municipality covers 3,225 km², with an altitude of 60 m. Its population is 66,764 thousand inhabitants [7], the climate is equatorial, with an average temperature of 25 °C throughout the year. It has a rainy season between the months of November and April and a dry season between May and October.

In order to conduct all stages of this study, two collection campaigns of the medicinal plant were undertaken in the Comunicações neighborhood, specifically along Fábio Lucena alley. Following collection, the gathered material was transported to the Chemistry Teaching and Research in Natural Products Laboratory, located at the Centro de Estudos Superiores de Tabatinga – LEQPPN/CESTB, where it underwent cleaning with detergent and running water. The botanical specimens collected were then divided into two portions, one allocated for the preparation of the exsicata and the other designated for extract preparation.

The preparation of the exsicata followed the methodology outlined by Machado and Barbosa (2010) [8], conducted entirely at LEQPPN/CESTB. After thorough washing, the material underwent a sanitation process using 70% alcohol spray to ensure cleanliness and disinfection. Subsequently, the most optimal sections of the plant were selected and carefully separated by diagonal cuts using pruning shears and a utility knife.

After this process, the plant pieces were arranged on newspaper and interleaved with cards to maintain their shape and protect them. Subsequently, the plant specimen was placed in a wooden press measuring 42 x 28 cm and pressed. The material was then transferred to an oven and dried at 35°C for 24 hours. Following this, the final assembly of the exsicata was conducted on cardboard paper with a white background, enclosed in a wooden paper envelope.

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2.2 Production of aqueous extracts of *A. chica*

To prepare the extract, the roots, stems, and leaves were individually separated and then dried in an oven at 40°C for 48 hours. Following the drying process, 3 grams of plant stems, roots, or leaves were precisely weighed using an analytical balance to produce the aqueous extracts.

Immediately after weighing, the measured portions were placed into separate Erlenmeyer flasks, with each flask receiving 100 mL of hot distilled water. The extraction process lasted 24 hours, performed in triplicate. After this period, the samples underwent filtration. Excess water was removed using a sand bath, and upon complete elimination of the liquid component, the yield of crude extracts was calculated. Subsequently, the samples were dispatched to the laboratories of the Escola Superior de Ciências da Saúde (ESA/UEA) and the Instituto Nacional de Pesquisas da Amazônia (INPA) for antioxidant activity determination and mass spectrometry analyses.

2.3 *Screening* of extracts *A. chica* with antioxidant potential and determination of EC_{50}

To assess samples for antioxidant activity, the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay was conducted, following a modified protocol from Molyneux (2004). The assay was performed in a 96-well microplate, with samples diluted in methanol to a concentration of 1 mg/mL. Subsequently, 30 µL of the sample and 270 µL of DPPH solution were added to each well. Gallic acid served as the control. Following incubation in darkness for 30 minutes, the absorbance was measured at 517 nm using a spectrophotometer.

Based on the antioxidant activity results, samples exhibiting antioxidant activity ≥ 80% were chosen to determine the Efficient Concentration (EC_{50}), representing the quantity of antioxidant required to reduce the initial concentration of DPPH by 50%.

The EC_{50} value was calculated using linear regression in Excel, following the methodology outlined by Arbos et al. (2013). This parameter serves as an indicator of the antioxidant agent's capability to scavenge 50% of the DPPH free radicals in solution, indicating the concentration required to neutralize half of the free radicals.

2.4 Identification of chemical compounds by mass spectrometry

To determine the chemical profile and identify the compounds, we utilized the TSQ Quantum Access mass spectrometer equipped with an ESI source (Thermo Scientific®) operating in both positive and negative modes. Mass spectrometry is a critical analytical technique in our research, allowing precise identification and quantification of chemical compounds within the plant extracts. The spectrometry data were processed using Xcalibur software, and the characterized molecular structures were illustrated with ChemDraw. This approach not only

