In vitro fungicidal effect of the floral extract of *Cattleya walkeriana* (Gardner, 1839) (Orchidaceae)

Carlos Alberto Carvalho Moreira[1], Thiago Henrique Lopes Barros[1], Elizabete Nunes da Rocha[1], Matheus Vinícius Abadia Ventura[1,2] & Antonio Carlos Pereira de Menezes Filho[1,3]

[1] UniBRAS University Center, Rio Verde, Goiás, Brazil

[2] Goiano Federal Institute, Rio Verde Campus, Rio Verde, Goiás, Brazil

[3] Federal University of Jataí, Jataí, Goiás, Brazil

Correspondence: Carlos Alberto Carvalho Moreira, Microbiology Laboratory, UniBRAS University Center, Rio Verde, Goiás, Brazil. E-mail: carlosalbertocarvalhomoreira@gmail.com

Received: May 14, 2025 DOI: 10.14295/bjs.v4i7.761

Accepted: June 19, 2025 URL: https://doi.org/10.14295/bjs.v4i7.761

Abstract

Some orchid groups have shown promising results regarding biological activity in inhibiting fungal growth. This study aimed to evaluate the antifungal activity of the hydroethanolic floral extract of *Cattleya walkeriana* against strains of *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, *C. gloeosporioides*, and *Aspergillus* spp. (*A. flavus*, *A. niger*, and *A. fumigatus*) at different concentrations through *in vitro* assays. The extract was prepared using a 70% (*v/v*) hydroethanolic solution and tested on *Petri* dishes containing potato dextrose agar (PDA) medium inoculated with fungal strains. Results were expressed in millimeters (mm) of fungal growth inhibition (antibiosis) at concentrations of 100%, 80%, 50%, 10%, and 1.25% (*v/v*). The study demonstrated that higher concentrations (80% and 100%) of the hydroethanolic floral extract of *Cattleya walkeriana* exhibited strong antifungal activity against the tested fungal strains.

Keywords: *Cattleya* genus, *Colletotrichum acutatum*, *Aspergillus flavus*, phytochemistry, antibiosis.

Efeito fungicida *in vitro* do extrato floral de *Cattleya walkeriana* (Gardner, 1839) (Orchidaceae)

Resumo

Alguns grupos de orquidáceas têm demonstrado bons resultados quanto à atividade biológica na inibição do crescimento fúngico. Este estudo teve como objetivo avaliar a atividade antifúngica do extrato floral hidroetanólico de *Cattleya walkeriana* sobre cepas de *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, *C. gloeosporioides* e do gênero *Aspergillus* (*A. flavus*, *A. niger* e *A. fumigatus*) em diferentes concentrações, por meio de ensaios *in vitro*. O extrato foi preparado com solução hidroetanólica a 70% (*v/v*) e testado em placas de *Petri* contendo meio BDA (batata-dextrose-ágar) e um inóculo fúngico. Os resultados foram expressos em milímetros (mm) de inibição do crescimento fúngico (antibiose), nas concentrações de 100%, 80%, 50%, 10% e 1,25% (*v/v*). O estudo demonstrou que as maiores concentrações do extrato floral de *Cattleya walkeriana* (80% e 100%) apresentaram forte ação antifúngica sobre as cepas testadas.

Palavras-chave: gênero *Cattleya*, *Colletotrichum acutatum*, *Aspergillus niger*, fitoquímica, antibiose.

1. Introduction

The Orchidaceae family stands out and captivates among higher plant genera with its majestic beauty. Most orchid species are epiphytic plants, exhibiting remarkable diversity in stem and leaf size and shape and the color and form of their flowers (Schneiders et al., 2012). Orchidaceae is one of the largest families among angiosperms, comprising approximately 20,000 species (excluding artificial hybrids), distributed across about 850 genera (Nardelli et al., 2020).

