Evaluation of the potential of the ethyl acetate extract of sucupira in the *in vitro* control of *Agroathelia rolfsii*

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ABSTRACT. Sustainable agricultural systems have advanced, promoting the reduction of dependence on pesticides through the responsible management of natural resources. Among the alternatives for the control of phytopathogens, plant extracts have been widely studied due to their antifungal potential. This study aimed to evaluate in vitro the antifungal activity of the fruit extract of *Pterodon emarginatus* against the fungus *Agroathelia rolfsii*. The experiment was conducted at the IF Goiano – Campus Posse Laboratory, using isolates of *A. rolfsii* from the mycotheca of Campus Urutaí. The extract was obtained by macerating 200 g of fruits in 1000 mL of ethyl acetate, followed by natural evaporation of the solvent for 33 days. To enable its application, the extract was previously emulsified in Eumulgin® CO 40 (PEG-40), at a ratio of 1:1 (v/v). After the solidification of the culture medium (20 mL per plate), the emulsified extract was applied on the surface of the Petri dishes, corresponding to the concentrations of 0.25; 0.375; 0.5; 1.0 and 2.0 µL mL^{-1} (0.025; 0.0375; 0.05; 0.10 and 0.20% v/v), considering the volume of pure extract of 5, 7.5, 10, 20 and 40 µL. The experimental design was completely randomized, with 11 treatments and five replications. Mycelial growth was evaluated by radial measurements on two orthogonal axes, and the averages were calculated for each treatment. From the obtained data, the percentage of mycelial growth inhibition (MGI) and the mycelial growth rate index (MGRI) were determined. The results indicated that the extract of *P. emarginatus* showed antifungal activity against *A. rolfsii*, with significant effects at the evaluated concentrations. Treatments containing only ethyl acetate and Eumulgin® CO 40 did not differ statistically from the control, suggesting that the inhibition may be associated with bioactive compounds present in the extract. It is concluded that the extract of *P. emarginatus* presents potential as an alternative agent in the control of *A. rolfsii*, contributing to the development of sustainable phytosanitary management strategies.

Key words: Phytopathogen, Biocontrol, Plant extract, *Pterodon emarginatus*.

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INTRODUCTION

The indiscriminate application of chemical pesticides in the management of fungal phytopathogens has caused several problems, such as the presence of residues in food, impacts on public health, environmental damage, and the emergence of resistant fungal strains (Santos et al. 2024). In this context, the use of plant extracts as a basis for the formulation of new biofungicides represents a promising alternative for the phytosanitary control of fungi in agriculture (Santos et al. 2024).

Plant extracts are natural products derived from plants, widely recognized for their multiple biological properties, including antifungal, therapeutic, bactericidal, and insecticidal activities (Ribeiro et al. 2014; Sá-Filho et al. 2021). The inhibitory action on the growth of phytopathogenic fungi is associated with the presence of secondary metabolites play multiple ecological roles, including defense, signaling, and environmental interaction (Pacheco and Alves, 2020; Sá-Filho et al. 2021). These natural products are often considered potentially less harmfull, depending on composition, dose, and

formulation, in addition may reduce selection pressure compared to single-site fungicides, although resistance development cannot be ruled out (Venturoso, 2009). In this scenario, it becomes essential to intensify research aimed at evaluating the potential of plant extracts so that they can be applied sustainably in the management of fungal diseases, such as those caused by *Agroathelia rolfsii* (Sacc.) Redhead & Mullineux (2023), formerly known as *Sclerotium rolfsii* Sacc. (1911) (Redhead and Mullineux 2023). The fungi *Agroathelia rolfsii*, a soilborne pathogen that survives primarily as sclerotia, causes diseases such as root rot and damping off. It is a soil-borne pathogen with a wide range of susceptible hosts, widely distributed in tropical and subtropical regions, including several regions of Brazil, affecting both small and large agricultural properties (Santos, 2010; Ablometi et al. 2025).

