Phytochemistry and antifungal activity of floral ethanolic extract of *Schultesia aptera* Cham. (Gentianaceae f.)

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Abstract

Schultesia aptera is a plant species belonging to the Gentianaceae family found in Cerrado areas of Brazil. S. aptera presents a low number of studies, mainly phytochemical. This study aimed to evaluate the qualitative phytochemical profile and antifungal activity of agricultural interest of the floral ethanolic extract of *S. aptera*. Flowers were collected in April 2024. The ethanolic extract was obtained by maceration and then freeze-drying. The antifungal assays were carried out at different concentrations (25, 50, 100, 200 and 300 µL mL^{-1}) on the fungal strains of *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*. Phytochemical prospecting demonstrated the presence of 10 groups of pharmaceutical, biotechnological and agricultural interest that have antifungal activities. The floral extract of *S. aptera* demonstrated effectiveness between the highest concentrations 100-300 µL mL^{-1} with inhibition rates between 33-71 for *C. acutatum* and between 31-67% for *C. gloeosporioides*. *S. sclerotiorum* and *R. stolonifer* strains were resistant to all concentrations. The floral ethanolic extract of *Schultesia aptera* demonstrated to be an antifungal agent on the genus *Colletotrichum* and can be used to control and inhibit *Colletotrichum acutatum* and *Colletotrichum gloeosporioides*.

Keywords: *Schultesia* genus, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, tannins.

Fitoquímica e atividade antifúngica do extrato etanólico floral de *Schultesia aptera* Cham. (Gentianaceae f.)

Resumo

Schultesia aptera é uma espécie vegetal pertencente a família Gentianaceae encontrada em áreas de Cerrado do Brasil. *S. aptera* apresenta um baixo número de estudos, principalmente fitoquímico. Este estudo teve por objetivo avaliar o perfil fitoquímivo qualitativo e atividade antifúngica de interesse agrícola do extrato etanólico floral de *S. aptera*. Flores foram coletadas em Abril de 2024. O extrato etanólico foi obtido por maceração e liofilizador em seguida. O ensaios antifúngica foi realizado em diferentes concentrações (25, 50, 100, 200 e 300 µL mL^{-1}) sobre as cepas fúngicas de *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides* e *Rhizopus stolonifer*. A prospecção fitoquímica demonstrou a presença de 10 grupos de interesse farmacêutica, biotecnológico e agrícola que apresentam atividades antifúngicas. O extrato floral de *S. aptera* demonstrou efetividade entre as maiores concentrações 100-300 µL mL^{-1} com taxas de inibição entre 33-71 para *C. acutatum* e entre 31-67% para *C. gloeosporioides*. As cepas de *S. sclerotiorum e R. stolonifer* foram resistentes para todas as concentrações. O extrato etanólico floral de Schultesia aptera demonstrou ser um agente antifúngico sobre o gênero *Colletotrichum* podendo ser usado no controle e inibição de *Colletotrichum acutatum* e *Colletotrichum gloeosporioides*.

Palavras-chave: gênero *Schultesia*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, taninos.

1. Introduction

Gentianaceae f. is distributed worldwide, except in Antarctica, and occupies widely diverse habitats (Struwe et al., 2002; Guimarães et al., 2003; Chi et al., 2021). The family includes 91 genera and approximately 1700 species classified into six tribes (Saccifolieae, Exaceae, Chironieae, Helieae, Potalieae, and Gentianeae), plus the genus *Voyria*, whose position is uncertain (Struwe et al., 2002; Gentian Research Network 2012).

Schultesia Mart. comprises 21 closely related taxa (Guimarães et al., 2013). The genus *Schultesia* has a Pantropical distribution with specimens in the Cerrado area of Brazil, where the species *Schultesia gracilis* Mart., *Schultesia heterophylla* Miq. and *Schultesia aptera* Cham. (Figure 1) by Resende et al. (2013) and Moreira et al. (2019).

Species of the genus *Schultesia* are closely related, and the characteristics used for their identification include the texture of the leaf, the morphology of the calyx, the dimensions of the pedicel and corolla, and the particularities of the androecium, gynoecium and capsule (Guimarães 2002). The morphological and structural characteristics of *S. aptera* are known, however, the phytochemical constitution of the floral organ is still unknown. We know that several phytochemical groups can be present in the flowers of different groups of vegetables that have different biological activities, such as antifungal.