The genus *Cattleya* is distributed from Mexico through Central America, along the foothills of the Andes, with a high concentration of species in Colombia, Venezuela, and Brazil. In Brazil, the main native species is *Cattleya walkeriana* Gardner, found predominantly in the Cerrado domain, with occurrence extending to the Amazon region, the Pantanal biome, and the Atlantic Forest biome, as described by Barros et al. (2010), Miller & Warren (1996), and Faria et al. (2002).

According to Houch & Mendes (1999), *C. walkeriana* exhibits a notable distribution in habitats such as rocks, trees, and areas near lakes, rivers, or swamps, and in our study, it was found in the leaf litter (serrapilheira). This species is relatively small for the genus *Cattleya*, reaching up to 15 cm in height. It belongs to the unifoliate group, with pseudobulbs rarely bearing two leaves. Native to South America, *C. walkeriana* is primarily distributed across Brazilian forests within the Cerrado domain. Its flowering period occurs between January and May, producing aromatic flowers in lilac hues (Silva; Milaneze-Gutierre, 2004).

Few studies have evaluated the potential of *C. walkeriana* as a source of bioactive phytochemicals with medical or biotechnological interest. In one of the few available investigations, Menezes Filho & Castro (2019) reported the presence of secondary metabolites in the leaf tissues of *C. walkeriana*, including organic acids, reducing sugars, alkaloids, flavonoids, saponins, coumarins, cardiac glycosides, condensed tannins, depsides, depsidones, and olefinic diphenols.

Regarding its antifungal potential, the floral extracts of *C. walkeriana* still lack robust scientific evidence. However, Menezes Filho et al. (2021) evaluated the antifungal effect of extracts from another orchid species, *Spathoglottis unguiculata*, against the phytopathogens *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, and *Colletotrichum gloeosporioides*, observing promising results. Similarly, Irimescu et al. (2020) investigated the antifungal and antibacterial activity of methanolic extracts from the leaves, stems, and roots of *Phalaenopsis* against microorganisms such as *Candida* spp. and *Staphylococcus* spp. Despite these advances, information on the phytochemical and biological effects of orchid floral organs remains scarce, highlighting the need for further studies focused on this plant structure.

In this context, the present study aimed to evaluate the antifungal activity of the floral extract of *Cattleya walkeriana* against *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, *C. gloeosporioides*, *Aspergillus flavus*, *A. niger*, and *A. fumigatus*.

2. Materials and Methods

2.1 Collection site

Flowers (300 g) of *C. walkeriana* were collected during the daytime, between 8:00 and 11:00 a.m., from April to May 2024, in a permanent preservation area located in the municipality of Santo Antônio da Barra, Goiás, Brazil, at the following coordinates: (17º31’17.5’’S and 50º42’02.3’’W) and (17º30’44.5’’S and 50º41’26.2’’W). A voucher specimen was deposited at the Laboratory of Botany and Plant Systematics of the UniBRAS University Center in Rio Verde under the voucher number RVUBRAS: 1005.

2.2 Preparation of the floral extract

A 70% (*v/v*) hydroethanolic floral extract was prepared by macerating 150 g of flowers in 200 mL of solvent. The material was homogenized until a liquid paste was obtained. The extract was then transferred to an amber glass bottle and kept at rest for 72 h, following the protocol described by Garcia et al. (2012). After this period, the extract was filtered using quantitative blue band filter paper (Unifil, C-42, Brazil). The resulting supernatant was collected and stored in an amber bottle under refrigeration at 4 °C until further analyses. The crude extract was considered as the 100% concentration, and all subsequent dilutions were prepared proportionally based on this reference.

2.3 Antifungal assay

The fungicidal assay was performed according to the method described by Menezes Filho et al. (2021), with adaptations described below. Fungal isolates of *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, *Aspegillus flavus*, *Aspergillus niger*, and *Aspergillus fumigatus* were used, all obtained from the agricultural mycological collection of the Microbiology Laboratory at the UniBRAS University Center in Rio Verde, Goiás, Brazil.