Considering the impact of *A. rolfsii* on agriculture, it is essential to adopt alternative and effective methods that provide sustainable control in the long term. Sustainable agricultural systems have advanced in this direction, promoting the reduction of dependence on pesticides through the rational management of natural resources, which contributes to environmental preservation and the maintenance of agricultural productivity (Bettiol, 2006). Among the control alternatives, plant extracts have been widely studied due to their effectiveness, acting as promising sources of biologically active substances with proven action against various microorganisms, such as bacteria, filamentous fungi, and yeasts (Oliveira, 2011).

White sucupira (*Pterodon emarginatus* Vogel [1837]), traditionally used in folk medicine due to its therapeutic properties, has attracted increasing scientific interest due to the antifungal and antioxidant activity of its fruit (Lemos et al. 2021; Alcântara et al. 2023; Seraphin et al., 2025). Phytochemical studies show that extracts and essential oils of *Pterodon emarginatus* have a high concentration of terpenes and sesquiterpenes, highlighting compounds such as β-caryophyllene, α-humulene, β-elemene, germacrene D, bicyclogermacrene, spathulenol, and farnesol. These secondary metabolites have been associated with relevant biological activities, including antimicrobial and antifungal properties against different phytopathogenic microorganisms, indicating the potential of this species as a source of bioactive compounds for the control of plant diseases (Froldi et al. 2023). However, to date, there are no records in the literature regarding the use of this plant extract in the control of *A. rolfsii*, which reinforces the originality and scientific relevance of this research. Thus, the present study aimed to evaluate the fungitoxic effect of the ethyl acetate extract of white sucupira on the *in vitro* growth of the phytopathogen *A. rolfsii*, contributing to the understanding of its properties and to the development of sustainable alternatives in the management of plant diseases.

MATERIAL AND METHODS

The experiment was conducted at the Multi-User Natural Sciences Laboratory (LMCN) of the Instituto Federal Goiano – Campus Posse. Mature fruits of *Pterodon emarginatus* Vogel (sucupira) were manually collected from a single tree on October 22, 2024. The collection was carried out at the Valdo Ramos agricultural exhibition park, located in the municipality of Iaciara, state of Goiás, Brazil, at the geographic coordinates 14°05'45'' South and 46°37'54'' West, with an average altitude of 819 m. The fruit collection region presented climatic conditions typical of the spring season, with a minimum temperature estimated at around 22 °C and a maximum that could reach 34 °C. Botanical identification was performed based on morphological characteristics and with the aid of specialized taxonomic literature, being confirmed by comparison with species descriptions.

Fruit collection was carried out directly from the ground, immediately after natural fruit drop, selecting only those that showed physical integrity and absence of apparent damage caused by insects or fungi. After collecting, the fruits were stored in paper bags and kept in a dry and ventilated place until the moment of plant extraction. A total of 50 g of fruits were weighed using a digital semi-analytical balance with a precision of 0.01 g (model AD, brand Marte) and placed in a glass container with a lid. The fruits underwent a disinfection process, being immersed in 100 mL of 5% sodium hypochlorite for 10 minutes, followed by 100 mL of alcohol for another 10 minutes. After this period, the fruits were washed three times with 150 mL of distilled water.

Next, with the aid of a sterile scalpel, the fruits were perforated to facilitate the release of the active compounds present inside them. The perforated fruits were placed in a glass reagent bottle and 250 mL of ethyl acetate P.A. (analytical standard) was added.

The choice of ethyl acetate is due to its ability to extract bioactive compounds of different polarities, such as terpenes, flavonoids, and phenolic compounds (Brito, 2019). This solvent is widely used in plant extractions because it presents good efficiency in removing these substances and because it evaporates easily, which facilitates the subsequent steps of the experiment (Brito, 2019).

A proportion of 200 g of fruits for each 1 liter of solvent was considered (Lucena, 2015). The contact between the solvent and the raw material was maintained for four days at room temperature ranging from 26 °C to 29 °C, protected from light and with daily agitation in an orbital shaker (model SL-180/D, brand Solab) at 90 rpm. After this period, the compound resulting from the mixture of the solvent with the raw material was filtered, discarding the fruits. The resulting liquid was kept at room

temperature (26–29 °C) for 40 days inside a fume hood, the solvent was removed under controlled conditions (e.g., rotary evaporation) to preserve bioactive compounds. At the end of the process, the concentrated sucupira extract was stored in a beaker covered with perforated aluminum foil (holes of approximately 1 mm in diameter), remaining for another 15 days in a glass desiccator containing silica at the bottom, for continued drying and stabilization of the extract. After complete evaporation of the solvent, a crude extract with a viscous consistency and dark-brown color was obtained, characteristic of the plant material used.