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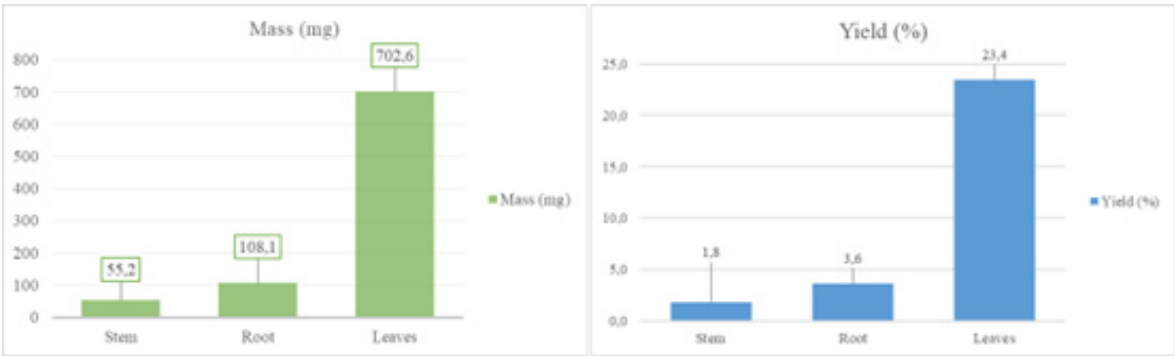
provides detailed insights into the chemical composition of the extracts but also underpins the broader pharmacological potential of the studied plant species.

3. RESULTS AND DISCUSSION

3.1 Sample yields and desiccation deposit

To determine the yield of the aqueous extract, a calculation was performed based on the ratio between the mass of the dry crude extract obtained and the initial mass of the botanical material (3 grams). The yield, based on the mass of the dry extract, was then expressed as a percentage, as illustrated in Figure 01.

Figure 1: Mass of the dry extract (A) and yield (B) obtained from the aqueous extract of A. chica. Source: Author.



In the depicted figure, it is noteworthy that A. chica leaves displayed the highest extract mass, presenting a significant disparity compared to both stems and roots (Figure 1A). Likewise, the yield of leaves surpassed that of stems and roots, as depicted in Figure 1B. Consequently, A. chica leaves emerge as the most promising subjects for scientific inquiry, owing to their pronounced accumulation of extractable compounds, a phenomenon vividly substantiated by the substantial extract yield. This assertion finds robust support in the extant literature, underscored by a plethora of investigations centering on leaf-based analyses, as extensively discussed in subsequent sections.

In a study conducted by Batalha (2017) [11], utilizing 300 g of A. chica leaves and 1L of distilled water for extract preparation, the yield did not surpass 8.61%. When contrasted with the outcomes of our current investigation, this figure proves inferior. Hence, it is plausible to infer that the methodology employed in our study for extract procurement demonstrated superior efficiency.

In a separate investigation conducted by Moura (2019) [12], focusing on the antibacterial activity of *A. chica* extract, 50.07 g of dry leaves and 25.04 g of dry stems were employed. The aqueous extracts from the leaves exhibited a maximum yield of 13.33%, a value surpassing

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those of stem and root treatments but falling short of the leaf treatment reported in the current study. Another study carried out by Santos (2015) [13], with a more complex characteristic, used supercritical extraction of *A. chica* leaves. As a result, the highest yield presented was 24%, showing that the methodology employed in the present study showed better results.

