Chemical constituents and antifungal potential of *Attalea geraensis* Barb. Rodr. (Arecaceae) palm leaves, a species native to the Cerrado of Brazil

Constituintes químicos e potencial antifúngico de folhas da *Attalea geraensis* Barb. Rodr. (Arecaceae), uma espécie nativa do Cerrado do Brasil

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Abstract

Fungal diseases, especially those that affect the root systems of plants, caused by *Rhizoctonia* and *Macrophomina* are limiting factors for achieving high crop yields. Alternatives to controlling fungi with chemical products drive the search for new options for bioactive compounds from plants. *Attalea geraensis*, a palm tree from the Brazilian Cerrado, is rich in flavonoids with antifungal actions. The objective of this work is to identify the chemical classes present in the ethanolic extract of green leaves of *A. geraensis* and determine the antifungal potential of the extract against isolates of *Macrophomina phaseolina* (Tassi) Goid. and *Rhizoctonia solani* JG Kühn. Phytochemical prospection, flavonoid dereplication, and antifungal activity were carried out of the ethanolic extract of the green leaves of *A. geraensis* harvested in the Cerrado area of Brazil. Steroids, triterpenes, saponins, and anthraquinones are described here for the first time for the leaves of *A. geraensis*. The flavonoids quercetin, isorhamnetin, 3,7-dimethylquercetin, quercetin 3-galactoside, 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-[[3,4,5-trihydroxy-6-(hydroxymethyl]) oxan-2-yl]oxy]-4H-chromen-4-one, rhamnazin 3-galactoside, keioside, and rhamnazin 3-rutinoside were identified. Of these, only quercetin and isorhamnetin had already been identified in the leaves of *A. geraensis*. The result of a synergistic action between fungus and plant or there could be an antagonistic effect between flavonoids and the other identified chemical classes.

Keywords: indaiá, palm trees without aerial stem, glycosylated flavonoids, phytopathogenic fungi.

Resumo

Doenças fúngicas, especialmente as que afetam os sistemas radiculares das plantas, causadas por Rhizoctonia e Macrophomina, são fatores limitantes para obtenção de grande produtividade das culturas. Alternativas ao controle dos fungos com produtos químicos impulsionam a pesquisa de novas opções de compostos bioativos oriundos de plantas. A Attalea geraensis, uma palmeira do Cerrado brasileiro, é rica em flavonoides com ações antifúngicas. O objetivo do trabalho foi identificar as classes químicas presentes no extrato etanólico das folhas verdes de A. geraensis e determinar o potencial antifúngico do extrato frente a isolados de Macrophomina phaseolina (Tassi) Goid. e Rhizoctonia solani J.G. Kühn. Realizou-se a prospecção fitoquímica, desreplicação de flavonoides e atividade antifúngica a partir do extrato etanólico das folhas verdes da A. geraensis, colhida em área de Cerrado do Brasil. Os esteroides, triterpenos, saponinas e antraquinonas estão sendo descritos pela primeira vez para as folhas de A. geraensis. Foram identificados os flavonoides quercetina, isoramnetina, 3,7-dimetilquercetina, quercetina 3-galactosídeo, 5,7-dihidroxi-2-(4-hidroxi-3-metoxifenil)-3-{[3,4,5-trihidroxi-6-(hidroximetil)oxan-2-il]oxi}-4Hcromen-4-ona, ramnazina 3-galactosídeo, keiosídeo e ramnazina 3-rutinosídeo. Destes, somente a quercetina e isorhamnetin já haviam sido identificadas nas folhas da A. geraensis. Os resultados indicam potencial fungistático para a espécie. Infere-se que a diversidade de flavonoides presentes nas folhas de A. geraensis pode ser resultado da ação sinérgica entre fungo e planta ou que haja um efeito antagonista entre os flavonoides e as demais classes químicas identificadas.

Palavras-chave: indaiá, palmeira acaule, flavonoides glicosilados, fungos fitopatogênicos.

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1. Introduction

Agricultural expansion in Brazil occurs in an accelerated and consistent way. The monocultures implemented have generated problems such as an excess of fungal diseases, especially those that affect the root systems of plants, leading to large production losses. Some of these fungi, such as Rhizoctonia and Macrophomina, are limiting factors for obtaining high productivity and economic sustainability. They cause diseases such as gray rot, which affect the root system and stems of soybean plants. In addition, they are difficult to control because they can develop in dry climates and high temperatures (Cruciol and Costa, 2018). Because of this, massive uses of pesticides are required. However, the use of these chemicals has become a problem, as it induces an increase in the resistance of microorganisms, thus requiring a gradual and greater number of applications (Ayora-Talavera, 2017). Consequently, there is a greater environmental contamination and food poisoning, among other problems, which generates public health concerns (Pignati et al., 2017).