The phtypathogen *Agroathelia rolfsii* was obtained by culturing sclerotia donated by the Mycological Collection of the Microbiology and Phytopathology Laboratory of IF Goiano – Campus Urutaí. The growth of the fungus was carried out in potato culture medium (PDA) in the Natural Sciences Laboratory of IF Goiano – Campus Posse.

For the preparation of the culture medium, 200 mL of potato broth, 20 g/L of glucose, and 7 g of agar were used, completing the volume to 1 L of culture medium. The medium was sterilized in an autoclave at 121 °C at 1 atm pressure for 20 minutes and then distributed into Petri dishes measuring 100 × 15 mm.

The sclerotia were cultured in the culture medium for 10 days at 28 °C and 60 % humidity. The morphology of the hyphae of *A. rolfsii* was verified by light microscopy, using mycelium slides stained with methylene blue.

For the growth inhibition tests of *Agroathelia rolfsii*, the crude extract, with a viscous consistency, was emulsified in Eumulgin® CO 40 (PEG-40), at a ratio of 1:1 (v/v) (extract:emulsifier), under manual agitation until complete homogenization, without prior dilution, in order to enable its application.

The extract was incorporated into molten PDA medium (45-50 °C) before pouring into Petri dishes containing 20 mL of culture medium, being uniformly distributed with the aid of a Drigalski spatula, using the poisoned food technique, adapted from Ferreira et al. (2020).

Volumes of 10, 15, 20, 40, and 80 µL of the emulsion were applied, corresponding to 5, 7.5, 10, 20, and 40 µL of pure extract per plate, respectively. The concentrations were calculated based on the actual volume of extract in relation to the volume of the culture medium (20 mL), being expressed in µL mL^{-1} and in % (v/v), corresponding to 0.25; 0.375; 0.5; 1.0 and 2.0 µL mL^{-1} (0.025; 0.0375; 0.05; 0.10 and 0.20% v/v). After the application of the treatments, a mycelial disk of 6 mm in diameter of *A. rolfsii* was transferred to the center of the plates.

The influence of the ethyl acetate solvent on fungal growth was also evaluated. For this, volumes of 10, 15, 20, 40, and 80 µL per plate were applied, corresponding to concentrations of 0.5; 0.75; 1.0; 2.0, and 4.0 µL mL^{-1} (0.05; 0.075; 0.10; 0.20, and 0.40% v/v), considering the volume of 20 mL of culture medium. The solutions were distributed on the surface of the PDA medium with the aid of a Drigalski spatula, and the mycelial disk was subsequently transferred to the center of the Petri dish.

The plates were incubated in a B.O.D. chamber under controlled temperature conditions (28 ± 1 °C) and absence of light. The plates were laterally sealed with plastic film to avoid contamination.

The evaluations of the experiment were carried out by measuring the diameter of the mycelial colony using a digital caliper (mm). From the center of the colony, the diameters were measured along the horizontal and vertical axes. The first evaluation was performed 48 hours after the installation of the experiment, and measurements were subsequently carried out daily until the plate was completely filled by the control treatment (Hillen et al., 2012). These data were used to estimate the percentage of mycelial inhibition (MGI%), according to Edgington, Knew, and Barron (1971), and the mycelial growth rate index (MGRI), according to Oliveira (1991).

The experimental design was completely randomized, consisting of 11 treatments, including a negative control (PDA medium without addition of extract or solvent), five treatments containing ethyl acetate, and five treatments containing emulsified sucupira extract. Each treatment consisted of five replications, considering each Petri dish as an experimental unit. Thus, the treatments were defined according to Table 1.

Table 1. Description of the treatments used in the experiment, including the volumes of crude extract and solvent (ethyl acetate), as well as the final concentrations expressed in µL mL^{-1} and % (v/v).