The *S. sclerotiorum* strain was isolated from sclerotia collected from soil in soybean cultivation fields;

Colletotrichum strains were obtained from papaya and strawberry crops; and *Aspergillus* strains were isolated from sugarcane bagasse. For the antifungal assay, 10 cm diameter *Petri* dishes were used. Potato dextrose agar (PDA; Kasvi, India) was the nutrient medium. Initially, the medium was autoclaved at 121 °C and 1 atm for 25 minutes. Then, the medium was poured into Petri dishes under sterile conditions in a laminar flow cabinet. After solidification, different concentrations of the ethanolic floral extract of *C. walkeriana* (100%, 80%, 50%, 10%, and 1.25%, *v/v*) were added and evenly spread using a *Drigalski* spatula.

Afterwards, a 7 mm diameter mycelial disc was transferred to the center of each *Petri* dish. A 70% hydroethanolic solution without floral extract was used as a negative control. As positive controls, the commercial fungicide Frowncide 500 SC at 10 μg mL^{-1} and Amphotericin B at 20 μg mL^{-1} were used.

The plates were incubated at 20 °C (*S. sclerotiorum*), 25 °C (*C. acutatum* and *C. gloeosporioides*), and 28 °C (*A. flavus*, *A. niger* and *A. fumigatus*), respectively. The antifungal activity evaluation was concluded when the control plates exhibited complete fungal growth.

Based on the results, the percentage of growth inhibition (PGI) was calculated according to Equation 1:

$$PGI\% = ((dtt - dtq) / dtt) \times 100 \qquad \text{Eq. (1)}$$

where:

dtt = diameter in the control treatment,

dtq = diameter in the chemical treatment.

For the measurement of the fungal inhibition halo, a digital caliper (Matrix, Model MTX-316119, China) with a 150 mm capacity and a measurement error of 0.03 mm was used.

2.4 Statistical analysis

For the antifungal assay, the experiment was conducted in quadruplicate, and results were expressed as mean ± standard deviation. Statistical analysis was performed using the *Scott-Knott* test at a 5% significance level. The statistical software used was SIVAR (Ferreira, 2019).

3. Results

3.1 Antifungal activity

Table 1 presents the results of the antifungal activity of the floral extract of *C. walkeriana* against fungal isolates of *S. sclerotiorum*, *C. acutatum*, and *C. gloeosporioides*. Strong inhibition was observed at the highest concentrations tested (80% and 100%), although it was lower than the standard commercial fungicide Frowncide 500 SC. Inhibition zones of 31 mm, 22 mm, and 32 mm were recorded for *S. sclerotiorum*, *C. acutatum*, and *C. gloeosporioides*.

Table 1. Antifungal activity of the hydroethanolic floral extract of *Cattleya walkeriana* against *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, and *Colletotrichum gloeosporioides*.

Microrganisms	*Cattleya walkeriana* floral extract					Frowncide 500 SC	CV (%)
	1.25%	10%	50%	80%	100%		
S. sclerotiorum	1.25 f	6.90 e	12.30 d	18.47 c	31.12 b	100.00 a	3.13
C. acutatum	0.00 e	0.00 e	7.02 d	19.40 c	22.47 b	100.00 a	3.94
C. gloeosporioides	0.00 f	6.42 e	13.15 d	28.27 c	32.60 b	100.00 a	5.36

Note: Means followed by standard deviation (SD) and the same letters do not differ statistically according to the *Scott-Knott* test at a 5% significance level. Results expressed in millimeters (mm) of fungal inhibition. Source: Authors, 2025.

Table 2 presents the results of the antifungal activity of the floral extract of *C. walkeriana* at different concentrations against strains of *A. flavus*, *A. niger*, and *A. fumigatus*, compared to the standard commercial antifungal Amphotericin B. Promising results were observed only at the highest concentration tested (100%), with inhibition zones of 13 mm for A. flavus, 13 mm for A. niger, and 18 mm for A. fumigatus.

Table 2. Antifungal activity of the hydroethanolic floral extract of *Cattleya walkeriana* against *Sclerotinia sclerotiorum*, *Colletotrichum acutatum*, and *Colletotrichum gloeosporioides*.