Studies with floral extracts are described with potential antifungal activity, Menezes Filho (2021) for the floral extract of *Jacaranda ulei* and by Menezes Filho et al. (2021a) for the floral extract of *Fridericia platyphylla* where they found good results of antifungal activity for both the genus *Candida*, species *C. krusei*, *C. tropicalis*, *C. guilliermondii* and *C. albicans*. Encouraging results were also described by Prado et al. (2022) for the control of fungi of agricultural interest by evaluating floral extracts of *Tabebuia roseoalba* and *Jacaranda cuspidifolia* on *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, *C. gloeosporioides* and *Rhizopus stolonifer*, and by Menezes Filho et al. (2021b) for *S. sclerotiorum*, *C. acutatum* and *C. gloeosporioides* evaluating the floral extract of *Spathoglottis unguiculata*.

Every year, a large number of complex fungal groups cause serious problems with agricultural losses in fruits, seeds and plants of ornamental interest. Among this group of fungal microorganisms that cause agricultural losses we have *Sclerotinia sclerotiorum* known as 'white mold' which attacks soybean plants leading to death. According by Polloni-Barros et al. (2022) soybean white mold (WM), caused by *S. sclerotiorum* (Lib.) de Bary, is a yield-limiting disease found in soybean that causes reductions in productivity as high as 60% to growers when environmental conditions are favorable (Cunha et al., 2010; McCaghey et al., 2017). This necrotrophic and polyphagous fungus is capable of infecting up to 400 different species (Boland; Hall, 1994).

The genus *Colletotrichum* presents several phytopathogenic fungi such as *C. acutatum* and *C. gloeosporioides* known as 'anthrachinosis' decimating fruit crops such as strawberries and papayas, avocados (Dowling et al., 2020), and in rice crops by *Rhizopus stolonifer* (Wang et al., 2019; Njoku et al., 2020; Khan et al., 2021). *R. stolonifer*, belongs to Zygomycetes, Mucorales, Mucoraceae, *Rhizopus* and *R. stolonifer*, is one of the most common and fastest-growing species in zygomycetes (Liu et al., 2024). The spores of *R. stolonifer* widely exist everywhere, can be spread by airflow and grow rapidly in humid environments, resulting in the decay of many fruits such as *Allium*, *Ananas*, *Cucurbia*, *Fragaria*, *Lycopersicon*, *Solanum* and so on (Liu et al., 2024).

Data on *S. aptera* are very scarce, and non-existent for phytochemical compounds and biological activities. In this sense, this study aimed to investigate the floral extract of *Schultesia aptera* regarding the phytochemical groups present in the floral extract and its antifungal activity on fungi of agricultural interest on *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides* and *Rhizopus stolonifer*.

Figuure 1. *Schultesia aptera* in the flowering period. Soure: Authors, 2024.

2. Materials and Methods

2.1. Species collection and identification

Flowers of *S. aptera* were collected in a rural area in the municipality of Rio Verde, Brazil, (17º43'08.0''S and 50º53'08.7''W) in April 2024. The species was identified by PhD. Isa Lucia de Morais Resende. An exsicata was herbalized and deposited in the Herbarium of the Plant Systematics Laboratory of the Postgraduate Program in Biodiversity and Conservation with Vouchers (HRV: 35087).

2.2. Production of floral ethanolic extract

The floral ethanolic extract was obtained using P.A. ethanol (600 g of flowers) as solvent and reflux in a Soxhlet-type apparatus until exhaustion for 12 h. The extract was then filtered through quantitative filter paper and lyophilized (Terroni, Mod. LS 3000, Brazil). The yield obtained was 14.3%.

2.3. Phytochemical screening

The phytochemical assay was carried out to detect the qualitative presence of the following phytochemical groups: organic acids, alkaloids, carbohydrates, azulenes, resins, flavonoids, coumarins, reducing sugars, non-reducing sugars, glycosides, cardiac glycosides, amino acids, tannins, phlobatannins, phlorotannins, Phytosterols, terpinoids, diterpenes, lignins, quinones, carboxylic acids, foamy saponins, hemolytic saponins, volatile oils, aromatic and aliphatic compounds, proteins, phenolics, triterpenoids, anthraquinones, anthocyanins, leucoanthocyanins, resins, fixed oil and oxylates were performed as described by Yadav & Agarwala (2011), Banu & Cathrine (2015) and Balamurugan et al. (2019). The results were based on visual observation of color change or precipitate formation after the addition of specific reagents.

2.4. Antifungal test

The agar (plate) diffusion method was used to determine the antifungal activity against *S. sclerotiorum, C. acutatum, C. gloeosporioides* and *R. stolonifer* as described by Toigo et al. (2022). The strains used are deposited and identified in the Mycological Bank of the Technological Chemistry Laboratory (SS12-21, CA15-67, CG 16-21 and RS 11-11) respectively, donated by the Natural Products Chemistry and Agricultural Microbiology Laboratory of Goiano Federal Institute, Rio Verde, Goiás State, Brazil. The fungal strains were cultivated at 20 °C for 10 days for *S. sclerotiorum* and 28 °C for 3-5 days for the other strains.