Treatment	Ethyl acetate volume (µL)	Pure extract volume (µL)	Concentration (µL mL^{-1})	Concentration (% v/v)
T1	–	–	–	–
T2	10	–	0.50	0.05
T3	15	–	0.75	0,075
T4	20	–	1.00	0.10
T5	40	–	2.00	0.20
T6	80	–	4.00	0.40
T7	–	5	0.25	0.025
T8	–	7.5	0.375	0.0375
T9	–	10	0.50	0.05
T11	–	20	1.00	0.10

The results obtained were subjected to analysis of variance (ANOVA) and, when the F test was significant, the means were compared using Tukey's test at the 5% significance level. All statistical analyses were performed using the R statistical computing software, version 4.4.3 (R Core Team, 2023).

RESULTS AND DISCUSSION

The mycelial growth of *Agroathelia rolfsii* over time showed differences among the evaluated treatments. The treatments containing *Pterodon emarginatus* extract, at concentrations of 0.25; 0.375; 0.5; 1.0; and 2.0 µL mL^{-1}, showed a consistent reduction in mycelial growth compared to the control and the treatments containing ethyl acetate.

It was observed that the control and the treatments with ethyl acetate (0.5 to 4.0 µL mL^{-1}) showed progressive growth throughout the experimental period, reaching larger mycelial diameters at the end of the evaluations. In contrast, the treatments with the plant extract maintained reduced growth over time, demonstrating a continuous inhibitory effect (Figure 1), which can also be visually observed in the culture plates (Figure 2).

The treatments containing only ethyl acetate did not differ statistically from the control in most of the evaluations, indicating that the solvent did not exert a significant effect on the mycelial growth of *A. rolfsii*. This result confirms that the observed inhibition is associated with the bioactive compounds present in the *P. emarginatus* extract, and not with the solvent used in the extraction process. The quantitative data for the mycelial growth rate index (IVCM), percentage of mycelial growth inhibition (PIC), and colony diameter confirm the antifungal effect of the plant extract (Table 2).

The treatments with the extract showed a significant reduction in the mycelial growth rate index (IVCM), with values ranging from 0.33 to 0.48 mm day^{-1}, compared to the control (0.95 mm day^{-1}). In addition, all treatments with the extract showed inhibition greater than 50%, with the concentrations of 0.10 and 0.20% (1.0 and 2.0 µL mL^{-1}) standing out, as they presented the highest percentages of mycelial growth inhibition. This result indicates that all tested concentrations showed inhibitory effects, although no clear dose-response relationship was observed of mycelial growth.

This antifungal effect may be attributed to the presence of bioactive compounds in the sucupira plant extract. Phytochemical studies of *P. emarginatus* highlight the presence of β-caryophyllene, farnesol, α-humulene, and vouacapane-type diterpenes, substances associated with antioxidant, antimicrobial, and antifungal properties (Froldi et al., 2023; Xavier, 2023).

According to Froldi et al. (2023), chromatographic and spectrometric analyses performed on preparations of *P. emarginatus* seeds confirmed the presence of β-caryophyllene as one of the major compounds, in addition to farnesol, both related to important biological activities. These compounds show antimicrobial potential and may act by destabilizing the cellular membranes of microorganisms, contributing to the inhibition of fungal growth. Among these metabolites, β-caryophyllene was identified as one of the major compounds in sucupira, showing antimicrobial activity against *Staphylococcus aureus* (Donati et al., 2015). Thus, the presence of these bioactive compounds in the plant extract may explain the antifungal effect observed in the present study, evidenced by the reduction in mycelial growth and in the growth rate of the pathogen.

The results indicate that the plant extract obtained from sucupira fruits has potential as an antifungal agent in the control of *A. rolfsii*. However, it was observed that the inhibitory effect showed a plateau, indicating absence of a clear dose-dependent response (T7 – 0.25 µL mL^{-1}), with no statistically significant differences observed among the other evaluated concentrations (Table 2).