Contamination, whether of soil and/or water resources, by-products used in agriculture is a threat to the entire ecosystem (Pignati et al., 2017). Therefore, the search for natural or bioactive compounds that are less toxic to the environment and people but still effective in controlling phytopathogens is paramount. Among the natural compounds with antifungal action, plant extracts, such as that from the palm tree *Attalea*, a genus of the family *Arecaceae*, can be used. These plants are rich in phenolic compounds, including flavonoids, which are promising metabolites due to their antifungal properties (Williams et al., 1985; Andrade et al., 2020; Mohammed and Fouad, 2022).

Among Attalea, there is the Attalea geraensis Barb. Rod. ("indaiá, coquinho do cerrado, catolé, or indaiá do campo") (Lorenzi et al., 2004), with typical occurrence in the Cerrado of Brazil, where there is a diversity of landscapes and great floristic richness. This places the flora of the Biome among the richest Biomes among the world's savannas (Fernandes et al., 2016). A. geraensis is an oil palm of great socioeconomic value for ornamental and food use. The fruits (chestnuts), palm hearts, and palm leaves are widely used by traditional Brazilian communities, such as the Caboclos, Indigenous, and riverine peoples (Milani et al., 2011).

A. geraensis is well adapted to acidic, nutrient-poor soils and the water stresses of the Cerrado. This can be seen in the anatomy of waxy leaves and deep underground stems accumulating reserve substances (Galetti et al., 2004). These characteristics are essential for the survival of this species to fires and deforestation that occur in the Brazilian Cerrado, allowing its maintenance and expansion in the area of such events. In addition, plants of the genus *Attalea* live in symbiotic associations with fungi, allowing monodominant formations in large areas, including anthropized areas (Souza and Martins, 2004; Damasceno et al., 2009; Pott et al., 2011), as it occurs with *A. phalerata*, in some regions of the Pantanal Sul-Mato-Grossense (Oliveira et al., 2023).

The occurrence of A. geraensis in restrictive environments produces several chemical compounds, such as flavonoids, which have been reported for 16 species of this genus. Apigenin, tricine, and *C*-glycosidic flavones are found in their leaves, in addition to guercetin and isorhamnetin (Williams et al., 1983; Williams et al., 1985). Species of Attalea are characterized by their flavonoid content, with heterogeneous substances such as 5- and 7- glycosides and C-glycosyl flavones (Williams et al., 1985). Flavonoids are identified as markers of the genus and are described for facilitating the adaptation process of Attalea species to the environment. C-glycosylated flavones, for example, can act as phytoalexins against fungi and insects (Chen et al., 2013). There is a description of apigenin and tricine helping in severe water deficits and high carbon dioxide environments (Jara, 2017).

In this context, the objective is to identify the chemical classes present in the ethanolic extract of green leaves of *A. geraensis* and determine the antifungal potential of the extract against isolates of *Macrophomina phaseolina* (Tassi) Goid. and *Rhizoctonia solani* JG Kühn.

2. Materials and Methods

2.1. Collection and identification of plant material

Green leaves of *A. geraensis* were collected from ten specimens randomly in a Private Natural Heritage Reserve located in the Cerrado Biome in the municipality of Corguinho (19°49' S, 54°50' W, 320 m altitude), State of Mato Grosso do Sul, Brazil. Collections were carried out in accordance with Brazilian legislation with prior authorization from the Brazilian Institute of the Environment and Renewable Natural Resources – IBAMA (74662). An exsiccate was deposited at the Herbarium of the Federal University of Grande Dourados (DDMS), under no. 6480.

2.2. Obtaining the ethanolic extract and phytochemical prospection

The green leaves (200 g) were ground with ethanol by turbolysis and macerated for 24 h. Subsequently, the solution was left for 30 min in an ultrasound bath, and the resulting extract was filtered. This procedure was repeated for seven consecutive days, and the product of each extraction was pooled and dried in a desiccator under reduced pressure, resulting in 9.88 g of extract.

