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Secondary metabolites in four forest species of Amazonian fragments

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ABSTRACT: The rapid loss of natural habitats in the Amazon increases the importance of research with plants of medicinal potential. The aim of this study was to identify abundant tree species in two Amazon forest fragments and to qualify their secondary compounds. Two forest areas (fragments I and II) in the southern Amazon region, were selected for this species identification. All arboreal individuals with diameter trunks greater than 10 cm were measured and identified in ten plots, five per fragment. The four most abundant species were identified, and their alkaloids, flavonoids, saponins and tannins were phytochemically screened. Seven hundred and seventy-four trees were sampled, with *Protium altissimum*, *Metrodorea flavida*, *Rinoreocarpus ulei* and *Cheiloclinium cognatum*. being the most abundant ones. All the metabolites evaluated were present in *P. altissimum* and *C. cognatum* in both fragments. Flavonoids were absent in *M. flavida* in fragment I, and *R. ulei* had saponin in both forest fragments and alkaloids only in the II. These four Amazonian tree species have secondary metabolites with medicinal importance, which can be extracted in sustainable management.

Key words: biodiversity; fragmentation; medicinal plants; phytochemistry

Metabólitos secundários em quatro espécies florestais de fragmentos amazônicos

RESUMO: A rápida perda de habitats naturais na Amazônia aumenta a importância de pesquisas com plantas de potencial medicinal. O objetivo deste estudo foi identificar espécies arbóreas abundantes em dois fragmentos florestais amazônicos e qualificar seus compostos secundários. Duas áreas florestais (fragmentos I e II) na região sul da Amazônia, foram selecionadas para a identificação destas espécies. Todos os indivíduos arbóreos com diâmetro de tronco maior que 10 cm foram medidos e identificados em dez parcelas, cinco por fragmento. As quatro espécies mais abundantes foram identificadas e seus alcalóides, flavonóides, saponinas e taninos foram qualificados fitoquimicamente. Setecentas e setenta e quatro árvores foram amostradas, sendo *Protium altissimum*, *Metrodorea flavida*, *Rinoreocarpus ulei* e *Cheiloclinium cognatum* Sm. as mais abundantes. Todos os metabólitos avaliados estavam presentes em *P. altissimum* e *C. cognatum* em ambos os fragmentos. Flavonóides estavam ausentes em *M. flavida* no fragmento I, e *R. ulei* apresentou saponina em ambos os fragmentos florestais e alcalóides apenas no II. Essas quatro espécies de árvores amazônicas possuem metabólitos secundários com importância medicinal, que podem ser extraídos em manejo sustentável.

Palavras-chave: biodiversidade; fragmentação; plantas medicinais; fitoquímica

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Introduction

Inadequate land use transforms continuous forest areas into isolated fragments, causing habitats losses and threatening the natural biodiversity, including that of plants with medicinal potential (Rosa et al., 2017). Monocultures are replacing native landscapes in the south of the Amazon, increasing the importance of understanding the biodiversity and the potentialities of its plant species (Barbosa et al., 2018). Accelerated losses of native habitats increases the importance of research on medicinal plants, because endemic species may be extinct before they are studied.

Active compounds from the plant secondary metabolites may be used to treat or cure diseases. Phytochemical analyses can support pharmacology searching of active principles to identify bioactive compounds, including alkaloids, flavonoids, saponins and tannins with composition and quantity varying with environmental factors (Aquino et al., 2019). The main functions of these phytochemical compounds are, for example, alkaloids and flavonoids of potential anti-inflammatory and antioxidant (Compaoré et al., 2016) and of saponins and tannins may indicate the antibacterial and antifungal activity (Biswas & Dwivedi, 2019).

Brazil with its wide floristic diversity, 116 species of Gymnosperms and 35772 species of Angiosperms (Flora do Brasil, 2023), presents only 4.5% that follow the precepts of the National Policy of Medicinal Plants and Herbal Medicines (Brasil, 2006) and are registered in the Botanical Collection of Medicinal Plants (CBPM, 2023). Therefore, there is immense potential for new discoveries of medicinal plant species native to the country.

The sustainable use of forest remnants allows collecting their natural resources and public policies are necessary to maintain these areas. The identification of arboreal species with pharmacological potential in forest remnants is important for their subsistence, protecting natural resources and the use of these plants for basic health needs (Rovedder et al., 2016). Abundant species in forest fragments have large number of individuals with more offspring ensuring the continued arrival of individuals with lower local extinction chances even when raw material is extracted (He et al., 2022).

