



# Chemical characterization and antioxidant potential of *Maytenus guianensis* from Alto Solimões

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**ABSTRACT.** *Maytenus guianensis* has several biological activities, including antioxidant, anti-inflammatory, antimicrobial and antiparasitic properties. Previous studies have shown that terpenes and flavonoids are the most prevalent compounds in its chemical composition. Despite its potential use in traditional medicine, its phytochemistry is little explored in the Amazon region, especially in the Alto Solimões. This study analyzed the chemical profile and antioxidant potential of *M. guianensis* collected in the Alto Solimões, and identified the probable compounds with antioxidant activity in the hydroethanolic extract by means of mass spectrometry. The botanical material was collected, sanitized, dried, fragmented, extracted with 70% ethanol, and filtered. The samples were filtered, dried, weighed, packaged, and sent to Manaus, AM, for antioxidant activity determination and mass spectrometry analysis. As a result, extract yield was highest in the leaves (21%), followed by the roots (13.6%), stem (8.5%), and branches (3.2%). The root extract and branches had the highest antioxidant activity at 99.7%. Mass spectrometry definitively characterized seven compounds in the root extract: kaempferol-3-O-galactosyl (1-2) rhamnoside, amyryl, and taraxerol; a glycosylated flavonol in the leaf extract; quercetin-3-O-galactosyl (1-2) rhamnoside in the root extract; lupeol/friedelin; and betulinic acid in the twig extract. These results clearly demonstrate the high pharmacological potential of *M. guianensis* and justify further research with medicinal plants in the Alto Solimões region, promoting the appreciation of this species.

**Keywords:** Antioxidant activity; flavonoids; *M. guianensis*; northern region; mass spectrometry; terpenes.

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## Introduction

*Maytenus guianensis* Klotzsch ex Reissek is a plant native to the Amazon region. It is popularly known as chichuá (or xixuá). This species has a long history in traditional medicine, where it is used as an anti-inflammatory, antirheumatic, aphrodisiac, and healing agent (Borrás, 2003; Prata & Mendonça, 2009; Meneguetti et al., 2016). Despite its therapeutic benefits, the scientific community has scarcely studied this plant, as evidenced by the paucity of studies available in scientific databases.

The most extensively studied and proven activities of *M. guianensis* are those related to parasitic, leishmanicidal, antibacterial and antilarvicidal activity. Research conducted by Meneguetti et al. (2016) has demonstrated the antiparasitic and antileishmanial activity of *M. guianensis* extracts. This antileishmanial activity was also assessed by Macedo et al. (2019) using the hexane eluate subfraction of *M. guianensis* bark incorporated into microparticles. Consequently, an inhibitory effect on *Leishmania amazonensis* was observed, manifesting as a prolonged and variable response over an extended period. This finding suggests the potential for utilization as a complementary or alternative therapeutic modality for cutaneous leishmaniasis. Antibacterial activity was described by Silva et al. (2018), who reported that *M. guianensis* exhibited inhibitory effects against *Staphylococcus aureus* and *Streptococcus pneumoniae*. Another activity presented by *M. guianensis* was the antilarvicidal activity, evidenced in the study by Martins et al. (2021), which used an ethanolic extract and triterpene (tingenone B) from the bark of *M. guianensis* against *Aedes aegypti*.

About the antioxidant potential, only one study has been published to date that proves this activity in *M. guianensis* (Bay-Hurtado et al., 2015), highlighting the need for further investigation into this species, commonly used in traditional Amazonian medicine, particularly in the Alto Solimões region. Given the

chemical and biological potential of *M. guianensis*, as well as its regional importance, the main of this study was to conduct a phytochemical analysis and evaluate the antioxidant potential of the extracts and compounds isolated from the leaves, stem, branches, and roots of *M. guianensis* from Alto Solimões.

## Materials and methods

### Description of the collection site and preparation of the *exsiccate*

The botanical material was collected on 3 December 2022 near Sitio Deus Me Deu in Alto Solimões, Amazonas, Brazil. Leaves, stems, twigs and roots of *M. guianensis* were gathered. Following collection, the *exsiccate* was prepared using the method described by Silva et al. (2019), with some adaptations. The samples were cleaned with 70 % ethanol, pressed and dried in an incandescent lamp oven at 35 °C for five days. After drying, the samples were placed on white cardboard, labelled and wrapped in acid-free tissue paper, all sized according to the specifications in the literature (Silva et al., 2019).

### Preparation of hydroethanolic extracts of *M. guianensis*

The extracts were prepared as follows. Initially, the samples were cleaned with distilled water and dried in an oven for 72 hours at 40 °C. After drying, the samples were cut into small fragments. Then, 3 g of each different anatomical part of the plant were weighed separately into 250 mL Erlenmeyer flasks. Subsequently, 100 mL of 70 % ethanol was added to each flask. The experiment was conducted at room temperature, in triplicate, for 24 hours. After the experimental period, filtration was performed, and the samples were dried in a sand bath for 72 hours at 40 °C. Once dry, the samples were weighed, and the extract yield was calculated.

### Determination of antioxidant activity and determination of the chemical profile of extracts

The antioxidant activity test was conducted in the Master's in Biotechnology Laboratories of the *Universidade Estadual do Amazonas*, situated at the *Escola Superior de Saúde* (MBT/UEA/ESA). The antioxidant activity was determined through the free radical capture reaction DPPH (2,2-diphenyl-1-picrylhydrazyl) in a microplate, according to Molineux (2004).

The assay was carried out in a 96-well elisa plate by applying 30 µL of samples diluted in HPLC grade methanol, at a concentration of 1 mg mL<sup>-1</sup>, and 270 µL of DPPH solution. The experiment was carried out in triplicate. For control, gallic acid was used. After 30 minutes of reaction, the plates were taken to the spectrophotometer device to determine the absorbance at  $\lambda = 517$  nm.

The chemical profile of the sample was obtained using a TSQ Quantum Access mass spectrometer, with an ESI source (Thermo Scientific) operating in positive and negative mode. To process the mass spectrometry data, the Xcalibur software was used, and the characterized structures were drawn in ChemDraw.