A disc of mycelium with a diameter of 7 mm was transferred to the center of *Petri* dishes with a diameter of 10 cm containing sterile potato, dextrose and agar (PDA) medium. Different concentrations of S. aptera floral extract were initially dissolved in 0.1% Tween 80 to yield doses between 25-300 $\mu L\ mL^{-1}$, 500 µL of the concentration was pipetted into each plate. The plates were transferred to an incubator at 20 °C (10 days) and

28 °C (3-5 days), respectively. The diameter of the inhibition zone was measured and recorded as an indicator of antifungal activity and expressed as a percentage (%) using a digital caliper. The commercial reference fungicide Frowncide 500 SC® was used as a positive control (dose of 5 $\mu L\ mL^{-1}$).

The Tween 80 emulsifier was also evaluated at the lowest dose under investigation (25 $\mu L\ mL^{-1}$). The agar diffusion assays applied to both floral extracts against the four fungi were performed in quadruplicate. Mycelial growth was measured daily until complete fungal growth separately on control plates. The percentage of mycelial growth inhibition (MGI) was calculated by the following formula 1:

$$MGI\% = (Control – Treatments)/Control))*100 \quad (1)$$

2.5 Statistical analysis

The experimental design was completely randomized. The data were subjected to analysis of variance (ANOVA) and treatment means were evaluated by the *Scott-Knott* test with 5% significance using the ASSISTAT statistical program.

3. Results

10 phytochemical groups were found in the floral ethanolic extract of *S. aptera* (Table1). In particular for alkaloids, azulenes, flavonoids, tannins and anthocyanins.

Table 1. Phytochemical prospection of floral extract of *Schultesia aptera*.

Group	Results
Organic acids	+
Alkaloids	+
Carbohydrates	-
Azulenes	+
Resins	-
Flavonoids	+
Coumarins	-
Reducing sugars	+
Non reducing sugars	+
Glycosides	-
Cardiac glycosides	-
Amino acids	-
Tannins	Blue
Flobotannins	-
Phlorotannins	-
Phytosterols	-
Terponoids	-
Diterpenes	-
Lignins	-
Quinones	-
Carboxylic acids	-
Foaming saponins	-
Triterpenoids	-
Anthraquinones	-

Anthocyanins	+
Leucoanthocyanins	-
Fixed oil	-
Oxylates	+
Aromatic and aliphatic compounds	Red

Note: (+) presence; (-) absence. (Blue) color hydrolyzable or gallic tannins. (Green) color condensed or catechetical. (Yellow) aliphatic carbonyl compound and (Red) aromatic carbonyl compound. Source: Authors, 2024.

For antifungal activity, the floral extract of *S. aptera* demonstrated greater mycelial inhibition activity for C. acutatum followed by *C. gloeosporioides* with average values of 71 and 67% for 300 µL mL^{-1} ($p < 0.05$) when compared to the standard fungicide Frowncide 100% inhibition. For *S. sclerotiorum* and *R. stolonifer*, no inhibition activity was observed ($p > 0.05$) (Table 2).

Table 2. Antifungal activity on agricultural phytopathogens in different concentrations of *Schultesia aptera* floral extract.

Fungi	Concentrations in µL mL^{-1} (%)				
	25	50	100	200	300
S. sclerotiorum	0b	0b	0b	0b	0b
C. acutatum	15f	19e	33d	45c	71b
C. gloeosporioides	0e	0e	31d	56c	67b
R. stolonifer	0b	0b	0b	0b	0b

Note: Frowncide fungicide 500SC® (100%) inhibition positive Control. Negative control Tween 80 (0%) inhibition. Average values with the same letter in the line do not differ statistically using the *Scott-Knott* test with 5% significance.Source: Authors, 2024.

4. Discussion

Several plants have biological activities with great pharmaceutical and agricultural potential for controlling diseases, phytonematodes, sulgating insects, etc. These phytochemical agents are produced from the special metabolism of plants and their concentrations can vary considerably. It is known that plants subjected to abiotic and biotic stresses can produce a higher content of some groups of phytomolecules than others, this production occurs for the preservation and physical and cellular integrity of the individual plant under stress (Verma; Singh, 2020; Bhalla et al., 2021).