For phytochemical prospecting, the methodology described by Matos (2009) was adopted. The results were read according to Fontoura et al. (2015), who classify them as partial intensities (\pm = 10%), low (+ = 25%), moderately moderate (++ = 50%), moderate (\pm ++ = 75%) and high (+++ = 100%), in addition to negative (- = 0%).

2.3. Flavonoid dereplication

The ethanolic extract (50 mg) of leaves of *A. geraensis* was fractionated using an SPE C-18 cartridge (2 g, elution volume: 6 mL). Solutions (100 mg mL⁻¹) of fractions F50 and F100, which were eluted with 1:1 acetonitrile: water and acetonitrile, respectively, were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS)

in an Agilent 6545 Q-TOF-MS system (Agilent Technologies, Santa Clara, CA, USA) using an Agilent Zorbax Eclipse Plus C18 column (Rapid Resolution HD 2.1×50 mm, 1.8 µm). The mobile phase was composed of water containing 1% formic acid (A) and acetonitrile (B), and the elution schedule was 0-4 min from 5-15% B (linear gradient), 4-15 min 15-60% B (linear gradient), 15-24 min 60-100% B (linear gradient), and 24-27 min 100% B (isocratic elution). The flow rate and injection volumes were 0.300 mLmin⁻¹ and 3.0 µL, respectively. The operating parameters were Gas temp. 300 °C; Gas Flow 12 L min⁻¹; 35 Psi Nebulizer; SheathGas Temp 350; SheathGas Flow 10; VCap 2500; Nozze Voltage 0 V; Shredder 110; Skimmer. The spectra of MS1 and MS2 were acquired in the ranges of m/z 100-1500 and 70-1500, respectively. Collision energies (MS²): 30, 35, 40, 45, 50, and 60 units for masses (Da) of 100, 300, 500, 700, 1,000, and 1.500, respectively.

Data from LC-MS/MS were processed using the MassHunder Workstation Software (B.08.00). The flavonoids were identified according to the spectra of MS¹ and MS², obtained by comparison with the literature and with the library contained in the equipment (databases: DrugBank; FooDB; PlantCyc; ChEBI; T3DB; STOFF; BLEXP; NPA; NANPDB; COCONUT; KNApSAck; PubChem; UNPD).

2.4. Antifungal analysis

A stock solution of ethanolic extract at a concentration of 2% (0.2 g of the shredded green leaves of *A. geraensis* in 100 mL of 99.5% ethanol) was prepared at concentrations of 800, 1,200, 1,600, 2,000, and 2,400 µg 100 ml⁻¹. As a control, only PDA (Potato-Dextrose-Agar) medium was used. The media at different concentrations was poured into 60x15-mm Petri dishes with a volume corresponding to 10 mL per dish. There were four replicates per concentration, each dish considered as an experimental unit. After solidification, a 5-mm diameter disc of mycelium of the phytopathogens *Macrophomina phaseolina* and *Rhizoctonia solani*, previously grown for seven days, was pealed.

The plates were covered and sealed with plastic film and kept in a growth chamber at 25 °C with a photoperiod of 12 h.

Mycelial growth was evaluated by daily measurements of the diameter of colonies until the control reached the edges of plates. Based on the data obtained, the percentage of growth inhibition (PGI) was calculated for each treatment by the formula: PGI = ([{control colony diameter – treatment colony diameter]/control colony diameter] * 100).

Data obtained as a function of doses were submitted to analysis of variance and, when significant by F test, were submitted to regression analysis up to 5% probability.

3. Results

3.1. Phytochemical analysis

The phytochemical results indicated a predominance of phenolic compounds, flavonoids, steroids, and coumarins with high intensity (100%). Triterpenes, cardiotonic heterosides, and reducing sugars showed a moderately moderate intensity (50%). On the other hand, tannins (25%) had a low intensity, and saponins (10%) and anthraquinones (10%) had a partial intensity. The other investigated classes were not evidenced in this study.

Phytochemical prospection, a fast technique for profiling raw plant extracts, generated information on the chemical composition of the ethanolic extract of green leaves of *A. geraensis*. Considering previous studies (Williams et al., 1983,1985), which describe flavonoids as chemical markers of the genus *Attalea*, it was decided to direct the study of dereplication to the group of flavonoids contained in the species.