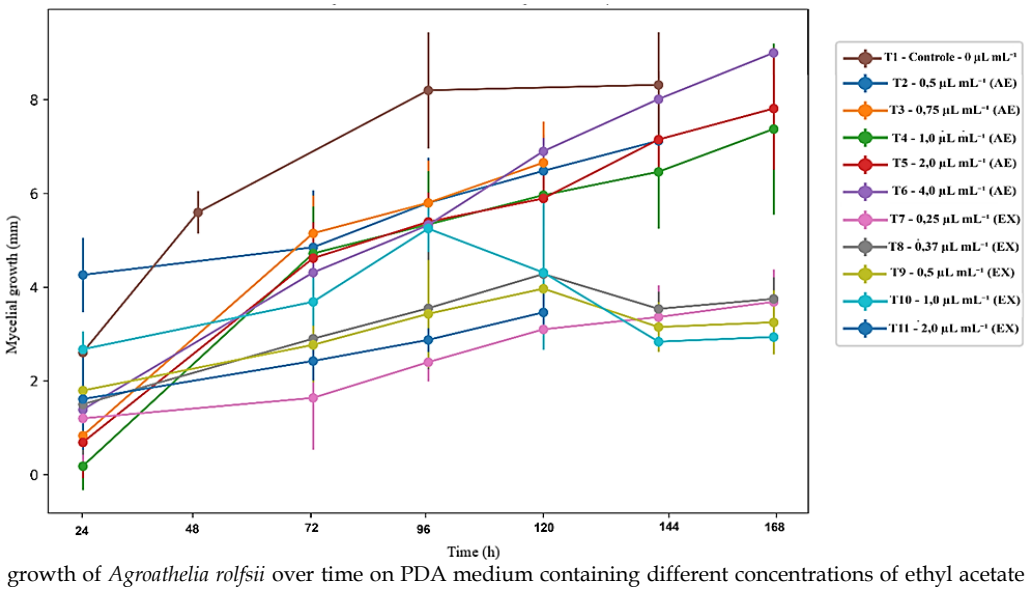

Figure 1. Mycelial growth of *Agroathelia rolfsii* over time on PDA medium containing different concentrations of ethyl acetate (EA) and *Pterodon emarginatus* extract (EX). The points represent the mean ± standard deviation of the experimental replicates

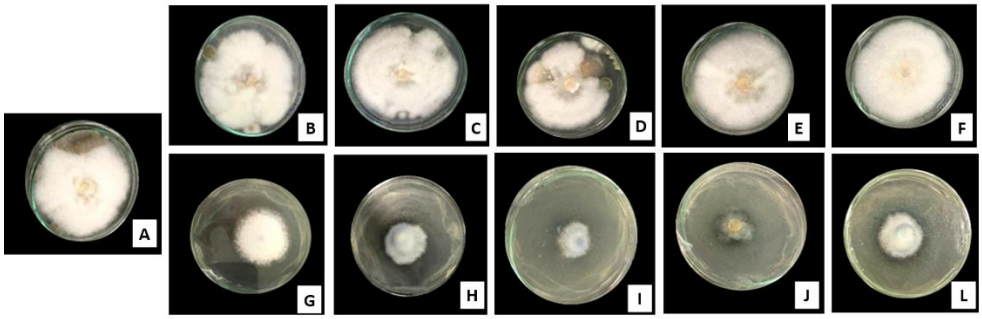

Figure 2. Mycelial growth of *Agroathelia rolfsii* on PDA medium after 168 hours of incubation under different treatments. A: T1 – control (0 µL mL^{-1}); B–F: treatments containing ethyl acetate (EA) at concentrations of 0.5; 0.75; 1.0; 2.0; and 4.0 µL mL^{-1}; G–L: treatments containing *Pterodon emarginatus* extract (EX) at concentrations of 0.25; 0.375; 0.5; 1.0; and 2.0 µL mL^{-1}.

Table 2. Effect of *Pterodon emarginatus* extract (EX) and ethyl acetate (EA) on the mycelial growth of *Agroathelia rolfsii*, expressed as the mycelial growth rate index (MGRI), percentage of mycelial growth inhibition (MGI), and colony diameter (mm).