The discovery and use of plants for medicinal purposes are of scientific and social interest. In addition, when these plants, with leaves as raw material, originate from native forests, they can allow the maintenance of these species and the sustainability of forest remnants. The aim of this study was to identify the most abundant tree species in two Amazon Forest areas in the southern Amazon, Alta Floresta municipality, Mato Grosso state, Brazil, and to qualify their secondary compounds.

Materials and Methods

Study area

The most abundant plant species were identified in two forest fragments in the southern Amazon, in the municipality of Alta Floresta, Mato Grosso state, Brazil. The area of the fragment I (09° 56' 58.6" S, 56° 03' 13.4" W) was 330.8 ha intercepted by an energy transmission line. The vegetation is occasionally suppressed beneath this line since 1995, increasing the edge effect and the number of clearings. The fragment II (09° 53' 19.07" S, 55° 59' 51.4" W) is a forest area with 539.1 ha, about 5.5 km distant from the number I. This second forest area is larger than the I, is in a good conservation state and has lower edge effect and higher canopy cover (Figure 1).

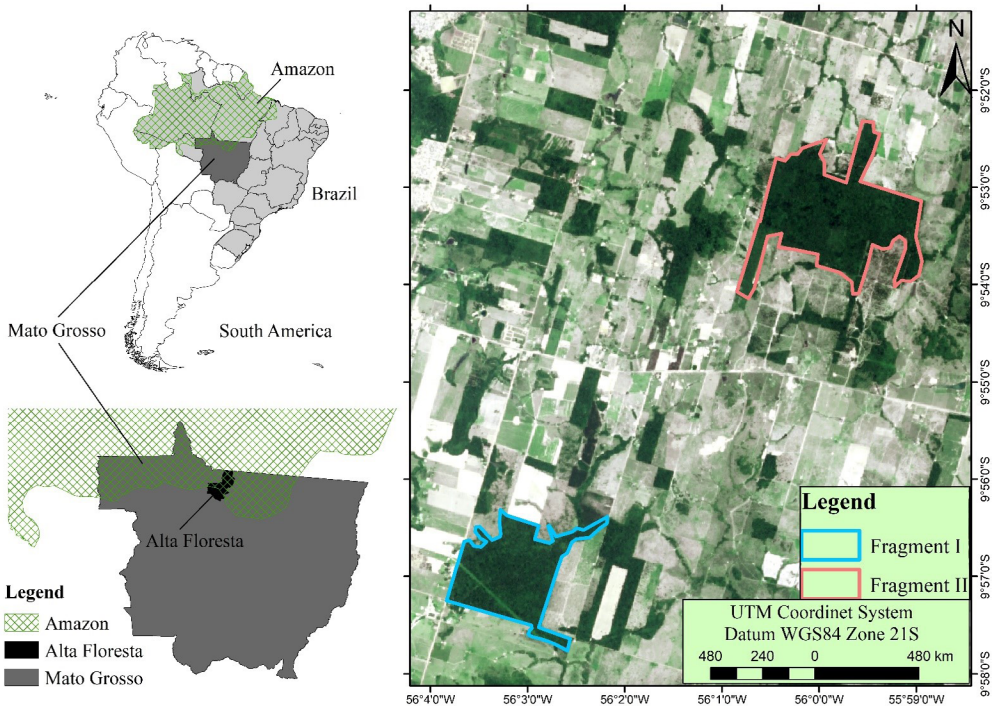

Figure 1. Forest fragments where the plants were collected in the Alta Floresta municipality, Mato Grosso state, Brazil.

The climate of the region is Tropical Monsoon (Am) in Köppen classification, with annual average temperature and precipitation of 27.6 °C and 3,000 mm year^{-1}, respectively (Alvares et al., 2013). The predominant soil is red-yellow acrisol. The relief of the municipality correspond to the Southern Plateau of Southern Amazonia, with dense and open ombrophilous forest types (Arruda et al., 2018).

Selection of plants for phytochemical analysis

The forest inventory of abundant species was carried out in ten plots (10 x 100 m each), five per fragment. Plants with diameter trunks greater than 10 cm at 1.3 m high were measured and identified at the lowest possible taxonomic level by taxonomists or parataxonomists. After identification, the documentation was incorporated into the HERBAM (Herbário da Amazônia Meridional-UNEMAT) collection: numbers 14,581 to 14,690.