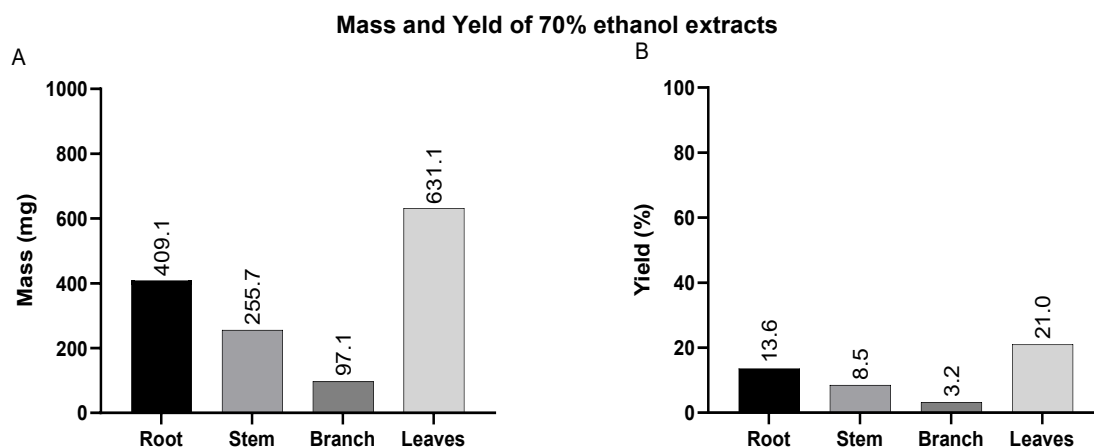
## Results and discussion

### Yield of extracts and deposition of *exsiccate*

The yield was calculated by using the initial mass of the sample (3 grams) and the mass of crude extract obtained from each anatomical part of the plant as references. This calculation was performed as a percentage, as depicted in Figure 1A, illustrating the yield of *M. guianensis* extracts in 70% ethanol obtained from various parts of the plant, including the root, stem, branch, and leaf.

As depicted in Figure 1, the highest yield was observed in the leaf extract, with 631.1mg of crude extract mass, corresponding to 21.0%. The root extract followed, yielding 409.1 mg (13.6%), while the stem extract yielded 255.7 mg (8.5%), and the branch extract yielded 97.1 mg (3.2%).

Comparing our results with those obtained by Dos Anjos et al., (2023), who extracted stem and leaf material from *M. evonymoides* using 80% ethanol, reveals noteworthy differences. Their study reported a leaf extract yield of 0.21% from 3.632 g of botanical material, and a stem extract yield of 0.14% from 3.074 g of the sample. Interestingly, despite starting with a smaller initial mass and using less extracting solution, our extraction process proved more efficient. Notably, the fragmented plant material resulted in higher yields, particularly evident in the leaf sample, which yielded 21% – a substantial improvement over the previously reported results. Additionally, the root, stem, and branch extracts in our study also yielded higher percentages compared to the study conducted by the author mentioned earlier.



**Figure 1.** (A) Mass of crude extracts obtained from different anatomical parts of *Maytenus guianensis* (leaves, roots, stem, and branches) after extraction with 70% ethanol. (B) Percentage yield of the extracts calculated based on the initial mass of the plant samples (3 g). Experiments were performed in triplicate, and results are expressed as mean  $\pm$  standard deviation.

Among the reports found in the literature involving work with species of the genus *Maytenus* and the use of extraction methods similar to those used in this work, Figueiredo (2021) stands out. The author obtained the ethanolic extract from the aerial parts of *M. erythroxylon*, subjecting 4.0 kg of the powder sample to maceration with 95% ethanol, which generated a mass of 404.0 g, resulting in a yield of 10.1%. Comparatively, our study, based on the aerial parts of the leaves, yielded 21%, indicating a significantly higher extraction efficiency.

Research conducted by Figueiredo (2021) involved preparing the ethanolic extract from the roots of *M. distichophylla*. The author used 3.620 kg of powdered roots subjected to maceration with 95% ethanol. The resulting hydroethanolic solution yielded 622.97g of crude ethanolic extract from the roots, with a calculated yield of 17.2%. In comparison, the root yield in our study was 13.6%, indicating a lower efficiency. However, it's important to note that Figueiredo (2021), started with a larger mass of material than in our study. Thus, the extraction method and quality of botanical material influenced the results, highlighting the significance of the percentage yields obtained in our study. Despite starting with a smaller mass, our study achieved efficient botanical yields for the species under investigation.

*Exsiccate* of *M. guianensis*, including leaves, twigs, stem, and roots, were meticulously prepared, as depicted in Figure 2. Subsequently, the *exsiccate* was thoroughly cataloged and archived in the Didactic Herbarium of CESTB/UEA, designated with the code NHPA02.



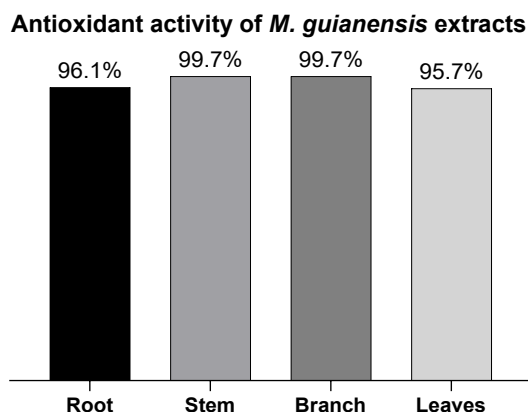
**Figure 2.** *Exsiccate* of *M. guianensis* deposited in the Didactic Herbarium of CESTB/UEA (code NHPA02).

### Antioxidant activity of extracts

The antioxidant activity of *M. guianensis* was quantified by reading the samples on the spectrophotometer. The results were promising, as seen in Figure 3. Below are the results regarding the antioxidant activity of the root, stem, twig and leaf extract of *M. guianensis* in 70% ethanol.