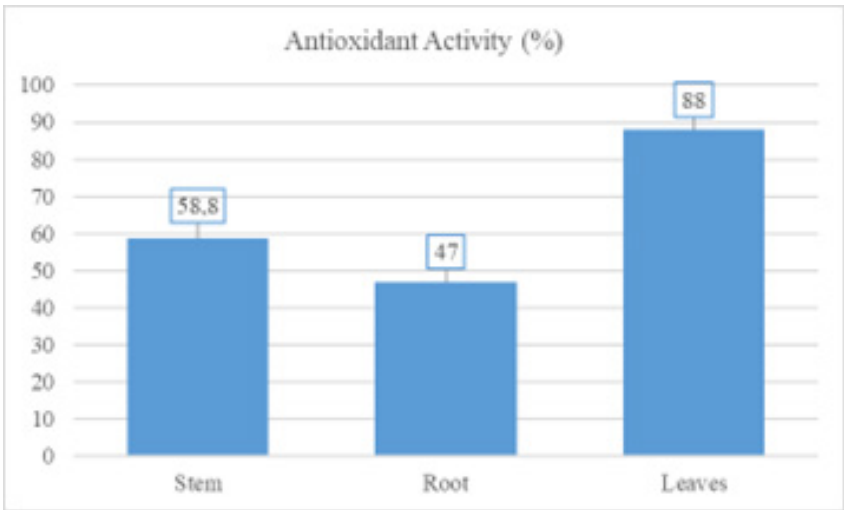
The exsicata of the species, as shown in figure 2, was deposited in triplicate in the UEA/CESTB teaching herbarium – Plants of the Alto Solimões Region, Fisheries Resources laboratory – Municipality of Tabatinga – Amazonas – Brazil. Importance of the origin of the exsicata.

Figure 2. The A. chica exsicata organized and ready for deposition at the Herbarium of CESTB/UEA. Source: Author.

3.2 Determination of antioxidant activity (AA%)

The antioxidant activity values for aqueous extracts of the different anatomical parts of *A. chica* are presented in Figure 03.

Figure 3. Antioxidant activity (AA) of extracts from different parts of A. chica λ = 517 nm. Source: Author.



Based on the AA% results of extracts from stems, roots, and leaves, it's evident that the species' antioxidant potential is highest in the leaves, followed by stems and roots. Moreover,

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as depicted in Figure 03, the leaf extract exhibits the greatest capacity to neutralize DPPH free radicals.

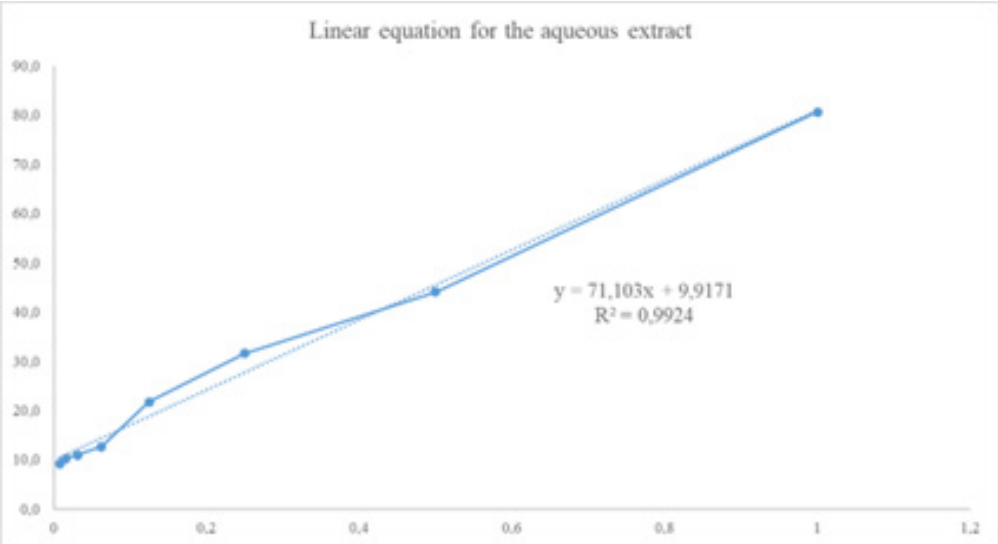
The *A. chica* extracts contain flavonoids in their composition. These compounds play a crucial role in the high antioxidant activity observed in the leaves, providing justification for the results obtained in the present study. Furthermore, in a study conducted by Alves (2010) [14], the antioxidant activity of the extract was primarily attributed to the presence of the flavonoids Escutelarein and Apigenin. This activity can be attributed to a combination of factors; in addition to Scutellarein and Apigenin, other phenolic constituents present in the plant extract may also contribute to the antioxidant activity.