3.2. Flavonoid dereplication

The compounds 1 to 8 (Figure 1, Table 1) were identified by LC-MS/MS, as follows: (1) quercetin, (2) isorhamnetin, (3) 3,7-dimethylquercetin, (4) quercetin 3-galactoside, (5) 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-4Hchromen-4-one, (6) rhamnazin 3-galactoside, (7) keioside, and (8) rhamnazin 3-rutinoside.

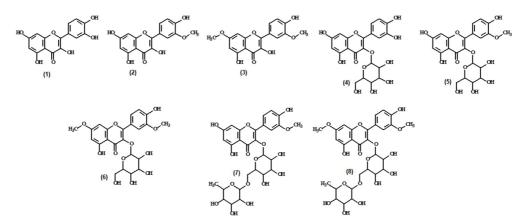


Figure 1. Chemical structures of flavonoids identified in green leaves of *Attalea geraensis*: (1) quercetin, (2) isorhamnetin, (3) 3,7-dimethylquercetin, (4) quercetin 3-galactoside, (5) 5,7-dihydroxy- 2-(4-hydroxy-3-methoxyphenyl)-3-{[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy}-4H-chromen-4-one, (6) rhamnazin 3-galactoside, (7) keioside, (8) rhamnazin 3-rutinoside.

The flavonoids identified in dereplication indicated the possibility that the ethanolic extract from leaves of *A. geraensis* to have antifungal potential. Therefore, the extract was evaluated at different concentrations against phytopathogenic strains.

3.3. Antifungal activity

There was mycelial inhibition (Figure 2) of both species of fungi evaluated confirmed by the growth in diameter of colonies (Figure 3). The results indicated a fungistatic potential for the ethanolic extract of leaves of *A. geraensis*. The doses used in the tests, up to the highest concentration of 2,400 µg 100 mL⁻¹, are acceptable on *R. solani* and *M. phaseolina* with fungistatic effect (Dilkin et al., 2024).

The increase in the concentrations evaluated led to a directly proportional relationship of inhibition of the mycelial growth of the fungi *M. phaseolina* and *R. solani*. Only the lowest concentration (800 µg 100 mL⁻¹) did not inhibit the growth of fungal species. Regarding *M. phaseolina*, inhibition started from 1,200 µg 100 mL⁻¹ and for *R. solani* from 1,600 µg 100 mL⁻¹. The inhibition percentages were between 5.3-25.0% and 4.6-24.9%, respectively (Figure 3).

Table 1. Data obtained in the analyses performed by LC-MS/MS of the ethanolic extract of green leaves of *Attalea geraensis* with the respective proposed substances and data described in the literature.

Retention time (min)	Proposed substances	Data [M+H]+ (m/z)	Fragments* (<i>m/z</i>)	Fragments literature	Literature
8.092	Quercetin 3-0-hexose	465	303 [Aglycone-162]+153 [Aglycone-162-150]+	463; 301	6
8.196	Quercetin	303	275 [Aglycone-28]+153 [Aglycone-150]+	303; 153	3
8.455	Rhamnazine 3-O-hexose	493	331 [Aglycone-162]+316 [Aglycone-162-15]+	515; 492; 353; 330	2
8.507	Isorhamnetin -3-O- rutinoside (keioside)	625	317 [Aglycone-308]+302 [Aglycone-308-15]+153 [Aglycone-308-15-149]+	625; 317	7
8.767	Isorhamnetin 3-O-hexose	479	317 [Aglycone-162]+302 [Aglycone-162-15]+153 [Aglycone-162-15-149]+	316; 301; 287	4
10.118-10.170	Rhamnazine 3-O-rutinoside	639	331 [Aglycone-308]+316 [Aglycone-308-15]+	639; 331	1
11.832-11.936	Rhamnazine	331	315 [Aglycone-15]+287 [Aglycone-15-28]+167 [Aglycone-15-28-120]+	330; 315; 301; 287; 167; 165; 151; 149	1;2
12.144-12.248	Isorhamnetin	317	302 [Aglycone-15]+274 [Aglycone-15-28]+153 [Aglycone-15-149]+	317; 153	3;5

1. Itokawa et al. (1981); 2. Barberá et al. (1986); 3. Wolfender et al. (2000); 4. Chang et al. (2003); 5. Hassan et al. (2014); 6. Quifer-Rada et al. (2015); 7. Joo et al. (2020). Literature data was obtained in a positive mode. *The fragmentation proposals are detailed in the Supplementary Material.