The graph depicts the antioxidant activity of *M. guianensis* extracts, obtained by extraction with 70% ethanol and evaluated using a DPPH free radical scavenging assay, demonstrating the plant's ability to

neutralize free radicals. Notably, the extracts exhibited robust antioxidant properties, with percentages above 90% for all parts studied, roots (96.1%), stem (99.7%), twigs (99.7%), and leaves (95.7%). These findings highlight *M. guianensis* as a promising reservoir of natural antioxidant compounds.



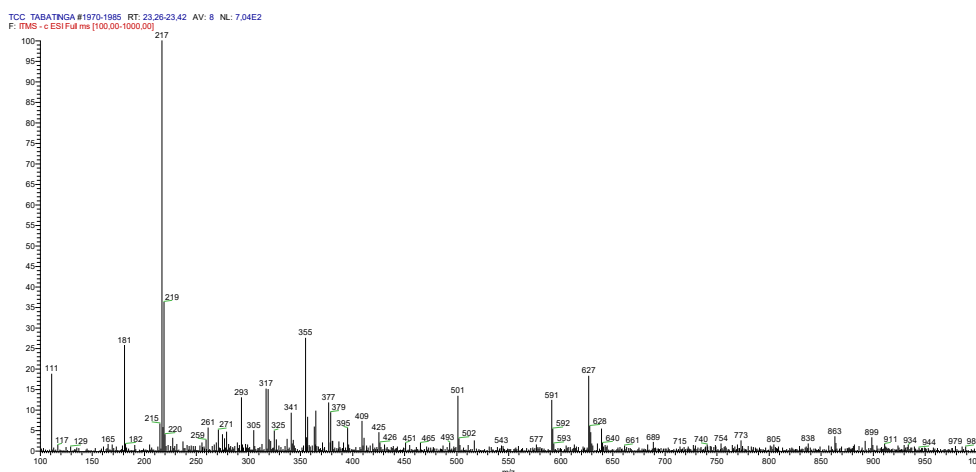
**Figure 3.** Antioxidant activity (%) of hydroethanolic extracts of *Maytenus guianensis* obtained from different anatomical parts (leaves, roots, stem, and branches).

In accordance with the research conducted by Bay-Hurtado et al., (2015), investigating the antioxidant capacity of *M. guianensis*, significant findings were observed, confirming its antioxidative potential. The study utilized various concentrations of ethanolic extract, ranging from 200  $\mu\text{g mL}^{-1}$  to 10  $\mu\text{g mL}^{-1}$ . Notably, at the highest concentration of 200  $\mu\text{g mL}^{-1}$ , the antioxidant activity reached 96.5%, followed by 95.9% at 150  $\mu\text{g mL}^{-1}$ , and 89.1% at 100  $\mu\text{g mL}^{-1}$ . Lower concentrations of 50 and 10  $\mu\text{g mL}^{-1}$  yielded antioxidant activities of 48.8 and 9.0%, respectively. The study's success in obtaining superior results may be attributed to the utilization of fractional and lower concentrations compared to our present investigation.

Comparing our findings with the analysis conducted by Haida et al., (2012), who assessed the antioxidant activity of ethanolic extracts from the leaves of *M. ilicifolia* and *M. aquifolium* using the DPPH capture method, notable differences emerge. In our study, the leaves of *M. guianensis* demonstrated superior antioxidant activity, with a recorded percentage of 95.7%. However, it is noteworthy that Haida et al., (2012) achieved commendable results despite using smaller sample masses. Specifically, *M. aquifolium* exhibited antioxidant activities of 91.19% at 1000  $\mu\text{g mL}^{-1}$ , 87.62% at 500  $\mu\text{g mL}^{-1}$ , 69.76% at 250  $\mu\text{g mL}^{-1}$ , and 47.14% at 125  $\mu\text{g mL}^{-1}$ . Similarly, *M. ilicifolia* displayed antioxidant activities of 90.30% at 1000  $\mu\text{g mL}^{-1}$ , 85.94% at 500  $\mu\text{g mL}^{-1}$ , 77.36% at 250  $\mu\text{g mL}^{-1}$ , and 53.73% at 125  $\mu\text{g mL}^{-1}$ . These findings, as reported by Haida et al., (2012), underscore the potential antioxidant inherent in species of the *Maytenus* genus and, by extension, in *M. guianensis*.

### Mass spectrometry analyzes

The chemical compounds were characterized based on the acquisition of mass spectra in both negative and positive modes. Figures 4, 5, 6 and 7 depict the mass spectra obtained in the negative mode.



**Figure 4.** Mass spectrum of the extract in 70% ethanol, root, ESI (-).

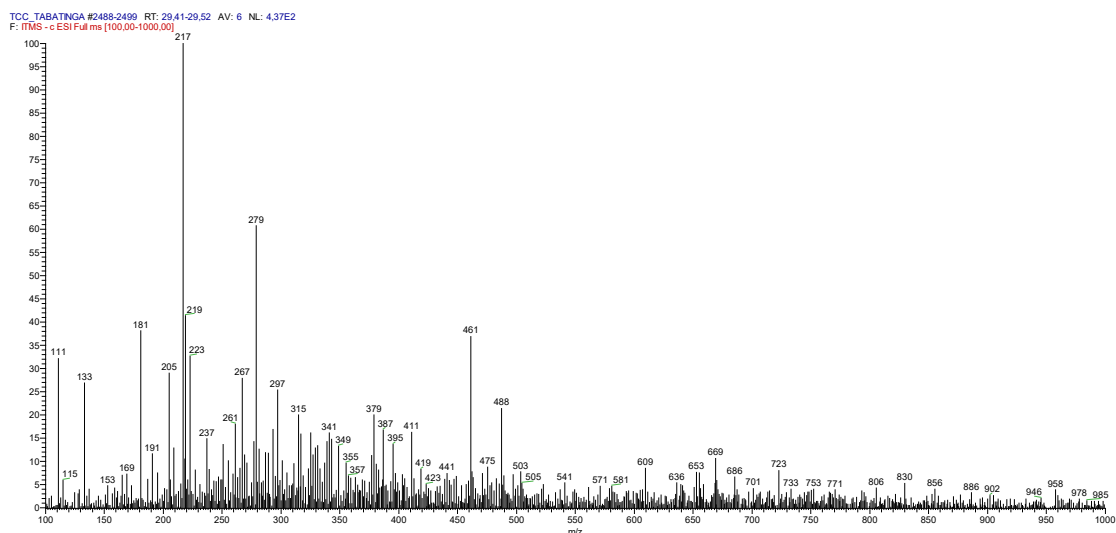


Figure 5. Mass spectrum of the extract in 70% ethanol, branches, ESI (-).