The antioxidant activity results presented align with those reported by Almog (2004) [15], who determined the antioxidant activity of *A. chica* leaves to be 88% in their study. Given the remarkable performance exhibited by the leaf extract concerning antioxidant outcomes, this sample was chosen for the analysis of EC50 determination.

3.3 Determination of EC_{50}

The 50% Efficient Concentration (EC_{50}) signifies the quantity of antioxidant necessary to diminish the oxidative action of a substance by half. This determination involves fitting experimental data to a first-order exponential curve, reflecting the inherent correlation between antioxidant concentration and the percentage of DPPH.

Figure 4. Linear equation describing the aqueous extract's EC50. Source: Author.



The EC_{50} analysis of the *A. chica* leaf extract, depicted in the preceding figure, reveals a linear relationship comprising eight data points. Typically, a minimum of five points is required for such analyses. The graph displays the equation of the straight line derived from spectrophotometric analysis, representing the relationship between absorbance concentration of the samples. The resultant EC_{50} value obtained from this analysis was 0.56 µg/mL.

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The curve fitted from the program's analysis offers a lucid visual depiction of the interplay between antioxidant concentration and DPPH reduction. This curve allows us to pinpoint the exact antioxidant concentration corresponding to 50% of the DPPH action. This value holds significant importance in comprehending the impact and efficacy of the antioxidant under scrutiny, serving as a valuable tool in assessing the antioxidant properties of substances under investigation [14].

The variation in EC_{50} values can be attributed to the polarity of the solvent, which influences the transfer of electrons and hydrogen atoms critical processes for antioxidant activity. Additionally, the presence of non-antioxidant compounds in the solutions tested may impact the EC_{50} value. These compounds can compete with antioxidants for the ability to scavenge free radicals, thereby diminishing the overall antioxidant activity of the solution.

3.4 Mass spectrometry analyzes

Following the chemical profile determination via EM, we acquired the chemical profiles of the aqueous extract sample of *A. chica* leaves in both positive and negative modes of ESI, depicted in Figures 05 and 06.

Figure 5. Base peak ESI (+) of the aqueous extract of A. chica. Source: Author.