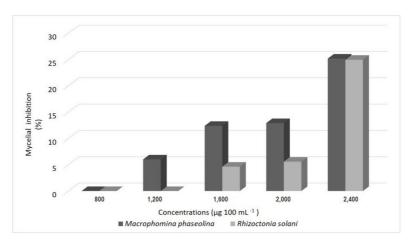


Figure 2. Inhibition of mycelial growth (%) of *Macrophomina phaseolina* and *Rhizoctonia solani* by the ethanolic extract of green leaves of *Attalea geraensis* at different concentrations.

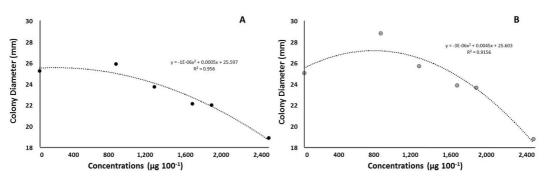


Figure 3. Inhibition of mycelial growth (%) of Macrophomina phaseolina (A) and Rhizoctonia solani (B) by the ethanolic extract of green leaves of Attalea geraensis at different concentrations.

4. Discussion

Steroids, triterpenes, saponins, and anthraquinones are described here for the first time for the leaves of *A. geraensis*. Steroids, triterpenes, and saponins had only been described for another species of the genus, *Attalea speciosa* Mart. ex Spreng, and for species of the same family (Arecaceae): *Acrocomia aculeata* (Jacq.) Lodd. Mart., *Mauritia flexuosa* L.f., *Phoenix dactylifera* L., *Paludosa phoenix* Roxb., and *Ravenea rivularis* Jum. & H.Perrier (Ávila et al., 2011; Matias et al., 2019; Mohammed and Fouad, 2022). Anthraquinones had only been described for *A. aculeata*.

Of the eight flavonoids identified in our study, six are described here for the first time for leaves of *A. geraensis*. Only the flavonoids quercetin and isorhamnetin had already been described for this species (Williams et al., 1983; Williams et al., 1985). On the other hand, for plants of the same family (Arecaceae), several flavonoids have already been described: chalcones, flavanol, and flavone derivatives (Mohammed and Fouad, 2022).

Flavonoids are already known for their antifungal potential; some of the substances identified in our study have already shown this potential. Quercetin, in association with other flavonoids, showed potential against *R. solani* (Ashmawy et al., 2020; Joaquín-Ramos et al., 2020). This substance can cause an irreversible process in the fungal cell because, when penetrating the phospholipid membrane, it damages its DNA (Pourakbar et al., 2021). On the other hand, isorhamnetin, in a dose-dependent manner, can generate oxidative species and increase the permeability of the pathogen's cell membrane (Tian et al., 2021).

In general, flavonoids protect plants against different biotic agents and abiotic stresses and among the many benefits to plants, they are considered signaling molecules in the defense against phytopathogens (Cesco et al., 2012). The fungicidal potential attributed to flavonoids, in terms of inhibiting fungal growth (fungicide effect) or limiting its development (fungistatic effect), or acting as elicitors, is often associated with the diversity and quantity of this class of polyphenols (Stanković et al., 2012; Hassan et al., 2021; Nofal et al., 2024).

Flavonoids are substances capable of creating a barrier in the plant as a form of defense against microorganisms (Costa et al., 2020) or of associating with plants in mycorrhizal symbiosis, as is the case of *A. geraensis*. In palm trees, in general, because they have lignified roots with few hairs, symbiosis facilitates access to nutrients and water in the soil and in flooded regions, such as the Pantanal Biome in Brazil, symbiosis contributes to the adaptation of plants to seasonal hydrological fluctuations (Bouamri et al., 2014).

There is a key factor for this association for palm trees' ecological success and predominance result from a probable symbiosis of A. geraensis with fungi. This is because luminescent fungi, for example, have a high incidence in palm tree areas. There are records of the bioluminescent fungus Neonothopanus gardneri in decaying leaves and at the base of the stem of Attalea oleifera Barb. Rod. (pindoba), at the base of the stem of A. speciosa Mart. ex Spreng. (babassu), and A. funifera Mart. ex Spreng. (piassava) (Capelari et al., 2011; Vieira et al., 2022). Ventura et al. (2021) found that N. gardneri has a predictable bioluminescence and growth pattern and is highly sensitive to Cd(II), 4-nitrophenol, phenol, and Cu(II) compounds. These characteristics may offer valuable advantages to plants, which, when receiving "signs" of the presence of toxic products in the environment, can somehow create defense mechanisms. Furthermore, the antitumor and antileishmanial bioactivities already reported for N. gardneri indicate the production of secondary metabolites that must be important for plants, such as defense and attraction.