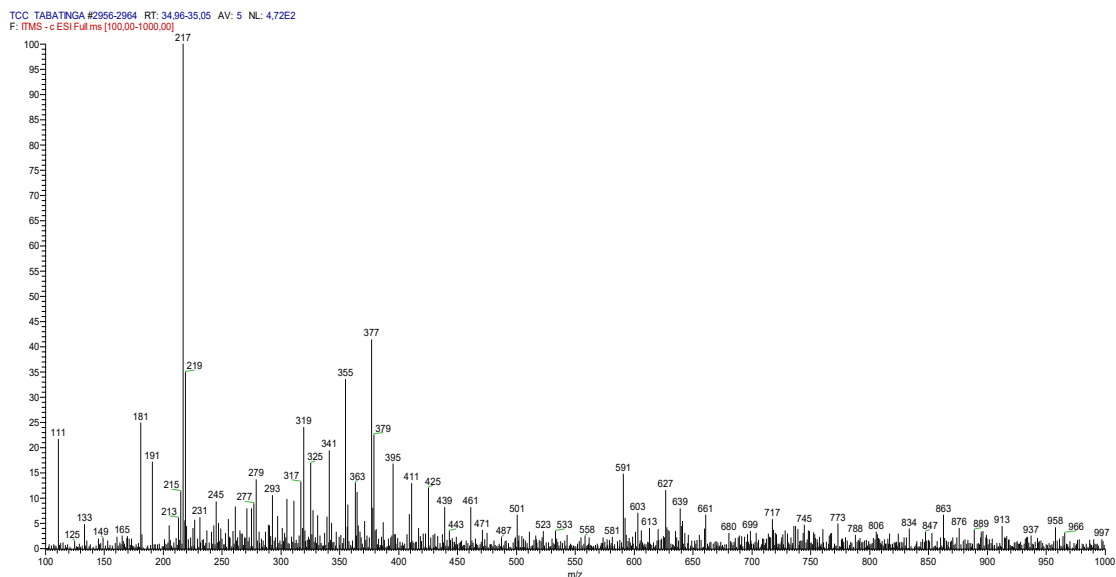


Figure 6. Mass spectrum of the extract in 70% ethanol, branch, ESI (-).

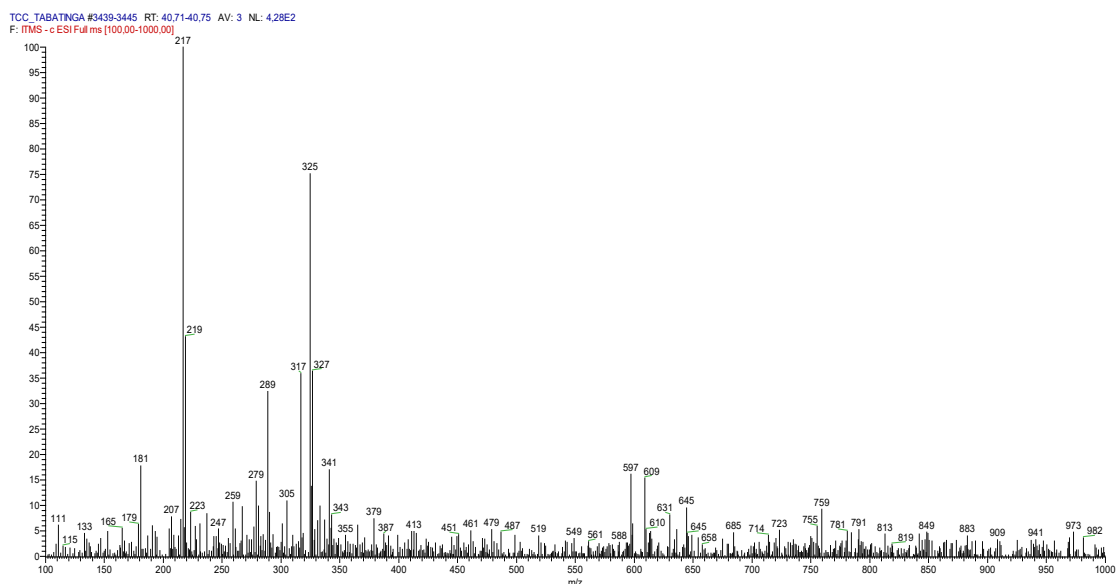


Figure 7. Mass spectrum of the extract in 70% ethanol, leaves, ESI (-).

In accordance with the data available in the literature and, through a thorough analysis of the chemical profile shown in the previous figures, four chemical compounds were negatively characterized, three of which belonged to the root extract in 70% ethanol and the other was detected in leaf extract in 70% ethanol. Of these four compounds, two flavonoids were recognized, with compound 01 presented in Figure 8 and two terpenes, compounds 02 and 03, presented in Figure 9.