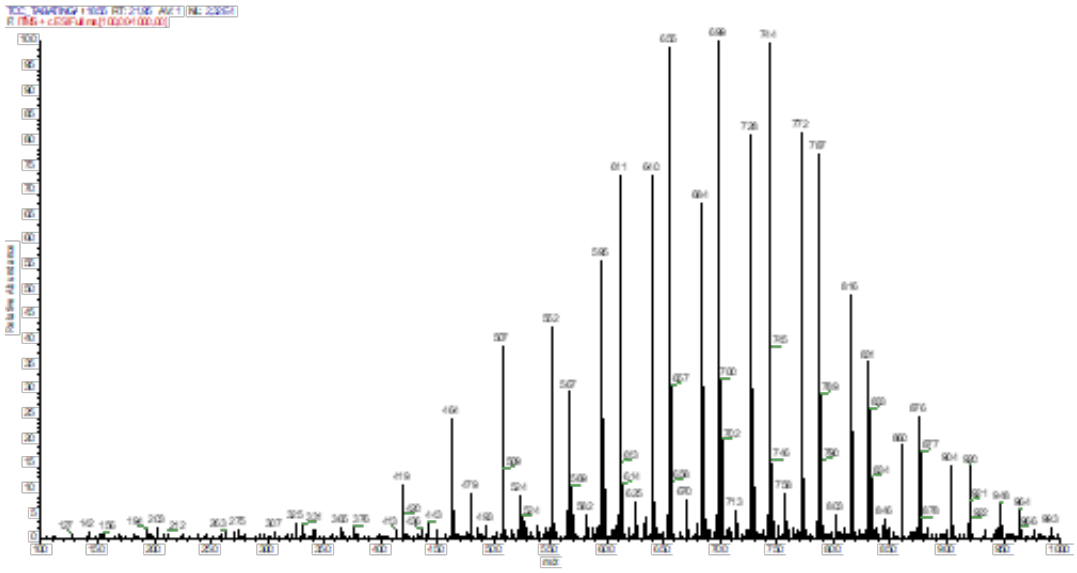

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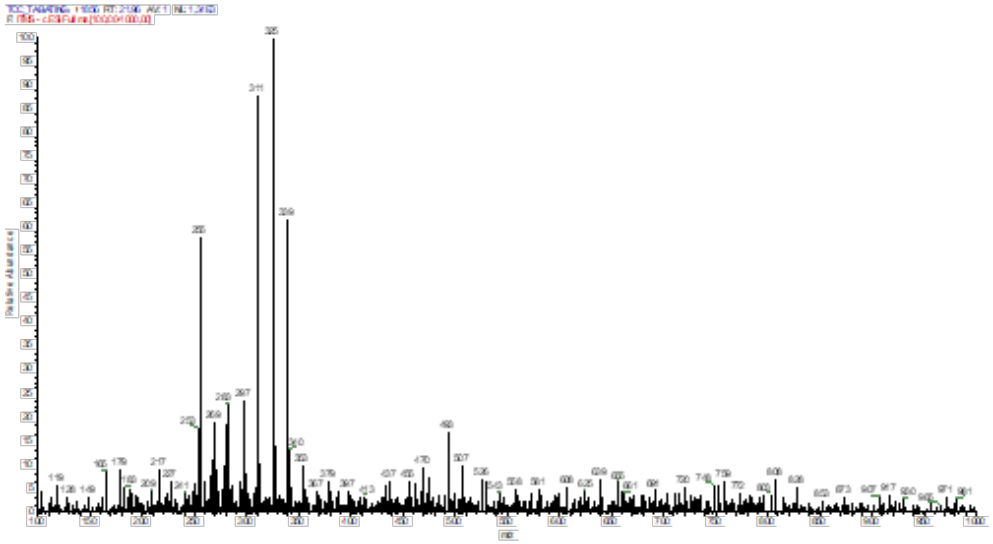
Figure 6. Base peak ESI (-) of the aqueous extract of A. chica. Source: Author



Six compounds were characterized by comparing mass spectrometry data obtained in positive mode with literature records. All detected compounds belong to the class of flavonoids. Compound 01 was identified as Vicenin II, evidenced by the presence of the ion m/z $[M+H]^+$ 595. Compound 02 was identified as Scutellarein-O-glucuronide with the ion m/z $[M+H]^+$ 463, further confirmed in negative mode by the ion m/z $[MH]^-$ 461. Additionally, ions 03 and 04 with m/z $[M+H]^+$ 479 and 493 respectively correspond to compounds 6-Hydroxyluteoline-O-glucuronide and 6-Methoxyluteoline-O-glucuronide. Furthermore, Escutelarein-O-(6"-Ocaffeoyl)-glucopyranoside and Carajurin were characterized, represented by the ions m/z $[M+H]^+$ 611 and m/z $[M+H]^+$ 299, classified as compounds 05 and 06, respectively. The mass spectra and chemical structures of the described compounds are shown in Figures 07-11. All compounds were characterized based on a study conducted by da Cruz et al., (2022) [17], which identified the flavonoids present in *A. chica* leaves using mass spectrometry.

Figure 7. Base peak of Vicenin II. m/z $[M+H]^+$ 595. Source: Author.