Some flavonoids of the flavonols class, such as quercetin, myricetin and kaempferol, are mycelial growth promoters in mycorrhizae and may be important for the establishment of *A. geraensis* in unfavorable edaphic conditions. This association between fungi and plants of the genus *Attalea* allows the species to develop in edaphic conditions with strong environmental restrictions (Bécard et al., 1992; Romero and Siqueira, 1996). Based on this information, it is possible to state that the diversity of flavonoids found in the leaves of *A. geraensis* in our study (Figure 1) may be related to these associations. This justifies the results obtained here (Figures 2 and 3), that is, a fungistatic effect at the highest concentrations (2,400 µg/100 mL) against *M. phaseolina* and *R. solani* (Figures 2 and 3), as there are synergistic actions between fungi and plants.

According to the results presented, it is suggested that there may be a symbiotic interaction between *A. geraensis* and fungi, an efficient strategy for the dominance of certain species in degraded environments. The presence of the *A. geraensis* in large areas, often in almost monodominance, may result from symbiotic interactions attributed to their chemical characteristics, facilitating colonization of a degraded Cerrado, as in Corguinho (area of Taboco), where the species was collected for this study.

A second hypothesis is that the different flavonoids in the leaves of A. geraensis may have had antagonistic actions, acting ineffectively on the organelle portions of the phytopathogens in vitro. Based on the above, it is suggested that, although flavonoids are a chemical class with great importance for A. geraensis and described as for their fungicidal potential, the small capacity of the extract to inhibit mycelial growth is due to antagonism of different classes of substances detected or their behavior in vivo, which in turn is related to the physiology of the palm tree itself (Wink, 2003; Casanova and Costa, 2017). However, some flavonoids, such as apigenin, quercetin-3-O-galactoside, and quercetin, are considered mycelial growth promoters in association fungi (mycorrhizae) (Bécard et al., 1992; Romero and Sigueira, 1996). Due to the effects on growth inhibition, the percentages obtained here were probably small, showing that synergistic processes occur between the different compounds found in leaves, which do not allow for effective inhibition of fungal development.

From an ecological and evolutionary point of view for this species, such synergistic processes for plant defense against pathogens and herbivory are important. However, antagonistic processes are described as important when most secondary chemicals have no other function than to generally contribute to non-adaptive but necessary responses that seek to increase the probability of producing some biologically active compounds to use when certain ecological circumstances arise (Rasmann and Agrawal, 2009). Thus, these metabolic interactions favor the colonization of microorganisms, mainly fungi, in a beneficial way for the plant. These conditions, in at least some parts of the system, are favorable for the collection of energy, while the association begins when the conditions are adverse (Trivedi et al., 2020).

In addition to the above, another species of the genus *Attalea*, *Attalea* phalerata Mart. ex. Spreng, is cited because it associates with edaphic factors; it has a physiological (metabolic interaction) and ecological strategy that allows its occurrence as a pioneer in degraded areas, forming a high population density (Pott et al., 2011). *A. geraensis* could show similar behavior, with physiological and ecological strategies that allow occupation in areas where other species would have more difficulty establishing themselves.

For the first time, for the leaves of *A. geraensis*, the classes of steroids, triterpenes, saponins, and anthraquinones are described, in addition to the flavonoids 3,7-dimethylquercetin, quercetin 3-galactoside, 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3-{[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxy}-4H-chromen-4-one, rhamnazin 3-galactoside, keioside, and rhamnazin 3-rutinoside.

There is a fungistatic potential of the ethanolic extract of leaves of *A. geraensis* because there is mycelial inhibition of *Macrophomina phaseolina* and *Rhizoctonia solani*, confirmed by diameter growth in colonies. Thus, the diversity of flavonoids present in the leaves of *A. geraensis* may be the result of a synergistic action between fungus and plant or there could be an antagonistic effect between flavonoids and the other identified chemical classes.

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Supplementary Material

Supplementary material accompanies this paper. Supplementary material. This material is available as part of the online article from https://doi.org/10.1590/1519-6984.271577