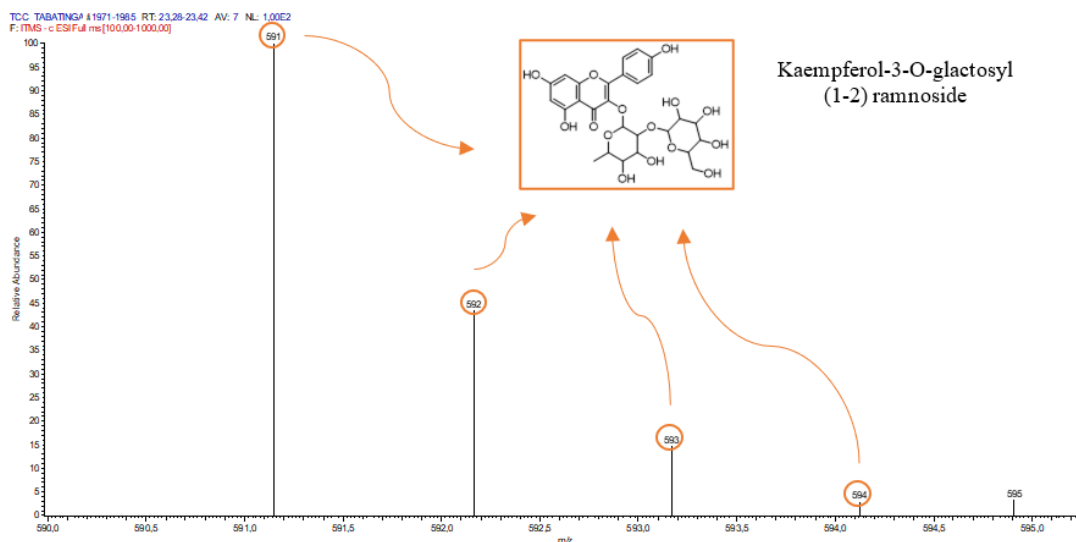


Figure 8. Compound 01 - Flavonoid characterized by mass spectrometry ESI (-).

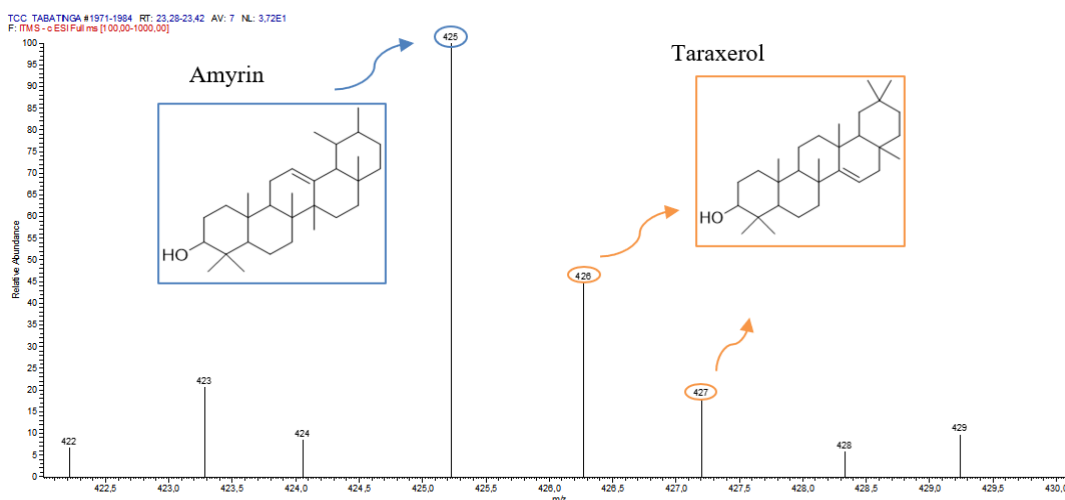


Figure 9. Compounds 02 (Amyrin) and 03 (Taraxerol) – Terpenes characterized by mass spectrometry in ESI (-).

Compound 01, identified as Kaempferol-3-O-glactosyl (1-2) rhamnoside with a mass of  $m/z$   $[M-H]^-$  595, was discovered in the leaves of *Maytenus ilicifolia*, as reported in the study by Souza et al., (2011). Compound 02, identified as Amyrin,  $m/z$   $[M-H]^-$  425. Compound 03, identified as Taraxerol,  $m/z$   $[M-H]^-$  426. It is noteworthy that Compounds 02 and 03 were also detected in the leaves of *M. aquifolium* and *M. ilicifolia*, as reported by Cordeiro et al. (1999).

Compound 04 was identified with an ion  $m/z$   $[M-H]^-$  609 and characterized as a glycosylated flavonol, as reported by Souza et al., (2008). Their study revealed that most flavonols possess galactose or glucose linked to an aglycone within their structure, and the ion  $m/z$   $[M-H]^-$  609 is characteristic of this compound type. Similarly, in the study conducted by Souza et al., (2008), Compound 04, with an ion  $m/z$   $[M-H]^-$  609, was detected in the leaves of *M. ilicifolia*, aligning with the findings of the present study, where this compound was also identified in the leaves of *M. guianensis*.

Mass spectrometry analyses were also conducted in positive mode, and the chemical profiles obtained are depicted in Figures 10, 11, 12 and 13.

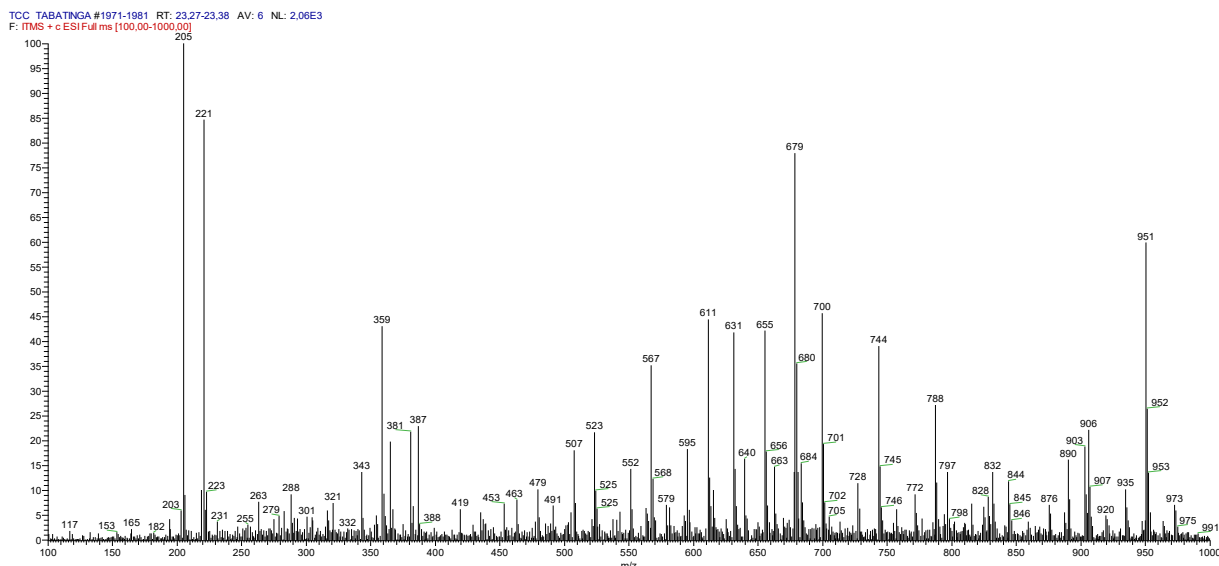


Figure 10. Mass spectrum of the extract in 70% ethanol, root, ESI (+).