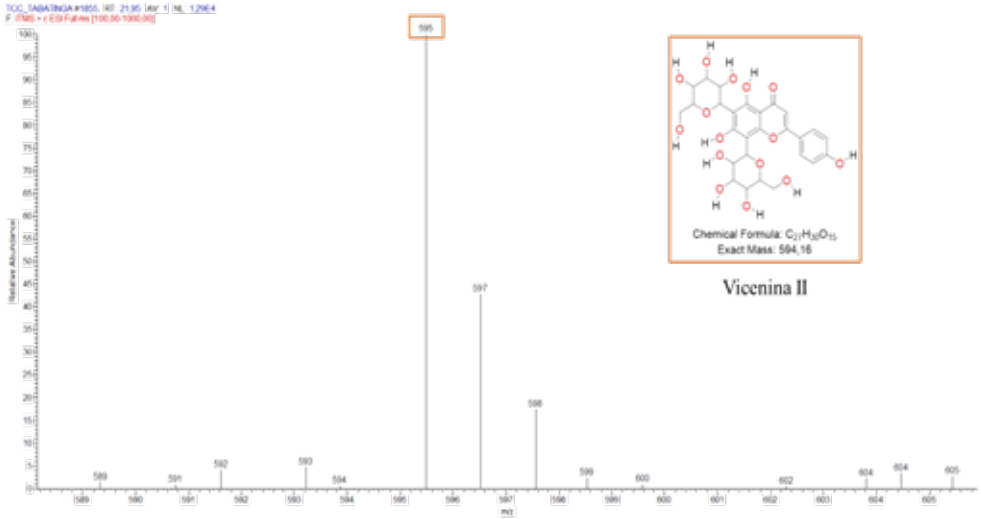


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Figure 8. Base peak of (A) Scutellarein-O-glucuronide m/z $[M+H]^+$ 463, (B) 6-Hydroxyluteolin-O-glucuronide m/z $[M+H]^+$ 479, and (C) 6-Methoxyluteolin-O-glucuronide m/z $[M+H]^+$ 493. Source: Author.

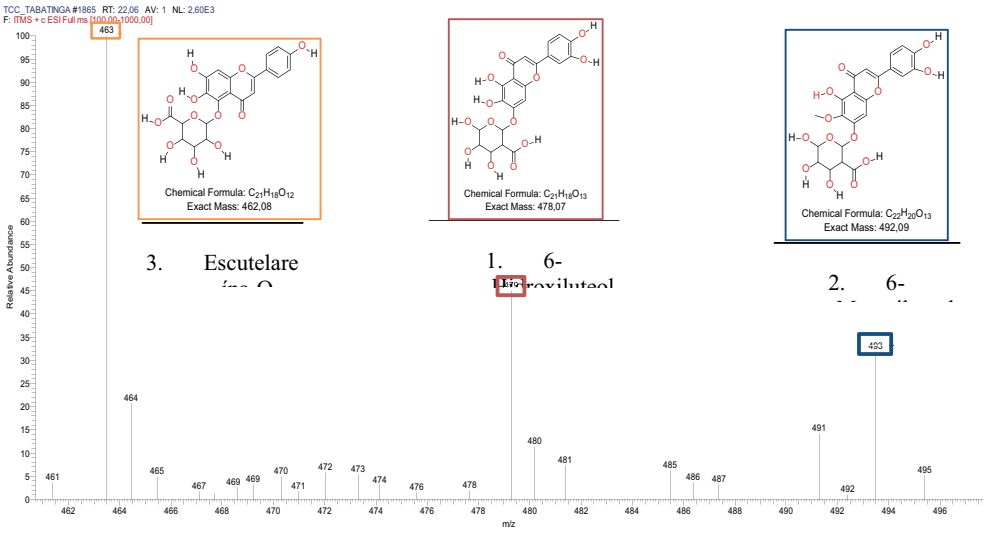

Figure 9. Base peak of Scutellarein-O-glucuronide m/z $[M-H]^-$ 461. Source: Author.