Microrganisms	*Cattleya walkeriana* floral extract					Anphotericin B	CV (%)
	1.25%	10%	50%	80%	100%		
A. flavus	0.00 d	0.00 d	0.00 d	6.40 c	13.25 b	100.00 a	4.91
A. niger	0.00 d	0.00 d	0.00 d	6.37 c	13.85 b	100.00 a	3.01
A. fumigatus	0.00 e	0.00 e	5.35 d	11.57 c	18.35 b	100.00 a	4.96

Note: Means followed by standard deviation (SD) and the same letters do not differ statistically according to the *Scott-Knott* test at a 5% significance level. Results expressed in millimeters (mm) of fungal inhibition. Source: Authors, 2025.

4. Discussion

The use of plant extracts has been widely investigated for their biological activity, particularly regarding the inhibition of various fungal groups, many of which are responsible for diseases in humans, animals, and agriculturally important plants. *Sclerotinia* species, for instance, cause stem rot in soybean crops, significantly impairing the yield of this economically important grain. Fungi from the *Colletotrichum* genus are major agents of anthracnose in fruit crops such as strawberry, avocado, passion fruit, and papaya, leading to considerable annual losses. Moreover, several *Aspergillus* species produce highly toxic mycotoxins, many of which are carcinogenic and potentially lethal to both humans and animals.

Some studies have reported the antifungal activity of extracts from orchid species such as *Spathoglottis* (Menezes Filho et al., 2021) and *Phalaenopsis* (Irimescu et al., 2020). Our findings show that Sclerotinia and *Colletotrichum* strains exhibit sensitivity to the floral extract of *C. walkeriana*, especially at the highest concentrations tested. In contrast, the *Aspergillus* strains demonstrated greater resistance, even at elevated concentrations (80% and 100%).

Menezes Filho et al. (2021) reported up to 31% inhibition for *S. sclerotiorum*, 10% for *C. acutatum*, and 12% for *C. gloeosporioides* using the hydroethanolic floral extract of *S. unguiculata*. Irimescu et al. (2020) found satisfactory antifungal activity against *Candida* species (*C. albicans*, *C. guilliermondii*, *C. parapsilosis*, and *C. krusei*) and antibacterial activity against *Staphylococcus aureus* and *S. epidermidis* using stem and root extracts of *Phalaenopsis* orchids.

Various phytochemical groups from the secondary metabolism of plants contain organic molecules with antimicrobial properties, capable of inducing sensitivity in different microorganisms such as fungi, bacteria, viruses, and protozoa. These various biological activities may be associated with studies that have reported the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids in orchid extracts (Kumari et al., 2017; Saikia; Thakur, 2024). The results of our study may suggest a promising new alternative to replace the use of synthetic antifungal drugs or agricultural products.

5. Conclusions

The floral extract of *Cattleya walkeriana* exhibited potential antifungal activity at the highest concentrations tested (100% and 80%), inhibiting the growth of *Colletotrichum acutatum*, *Colletotrichum gloeosporioides*, and *Aspergillus flavus*.

6. Authors' Contributions

Carlos Alberto Carvalho Moreira: conceptualization, methodology, investigation, data curation, writing – original draft. *Thiago Henrique Lopes Barros*: writing – original draft, investigation, methodology, formal analysis. *Elizabete Nunes da Rocha*: conceptualization, writing – original draft, writing – review & editing.

Matheus Vinícius Abadia Ventura: formal analysis, supervision (co-supervision). *Antonio Carlos Pereira de Menezes Filho*: conceptualization, methodology, investigation, resources, writing – review & editing, supervision, project administration, validation, visualization.

7. Conflicts of Interest

No conflicts of interest.

8. Ethics Approval

Not applicable.

9. References

Barros, F., Vinhos, F., Rodrigues, V. T., Barberene, F. F. V. A., & Fraga, C. N. (2010). Orchidaceae: Lista de Espécies da Flora do Brasil. Jardim Botânico do Rio de Janeiro. 2010. Available on <http://floradobrasil.jbrj.gov.br/2010/FB000179>. Accessed on June 10, 2025.