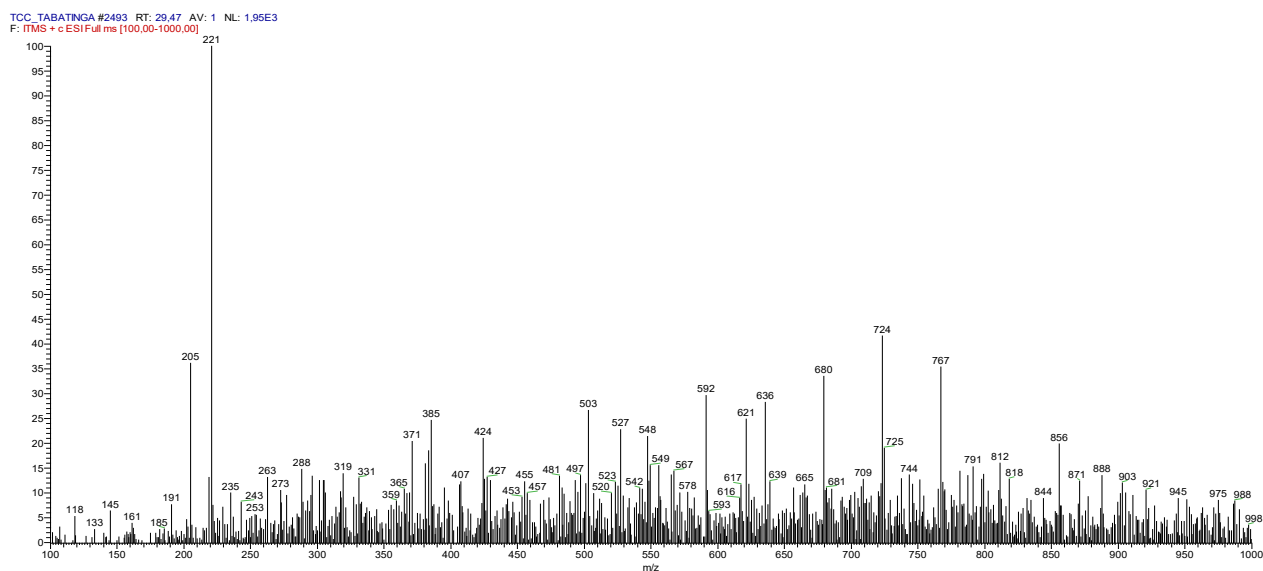


Figure 11. Mass spectrum of the extract in 70% ethanol, stem, ESI (+).