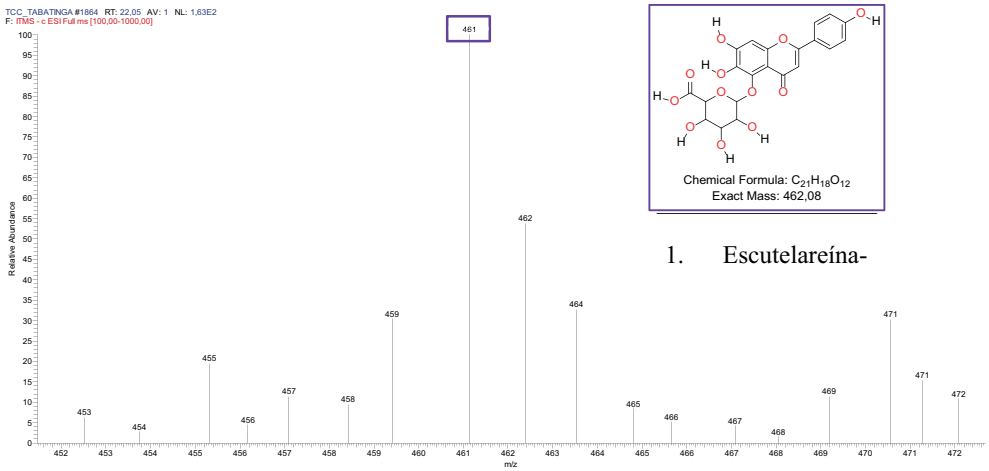

Figure 10. Base peak of Scutellarein-(6”-Ocaffeoyl)-glucopyranoside m/z $[M+H]^+$ 611. Source: Author.

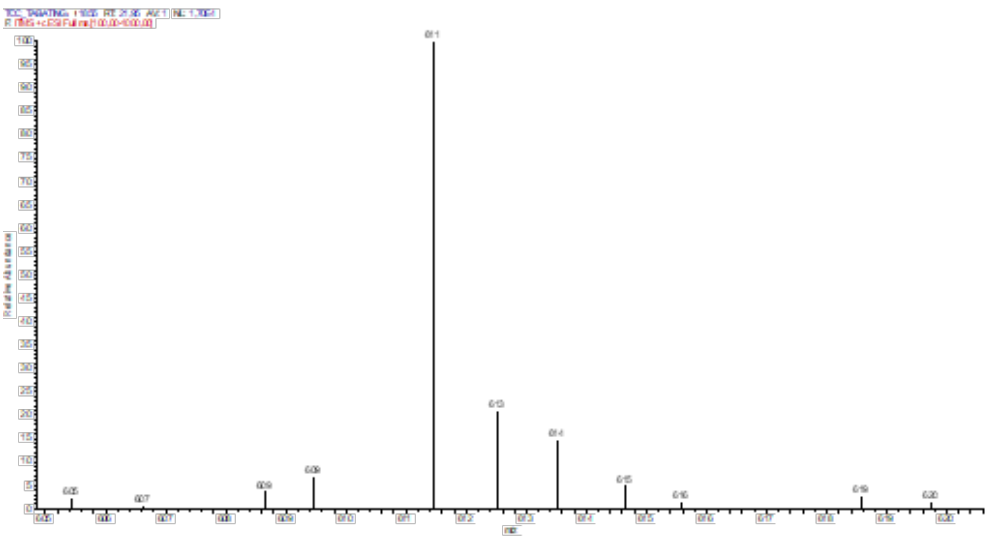

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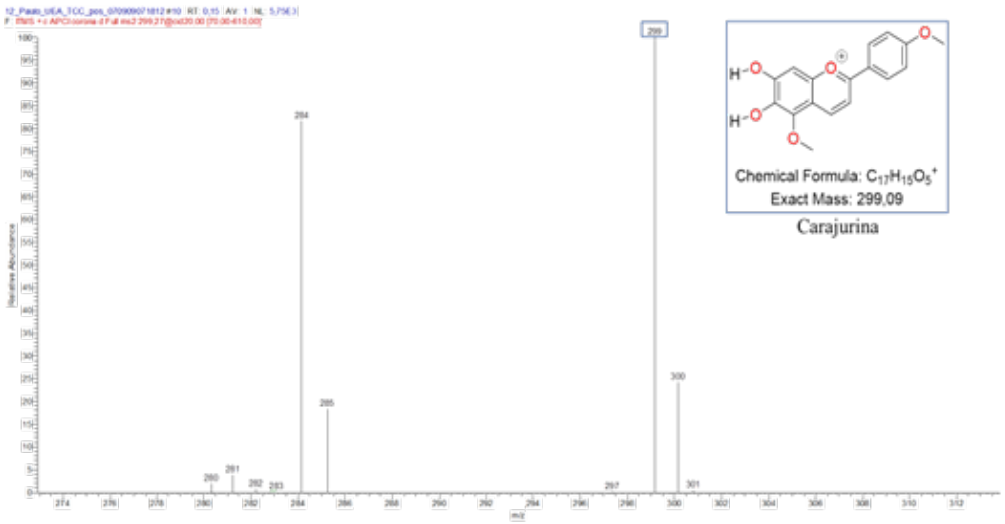
Figure 11. Base peak of Carajurin m/z $[M+H]^+$ 299. Source: Author.