As observed, the floral extract of *S. aptera* demonstrated a variety of phytogroups, making this the first study for this species evaluating the floral organ. Next, a preview of the main characteristics of the groups of greatest importance for medicine, pharmacy, biotechnology and agriculture is discussed, where we can verify that plant extracts have potential antifungal activity, which is determined by the set of phytochemical groups that enhance this activity (Parekh; Chanda, 2007; Sabir et al., 2021).

Some groups, especially alkaloids, flavonoids, tannins, saponins, steroids, terpenoids and phenolic compounds, have formidable biological activities. Alkaloids are one of the main and largest components produced by plants, and they are metabolic by products that are derived from the amino acids (Naseem 2014; Menezes Filho et al., 2020). Flavonoids consist of a large group of polyphenol compounds having a benzoyl-γ-pyrone structure and are ubiquitously present in plants (Agidew, 2022). The flavonoids are synthesized by the phenylpropanoid pathway. Several studies report this important phytochemical group where special metabolites of a phenolic nature, including favonoids, are responsible for a wide variety of pharmacological activities (Mahomoodally et al., 2005; Pandey, 2007). According to Dixo et al. (1983), flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection.

The group tannin is widely applied to a complex large biomolecule of polyphenol nature having sufcient hydroxyls and other suitable groups such as carboxyl to form strong complexes with various macromolecules

(Navarrete 2013; Baliyan et al., 2022). According by Boroushaki et al. (2016), tannins are generally used in the tanning process and used as healing agents in infammation, burn, piles, and gonorrhea. Saponins are an important group of plant special metabolites that are widespread throughout the plant kingdom. Saponins are basically phytochemicals that are found in most vegetables, beans, and herbs (Francis et al., 2002; Haralampidis et al., 2002). Terpenoids are small molecular products synthesized by plants and are probably the most widespread group of phytomolecules. Terpenoids show signifcant pharmacological activities, such as antiviral, antibacterial, antimalarial, anti-infammatory, inhibition of cholesterol synthesis, antitumoral and anti-cancer activities (Boroushaki et al., 2016).

Phenolic compounds are special metabolites, which are produced in the shikimic acid of plants and pentose phosphate through phenylpropanoid metabolization (Derong et al., 2016; Yang et al., 2021). Terpenoids are small molecular products naturally synthesized by plants and are probably the most widespread group of natural products. Terpenoids show signifcant pharmacological activities, such as antiviral, antibacterial, antimalarial, anti-infammatory, inhibition of cholesterol synthesis, and anti-cancer activities (Boroushaki et al., 2016).

Regarding antifungal activity, our results demonstrated significant effects for *C. acutatum* and *C. gloeosporioides*, especially for concentrations 100-300 µL mL^{-1}. Similar mycelial inhibition effects for both *Colletotrichum* species were also described by Prado et al. (2022) evaluating the floral extracts of *T. roseoalba* and *J. cuspidifolia* where at concentrations 100-300 µL mL^{-1} the average values were 62-77 and 44-56% and between 51-100 and 93-100%, respectively. Although the floral ethanolic extract of *S. aptera* did not show an inhibitory effect on *S. sclerotiorum* and *R. stolonifer*, Prado et al. (2022) found strong inhibition activity for these two strains that demonstrated to be sensitive to floral extracts of *T. roseoalba* and *J. cuspidifolia*.

Extracts of *Tabebuia serratifolia*, *Tabebuia impetiginosa* and *Jacaranda mimosifolia* have been shown in studies to be excellent options for inoculation and treatment of agricultural and native seeds to control phytopathogens from the *Colletotrichum* sp groups. and *Fusarium* sp., presented by Melo et al. (2016) and Naz et al. (2021). Menezes Filho et al. (2022) found encouraging results for the floral extract of *Miconia chamissois* with antifungal and antibacterial activity against *C. albicans*, *C. guilliermondii*, *C. tropicals*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella serovar Enteritidis*.

5. Conclusions

The floral ethanolic extract of *Schultesia aptera* presented several phytochemical groups in the qualitative analysis and potential antifungal activity on *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* in concentrations greater than 100 µL mL^{-1}.

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7. Authors' Contributions

Lucas Gomes Silva: study writing, plant collection, extract production, phytochemical analysis. Luis Eduardo Rodrigues de Carvalho: study writing, antifungal analysis and discussion. *Porshia Sharma*: translation, corrections, study writing and discussion. Aurélio Ferreira Melo: phytochemical analysis, antifungal activity, publication text standards. *Antonio Carlos Pereira de Menezes Filho*: translation, text writing, final review and publication. *Matheus Vinícius Abadia Ventura*: statistical analysis, writing, post-evaluation corrections and publication.

8. Conflicts of Interest

No conflicts of interest.

9. Ethics Approval

Not applicable.

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