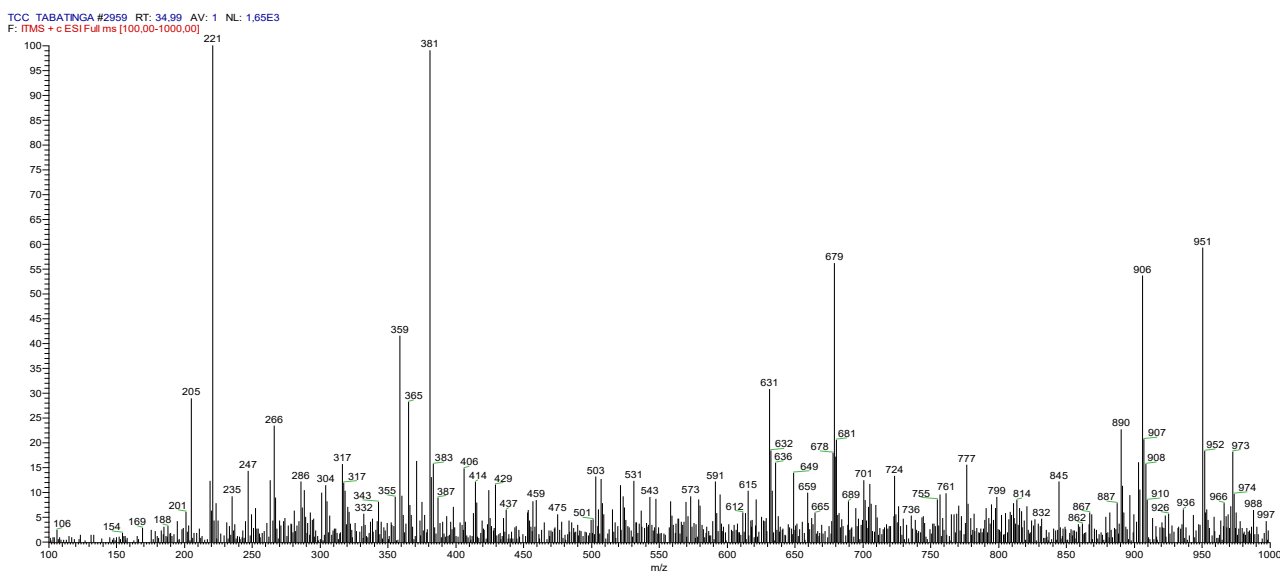
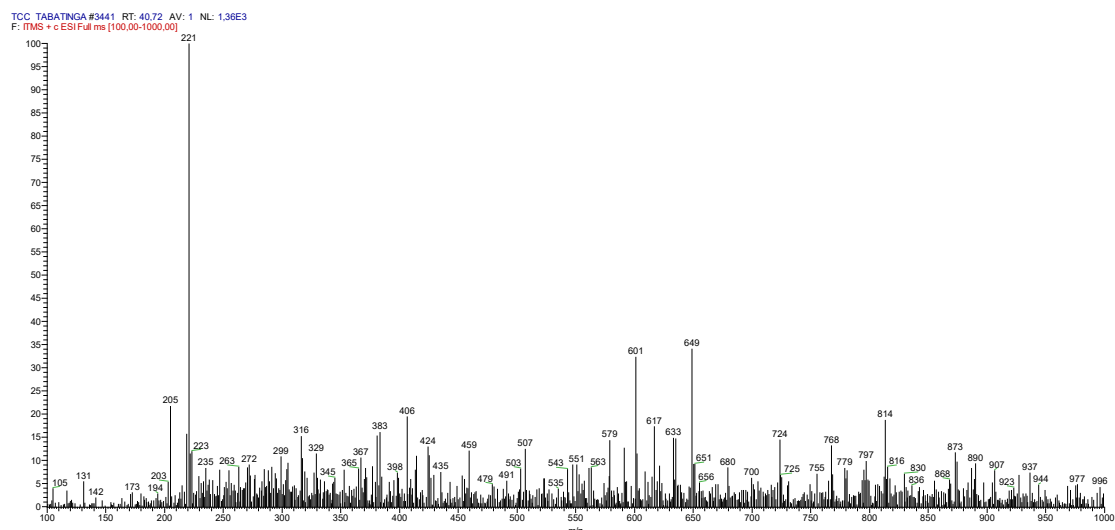
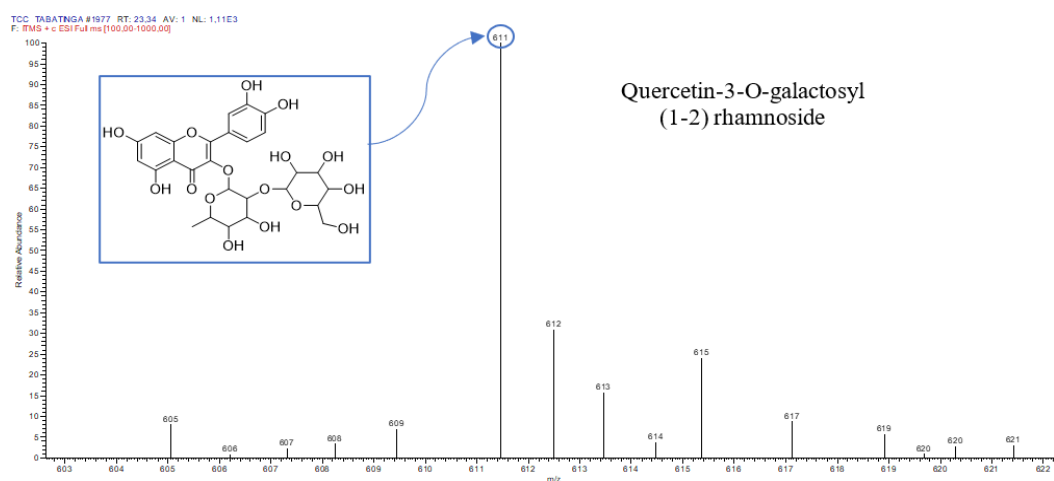


Figure 12. Mass spectrum of the extract in 70% ethanol, branch, ESI (+).

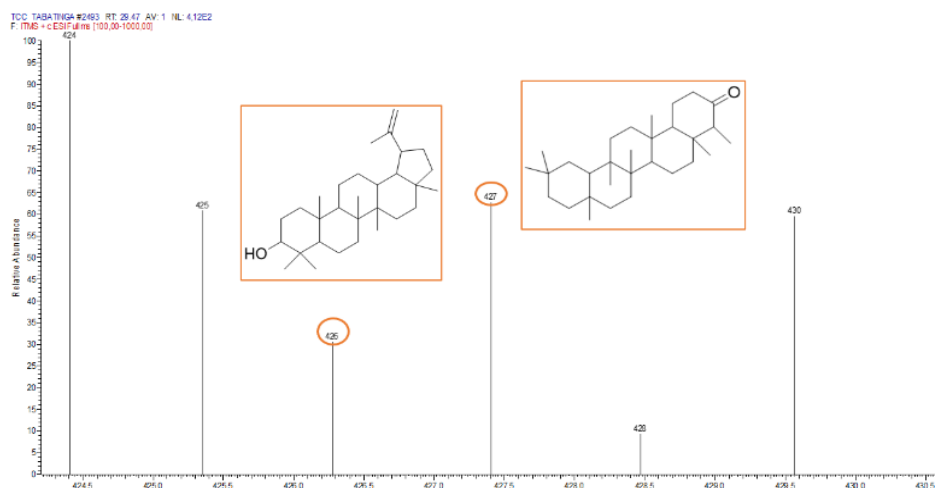


**Figure 13.** Mass spectrum of the extract in 70% ethanol, leaves, ESI (+).

In positive mode, three compounds were identified through data from literature and specific analyses of the chemical profile presented in the previous figures. Among these compounds, one was detected in the root extract in 70% ethanol, while the other two were found in the twig extract in 70% ethanol. Respectively, these identified compounds are a flavonoid (Compound 05) and two terpenes (Compounds 06 and 07), as illustrated in Figures 14, 15 and 16.