The four species with the greatest abundance of individuals in the ten plots sampled were selected with FITOPAC software 2.1. Phytochemical analysis of these species was performed by preliminary prospecting (Matos, 1997), with tests on hydroethanolic extracts for alkaloids, flavonoids, saponins and tannins. Abundant species have been selected because they produce more offspring, ensuring the continued arrival of individuals and reducing chances of local extinctions (He et al., 2022).

Experimental protocol

Leaf samples of each of the four most abundant tree species in both fragments were harvested in July and August 2015, dried at 40 °C and ground to obtain a powder. The extracts were obtained with 20 g of the powder from each plant and 100 mL of ethanol (92.8%). The mixture was kept in a thermostatic bath for 15 minutes and filtered. After that, the preliminary prospecting was performed according to Matos (1997):

Alkaloids: Fifteen drops of 1% sodium hydroxide and 2 mL of distilled water were added to 2 mL of the plant extract, followed by 2 mL of chloroform, when the aqueous phase was discarded. Then, 15 drops of 1% hydrochloric acid and 2 mL of water were added to the chloroform phase, which was discarded by pipetting. Three drops of the Dragendorff and Mayer reagent were added to this solution. The formation of insoluble and flocculent precipitate confirmed the alkaloid presence.

Flavonoids: Two milliliters per extract were added to 0.5 cm of magnesium on a tape and 2 mL of concentrated hydrochloric acid, being positive for the presence of flavonoids when the color changed from brown to red.

Saponins: Two milliliters of chloroform and 5 mL of distilled water were added to 2 mL of the extract from each plant and the solution stirred for five minutes. The appearance of persistent foam for more than one minute indicated the saponin presence.

Tannins: Three drops of $FeCl_3$ alcohol solution were added to 2 mL of the extract and the formation of a precipitate indicated the tannin presence.

The tests were done in duplicates and the control had only distilled water. The results were described with a cross system (+) to specify the presence or absence of each metabolite, using the following ratings: substantial presence (+++), notable presence (++), mild presence (+) and absence (-).

Results and Discussion

Seven hundred and forty-four plants were identified in the 10 plots (1 hectare) of the two forest fragments. *Protium altissimum* (Aubl.) Marchand, *Metrodorea flavida* K. Krause, *Rinoreocarpus ulei* (Melch.) Ducke and *Cheiloclinium cognatum* (Miers) ACSm. were the most abundant species, with 115, 58, 26 and 21 individuals, respectively (Table 1).

The total number of plants per hectare was within the range sampled per hectare in the Amazon (341-771 individuals per hectare). The highest abundance of *P. altissimum* confirms reports from other areas in the Amazon and Amazon-Cerrado transition areas (Lima et al., 2022; Pires et al., 2021). *Metrodorea flavida* is fairly widespread, and *R. ulei* and *C. cognatum*, commonly, found in the Amazon rainforest (Lima et al., 2022). The adaptation of these plants to the lighting, photoperiod and soil conditions of the Amazonian ecosystems explains their higher density in the two forest fragments.

The graphical representation of the procedures and indication of the presence of secondary metabolites in each fragment are in Figure 2. Metabolites of all classes were recorded in *P. altissimum* in both fragments, with mild alkaloid presence in both fragments, a substantial level of flavonoids in the fragment II and a notable level in the I, besides a notable presence of the tannin and saponin classes in the fragment II and a mild presence in the I (Table 1; Figure 2).

Table 1. Classes of metabolites secondary of the four plant species with the greatest number of individuals (NI) in the forest fragments I and II in the southern Amazon, municipality of Alta Floresta, Mato Grosso state, Brazil.

Family/Species	NI	Classes	I	II
Protium altissimum (Sapindales, Burseraceae)	115	Alkaloid	+	+
		Flavonoid	++	+++
		Saponin	+	++
		Tannin	+	++
Metrodorea flavida (Sapindales, Rutaceae)	58	Alkaloid	+	++
		Flavonoid	-	++
		Saponin	+	++
		Tannin	+	+++
Rinoreocarpus ulei (Malpighiales, Violaceae)	26	Alkaloid	-	++
		Flavonoid	-	-
		Saponin	++	++
		Tannin	-	-
Cheiloclinium cognatum (Celastrales, Celastraceae)	21	Alkaloid	+++	++
		Flavonoid	++	+++
		Saponin	++	++
		Tannin	+	++

Substantial presence (+++), notable (++), mild (+) and absence (-).