Faria, R. T., Santiago, D. C., Saridakis, D. P., Albino, U. B., & Araújo, R. (2002). Preservation of the brazilian orchid *Cattleya walkeriana* Gardner using in vitro propagation. *Crop Breeding and Applied Biotechnology*, 2(3), 489-492.

Ferreira, D. F. (2019). SISVAR: A computer analysis system to fixed effects split plot type designs. *Brazilian Journal of Biometrics*, 37(4), 529-535. https://doi.org/10.28951/rbb.v37i4.450

Garcia, R. À., Juliatti, F. C., Barbosa, A. G., & Cassemiro, T. A. (2012). Atividade antifúngica de óleo e extratos vegetais sobre Sclerotinia sclerotiorum. *Bioscience Journal*, 28(1), 48-57. https://seer.ufu.br/index.php/biosciencejournal/article/view/8174

Houch, P. R., & Mendes, M. A. (1999). *Cattleya walkeriana*. *O Mundo das Orquídeas*, 2, 13-15.

Irimescu, L-S., Diguţă, C. F., Encea, R. Ş., & Matei, F. (2020). Preliminary study on the antimicrobial potential of *Phalaenopsis* orchids methanolic extracts. *Scientific Bulletin, Series F. Biotechnologies*, XXIV(2), 149-153.

Kumari, R., Shakula, K., & Debasish, S. (2017). Extraction and screening of bioactive compounds of some common hydrophytic and wetland plants from East Singbhum. *IOSR Journal of Pharmacy*, 7(11), 23-29.

Menezes Filho, A. C. P., Castro, C. F. S., Silva, A. P., & Cruz, R. M. (2021). Avaliação físico-química, fitoquímica e atividades biológicas do extrato hidroetanólico floral de *Spathoglotis unguiculata* (Labill.) Rchb. f. (Orchidaceae). *Arquivos Científicos*, 4(1), 79-87. https://arqcientificosimmes.emnuvens.com.br/abi/article/view/509

Menezes Filho, A. C. P., & Castro, C. F. S. (2019). Análise fitoquímica preliminar de extratos foliares de orchidaceas (*Cattleya walkeriana* Gardner), (*Oncidium cebolleta* Sw.), (*Encyclia linearifolioides* Kraenzl.) e (*Polystachya concreta* (Jacq.) Garay & H. R. Sweet. *Revista Ensaios e Ciência*, 23(10, 16-23. https://doi.org/10.17921/1415-6938.2019v23n1p16-23

Miller, D., & Warren, R. (1996). O gênero *Catleya* Lindl. p. 256. *In*: Orquídeas do Alto da Serra. Lis, São Paulo.

Nardelli, M. S., Viana, A., Montiel, C. B., Kuhn, S. B., Liesenfeld, V., & Fortes, A. M. T. Desenvolvimento de plântulas de *Cattleya walkeriana* Gardner em diferentes meios de cultura. *Journal of Agronomic Sciences*, 9(1), 61-72.

Saikia, J., & Thakur, D. (2024). A review on endophytic bacteria of orchids: functional roles toward synthesis of bioactive metabolites for plant growth promotion and disease biocontrol. *Planta*, 260. https://doi.org/10.1007/s00425-024-04501-3

Silva, C. I., & Milaneze-Gutierre, M. A. (2004). Caracterização morfo-anatômica dos órgãos vegetativos de Cattleya walkeriana Gardner (Orchidaceae). *Acta Scientiarum. Biological Sciences*, 26(1), 91-100.

Schneiders, D., Pescador, R., Booz, M. R., & Suzuki, R. M. (2012). Germinação crescimento e desenvolvimento *in vitro* de orquídeas (*Cattleya* spp, Orchidaceae). *Revista Ceres*, 59(2), 185-191.

Funding

Not applicable.

Institutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.