Treatment	Concentration (µL/ mL^{-1}; % v/v)	MGRI (mm/day)	MGI %	Mycelium (mm)
T1	0 µL mL^{-1}; 0% v/v	0.9518 ± 0.20 a	-	8.31 ± 1.13 a
T2	0.5 µL mL^{-1} (0.05% v/v) de AE	0.5619 ± 0.15 b	25.64 ± 2.18 bc	7.0 ± 0.50 ab
T3	0.75 µL mL^{-1} (0.075% v/v) de AE	1.2286 ± 0.18 a	18.31 ± 3.54 bc	7.48 ± 0.34 ab
T4	1.0 µL mL^{-1} (0.10% v/v) de AE	1.0622 ± 0.22 a	30.33 ± 14.6 b	6.37 ± 1.28 b
T5	2.0 µL mL^{-1} (0.20% v/v) de AE	1.0429 ± 0.19 a	23.32 ± 7.85 bc	7.1 ± 0.80 ab
T6	4.0 µL mL^{-1} (0.40% v/v) de AE	1.0857 ± 0.02 a	13.60 ± 5.12 c	7.9 ± 0.51 a
T7	0.25 µL mL^{-1} (0.025% v/v) de EX	0.3816 ± 0.18 b	56.92 ± 5.7 a	3.79 ± 0.62 c
T8	0.375 µL mL^{-1} (0.0375% v/v) de EX	0.4816 ± 0.14 b	56.19 ± 1.74 a	3.85 ± 0.38 c
T9	0.5 µL mL^{-1} (0.05% v/v) de EX	0.3622 ± 0.11 b	63.09 ± 7.84 a	3.32 ± 0.71 c
T10	1.0 µL mL^{-1} (0.10% v/v) de EX	0.4520 ± 0.10 b	65.55 ± 3.88 a	2.92 ± 0.09 c
T11	2.0 µL mL^{-1} (0.20% v/v) de EX	0.3371 ± 0.15 b	57.16 ± 8.9 a	3.62 ± 0.48c

Values represent mean ± standard deviation (n = 5) a, b, and c: different letters indicate statistical differences among means according to Tukey's test ($p \leq 0.05$).

The PIC values confirm the high antifungal activity of the extract with inhibition greater than 50% at all tested concentrations. The highest rates were observed at concentrations of 0.10% and 0.20%. with approximately 63% and 65% inhibition, respectively. These results are consistent with studies that highlight the effectiveness of plant extracts as natural alternatives to conventional fungicides, contributing to the development of more sustainable agricultural practices (Araújo et al., 2014). The use of plant extracts may also reduce the risk of resistance development by pathogens and minimize environmental impacts, establishing itself as a promising tool in integrated disease management.

The use of plant extracts as an alternative for the control of microorganisms has been increasingly valued, especially in response to the growing demand for natural and sustainable solutions. In this context, compounds extracted from sucupira (*Pterodon emarginatus*) stand out for presenting significant antifungal activity, confirmed by different studies and evaluation methods (Martins & Muraishi, 2019; Batalini et al., 2020; Xavier, 2023).

The antifungal action of the *P. emarginatus* extract is attributed to the presence of bioactive compounds that act in different ways, from destabilizing the fungal cell wall to inhibiting spore germination (Batalini et al., 2020). In addition, these secondary metabolites may induce resistance responses in plants, also functioning as elicitors, which further increases their potential in integrated disease management (Martins & Muraishi, 2019).

CONCLUSION

The results showed that the plant extract of *P. emarginatus* presented significant antifungal activity against *A. rolfsii*, promoting a marked reduction in mycelial growth at all tested concentrations. The highest inhibition rates were observed at concentrations of 1.0 and 2.0 µL mL^{-1}, which showed the highest percentages of inhibition of the pathogen's mycelial growth.

Thus, the plant extract of *P. emarginatus* shows potential as a natural alternative to the use of synthetic fungicides and may contribute to more sustainable strategies for the management of *A. rolfsii* within the context of integrated disease

management. However, additional studies are needed to elucidate the mechanisms of action of the bioactive compounds present in the extract, as well as to evaluate its effectiveness under greenhouse and field conditions, in addition to investigating possible phytotoxic effects and the development of formulations that enable its agricultural application.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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