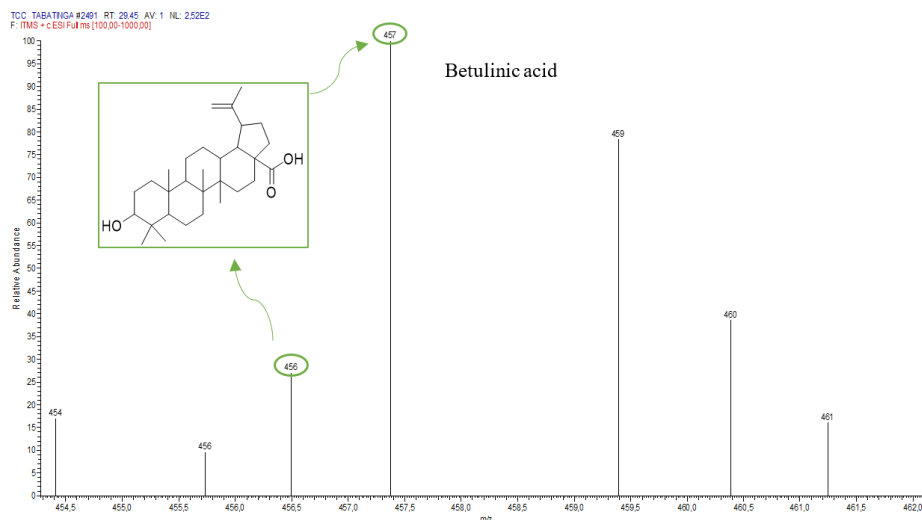


**Figure 14.** Compound 05 - Flavonoid characterized by mass spectrometry in ESI (+).



**Figure 15.** Compound 06 – Terpene characterized, Lupeol or Friedelin, by mass spectrometry in ESI (+).





**Figure 16.** Compound 07 – Terpene characterized by mass spectrometry in ESI (+).

Compound 05, identified as Quercetin-3-O-galactosyl (1-2) rhamnoside, with a mass of  $m/z$   $[M-H]^+$  611, was discovered in the leaves of *M. ilicifolia*, as detailed in study Souza et al. (2011). Compound 06 could be either Lupeol or Friedelin, sharing the same mass of  $[M-H]^+$  427, as reported in Pereira et al. (2022). Both compounds were found in the leaves of *M. ilicifolia*. Lastly, Compound 07 was characterized as Betulinic acid, with a mass of  $m/z$   $[M-H]^+$  456, detected in the leaves of *M. ilicifolia*, according to Pereira et al. (2022) findings.

Flavonoids, a category of phenolic compounds found in numerous plants and fruits (Pereira et al., 2022), exhibit diverse biological functions, including antiviral, antifungal, antibacterial, antiparasitic, antimicrobial, anti-inflammatory, immunomodulatory, and antioxidant properties. Among these, antioxidant activity is particularly notable, owing to the structural features of flavonoids and the abundance of phenolic radicals (Cataneo, 2019; Moraes, 2022).

These compounds have garnered increased attention in recent years within scientific circles due to their robust antioxidant capabilities, which hold promise for advancements in medicine. Researchers speculate that these compounds may hold potential for the development of novel medications and therapies targeting conditions such as Alzheimer's, Parkinson's, cardiovascular ailments, and cancer (Cataneo et al., 2020)

Terpenes are natural organic compounds notable for their diversity, with over 80,000 terpenoids identified. Their complex structures, derived from isoprene (a five-carbon unit), form various classes including monoterpenes, sesquiterpenes, diterpenes, triterpenes, tetraterpenes, and polyterpenes. Each class possesses specific characteristics and functions, ranging from providing aromas and perfumes to regulating plant growth and offering pest protection. Found in fruits, vegetables, flowers, and even insects, terpenes offer a wide range of possibilities for both science and industry. They have applications in medicine, cosmetics, and biofuels, making them promising compounds across multiple fields (Shohaib, 2011; Christianson et al., 2017; Forezi et al., 2022).

### Applications of flavonoids and terpenes

Flavonoids and terpenes, the major bioactive constituents found in *M. guianensis*, exhibit a wide range of pharmacological properties. Flavonoids such as kaempferol and quercetin are well known for their antioxidant and anti-inflammatory effects, which contribute to cardiovascular protection, neuroprotection, and potential anticancer activities (Mileo & Miccadei, 2016; Wang et al., 2018). Their free radical scavenging ability suggests promising applications in preventing oxidative stress-related diseases, such as neurodegenerative disorders and atherosclerosis (Li et al., 2020). Additionally, flavonoids play a significant role in immune modulation and antimicrobial defense, making them valuable for alternative treatments against bacterial and viral infections (Wong et al., 2019).

Terpenes, including amyrin, taraxerol, and betulinic acid, exhibit anti-inflammatory, analgesic, antimicrobial, and wound-healing properties (Nascimento et al., 2018; Rios et al., 2012). These compounds have been studied for their potential in treating skin conditions, respiratory diseases, and metabolic disorders, with some showing cytotoxic effects against tumor cells, suggesting applications in cancer therapy (Yadav et al., 2021). Moreover, terpenes have demonstrated neuroprotective activity by modulating inflammatory

pathways in the central nervous system, indicating possible benefits for neurodegenerative disease management (Nunes et al., 2020).

The identification of these compounds in *M. guianensis* underscores its potential as a source of bioactive molecules for pharmaceutical, cosmetic, and nutraceutical applications. Further research should focus on optimizing extraction methods, evaluating synergistic effects, and conducting in vivo studies to validate their clinical efficacy. These findings reinforce the importance of integrating traditional knowledge with modern pharmacological approaches for the sustainable development of natural therapeutic agents.

### Future implications

This study contributes to the scientific understanding of *Maytenus guianensis*, particularly its chemical composition and pharmacological potential. The identification of bioactive compounds such as flavonoids and terpenes highlights its relevance for developing new natural antioxidant agents, functional foods, and phytotherapeutic products. Furthermore, these findings support biodiversity conservation efforts and encourage further research on the isolation and standardization of these compounds for medicinal use. Future studies should focus on preclinical and clinical evaluations to validate their therapeutic applications and assess their safety and efficacy for pharmaceutical development.

### Conclusion

The present study highlights the significant potential of *M. guianensis* as a valuable plant species. The high percentages obtained in the antioxidant activity tests confirmed the efficacy of the root, twig, stem, and leaf extracts in neutralizing free radicals. Secondary metabolites, specifically flavonoids and terpenes, were characterized using mass spectrometry, corroborating the antioxidant activity of the species and indicating their promise for future applications in medicine and nutrition. Disseminating these findings will undoubtedly enhance public awareness regarding the use of chichuá and its potential health benefits. Notably, this is the first study to investigate the antioxidant activity of *M. guianensis* in the Alto Solimões Region, marking a significant contribution to the scientific knowledge of this region's flora.

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