As highlighted by Batalha (2017) [11], the extract of this plant is abundantly rich in antioxidants, particularly flavonoids. The six identified compounds all belong to the flavonoid class, underscoring the remarkable diversity of flavonoid compounds in the *A. chica* leaf extract. Consequently, more comprehensive and specific studies are necessary to fully characterize the substances present in the extracts of this plant. Flavonoids are considered one of the most relevant and diverse groups among plant-derived products, being widely distributed in the plant kingdom. These compounds are abundantly present in angiosperms, exhibiting a vast structural diversity within this group [18].

In agreement with da Silva and Queiroz (2003) [19], flavonoids are natural substances that encompass a range of polyphenolic structures found in various plants, known for their diverse beneficial activities for human metabolism. The distribution of these compounds in plants varies according to the phylum, order, and family, as well as between species. Generally, the flavonoids present in leaves may differ from those in flowers, twigs, roots, and fruits. Additionally, the concentration of the same substance can vary depending on the plant organ in which it is found [18].

Secondary metabolites, such as flavonoids, are of significant economic interest due to their diverse properties. In addition to their antioxidant activity, these compounds exhibit antimicrobial, antiviral, antiulcerogenic, antineoplastic, cytotoxic, antihypertensive, hypolipidemic, and anti-inflammatory properties [8].

The antioxidant capacity of flavonoids stems from their redox properties, which play a crucial role in the absorption and neutralization of free radicals. Consequently, these compounds are highly effective in mitigating various oxidizing molecules linked to DNA damage and tumor promotion [19].

The species *A. chica* is known for its diverse array of flavonoid compounds, among which the anthocyanin Carajurin stands out (Figure 11) as the primary pigment found in this plant.

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Anthocyanins are phenolic compounds widely distributed in nature, responsible for the blue, violet, and red colors seen in flowers, fruits, and plant roots. However, according to Takemura et al. (1995) [20], Carajurin is specifically restricted to the species *A. chica*.

In studies conducted by Santos (2015) [13], the Carajurin compound was not found in its aqueous extracts. However, in this study, the compound in question was identified and characterized, suggesting that infusion might be the most effective method for extracting Carajurin.

4. FINAL CONSIDERATIONS

This study aimed to explore the pharmacological potential of *A. chica*, particularly its antioxidant activity, drawing on its traditional use by indigenous populations for various ailments. The selection of methodology and solvents was deliberate, aiming to ensure accessibility to the plant's chemical components and their benefits. The adoption of a simple method like infusion facilitates the preparation of natural medicine for the population in a practical and accessible manner.

The results underscore the significant potential of *A. chica* as a reservoir of compounds with antioxidant activity, exemplified by the presence of Vicenin II, Scutellarein-O-glucuronide, 6-Hydroxyluteolin-O-glucuronide, 6-Methoxyluteolin-O-glucuronide, Scutellarein-(6"-Ocaffeoyl)-glucopyranoside, and Carajurin. This activity is attributed to secondary metabolites such as flavonoids and tannins, known for their ability to combat free radicals and safeguard cellular integrity against oxidative stress.

While the findings are promising, further large-scale studies are warranted not only in the Alto Solimões region but across the state of Amazonas. Expanding research efforts in this region is pivotal for advancing scientific understanding and ensuring that local communities are informed about the benefits and potential applications of *A. chica* products, thus fostering appreciation for this species.

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