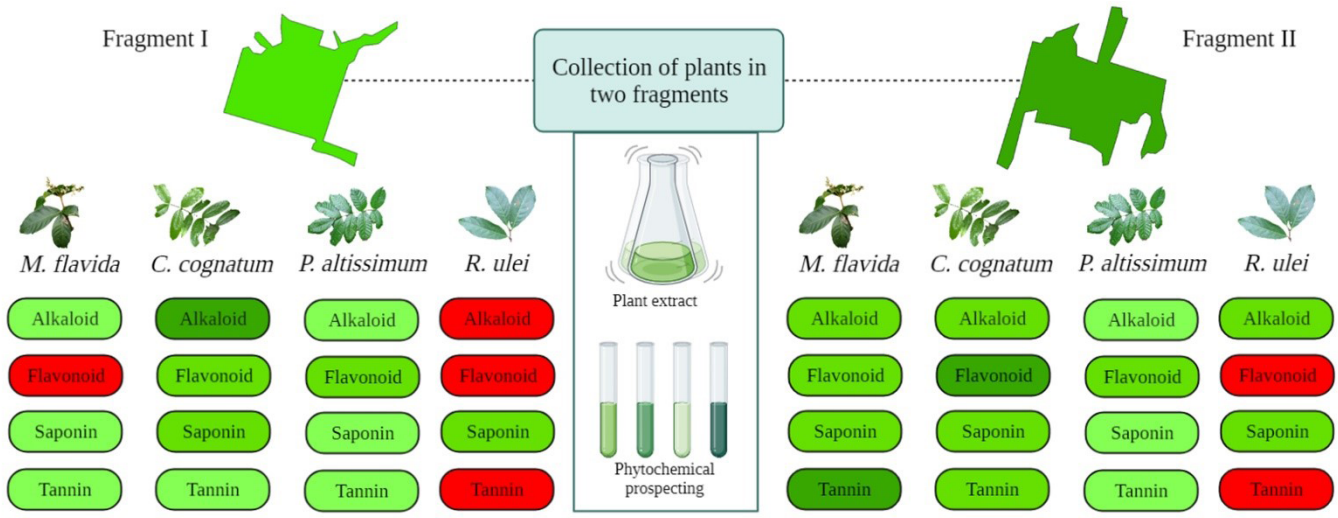

Figure 2. Graphical indication of the presence of metabolites in each fragment. where: (-) = color RGB: 255;0;0; (+) = color RGB: 133;255;8; (++) = color RGB: 101;224;0; (+++) = color RGB: 56;168;0. Figure made in BioRender.

The presence of alkaloids, flavonoids, saponins and tannins in *P. altissimum* shows that this species, like others of the Burseraceae family (ex. genus *Protium*), can be included in the searching for new drugs, since these metabolites have pharmacological properties (Silva et al., 2019). The extract of *Commiphora africana* (A. Rich.) Engl., a plant of this family, has medicinal potential with antioxidant and anti-inflammatory activities (Compaoré et al., 2016). Flavonoids, with a substantial presence in this plant, have potential for antioxidant, anti-inflammatory, and anti-proliferative processes (Wang et al., 2018). The influence of temperature and soil nutrients in the plant tissue and metabolite production can explain the flavonoid, saponin and tannin concentrations in *P. altissimum* leaves from the two forest fragments as reported for *Tithonia diversifolia* in different states (Sampaio et al., 2016) and *Lafoensia pacari* in different municipalities (Sampaio et al., 2011) of Brazil.

The presence of alkaloids, saponins and tannins was positive for *M. flavida* in both fragments, but flavonoids were only present in the fragment II (Table 1; Figure 2). The results for saponins in *R. ulei* were notable in both fragments, as well as that of alkaloids in the fragment II (Table 1; Figure 2).

The positive result for alkaloids, flavonoids, saponins and tannin in *M. flavida* agrees with reports for this plant in the municipality of Paragominas, Pará state, Brazil (Baetas et al., 1999). Thirteen alkaloids were isolated from the roots of *Toddalia asiatica* of the Rutaceae family (Chen et al., 2021), such as *Zanthoxylum nitidum* and genus *Fagaropsis* (Rutaceae) (Mutinda et al., 2022) had phytochemical compounds isolated. This is important because these compounds have anti-inflammatory and antioxidant activity (Mutinda et al., 2022). Differences in the presence of alkaloids, flavonoids, saponins and tannins in *M. flavida* between the two fragments may be due to variations in temperature, humidity, light, water and minerals affecting this plant growth and the production of secondary metabolites (Sampaio et al., 2011; Wang et al., 2022). The metabolite concentrations depend on the growth conditions and impact the pathways responsible for their accumulation in the plant (Wang et al., 2022).

The presence of saponins in the *R. ulei* extract may indicate pharmacological potential for this plant, since compounds of this group have antibacterial, antifungal, anti-inflammatory, antimicrobial, antiparasitic, antitumor, antiviral and cytotoxic activity (Biswas & Dwivedi, 2019). Saponins of *Hybanthus ipecacuanha* (L.) Bail., also of the same family as *R. ulei*, have bronchodilator, anti-inflammatory and antinociceptive activity, justifying their use to treat respiratory diseases (Leal et al., 2000). Isolated compounds of *Viola* spp., also of the same family as *R. ulei*, are used to treat cough, cold, flu, fever and malaria, and also as an anticancer drug (Chandra et al., 2015). Variations in the accumulation of secondary metabolites in this plant between the two forest fragments show the effect of environmental factors such as light, temperature, water and soil fertility and salinity, where rising temperatures at lower altitudes may have increased the metabolite content in plants of the fragment II, as recorded for aspen in Finland (Maja et al., 2016). Changes in one of these factors may affect the secondary metabolite contents of the plants, even with the other factors remaining constant. The biosynthesis of secondary compounds is a key component for the adaptation of plants to the conditions of biotic and abiotic stress (Wang et al., 2022).

All classes of metabolites were positive for *C. cognatum*, with a substantial presence of alkaloids in the fragment I and flavonoids in the II. Saponin was notable in this species in both fragments, and tannins were mild in fragment I and notable in the II (Table 1; Figure 2).

The positive results for alkaloids and flavonoids in *C. cognatun* confirm reports for this plant (Jeller et al., 2004) and others of the Celastraceae family, such as *Loeseneriella africana* (Willd.) (Compaoré et al., 2016), with compounds of potential anti-inflammatory and antioxidant effects in their extracts. The presence of saponins and tannins may

indicate the antibacterial, antifungal, anti-inflammatory, antimicrobial, antioxidant, antiparasitic, antitumor, antiviral, cardio-protective and immunomodulatory potential and the cytotoxic activity of this plant (Biswas & Dwivedi, 2019). Differences in the concentration of metabolites in *C. cognatun* between the two forest fragments may be due to environmental factors such as soil salinity, which increased the biosynthesis of these compounds. This may be related to the differences in antioxidant activity in *Cichorium intybus* plants collected in a saline habitat and two non-saline ones in Serbia (Zlatić & Stanković, 2017).

The methodologies used in the extraction and identification of phytochemical compounds include different parts of plants, such as branches with leaves (Compaoré et al., 2016) and roots (Chen et al., 2021). In addition, different chemical compounds (Compaoré et al., 2016; Sampaio et al., 2011) and devices (Compaoré et al., 2016; Sampaio et al., 2016) can be used to investigate the chemical composition and verify the medicinal activity of each of these compounds.

These phytochemicals indicate a promising research area to develop drugs to treat and prevent diseases (Aquino et al., 2019). The practical applicability of this study will be the verification of the medicinal activity of these plants for the various existing diseases.

Conclusions

Protium altissimum, *M. flavida*, *R. ulei* and *C. cognatum* are most abundant plants in the areas sampled in the two Amazon forest fragments. The presence of the secondary compounds alkaloids, flavonoids, saponins and tannins indicate the medicinal potential of these plants. These compounds with pharmacological importance can be extracted in sustainable management programs highlighting the importance of conserving these habitats for the Amazonian flora and to the human health.

The manuscript serves as an insight for new prospection studies of metabolites in Amazonian species. The results are of interest to the most diverse public, from entities that aim at the sustainability of native forests with its biodiversity to professionals and researchers in the health area, as well as the ethnobotanical society with its culture of using natural products.

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Compliance with Ethical Standards

Author contributions: Conceptualization: AGS, MPP, CRASL; Data curation: AGS, MPP; Formal analysis: AGS; Funding acquisition: MPP, CRASL; Investigation: AGS, MPP; Methodology: AGS, MPP; Project administration: MPP; Supervision: MPP, CRASL; Validation: AGS, MPP, CRASL, JCZ; Visualization: AGS, MPP; Writing – original draft: AGS; Writing - review & editing: AGS, MPP, CRASL, JCZ.

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