

Agricultural fungicidal effect of floral extracts of *Bauhinia variegata*, *B. forficata* and *B. purpurea*

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Abstract

Bauhinia is a botanical genus widely distributed in parts of Asia and South America. The plant bears beautiful and aromatic flowers that add ornamental beauty to parks and gardens, in addition to being used in medicine and *ayurveda*. This study aimed to evaluate the floral ethanolic extract of *B. variegata*, *B. forficata* and *B. purpurea* in fungal inhibition against *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*. Flowers were harvested in 2021 and 2022. The floral ethanolic extract was produced by the static maceration method. The antifungal activity was performed by the agar diffusion method at different extract concentrations. *Bauhinia* floral extracts showed a potential fungicidal effect on fungal species evaluated in particular for *Sclerotinia sclerotiorum*, followed by *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*.

Keywords: *Bauhinia* genus, *Colletotrichum*, *Sclerotinia*, antifungal activity, inhibition concentration of floral extract.

Efeito fungicida agrícola dos extratos florais de *Bauhinia variegata*, *B. forficata* e *B. purpurea*

Resumo

Bauhinia é um gênero botânico amplamente distribuído na Ásia e na América do Sul. Apresenta belas e aromáticas flores sendo utilizadas na arborização de parques e jardins, além de serem empregadas na medicina e ayurveda. Este estudo teve por objetivo, avaliar o extrato etanólico floral de *B. variegata*, *B. forficata* e *B. purpurea* quanto a capacidade de inibição fúngica sobre *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* e *Colletotrichum acutatum*. Flores foram colhidas em 2021 e 2022. O extrato etanólico floral foi produzido pelo método de maceração estática. A atividade antifúngica foi realizada pelo método de difusão em ágar em diferentes concentrações de extrato. Os extratos florais de *Bauhinia* demonstraram efeito fungicida sobre as espécies fúngicas avaliadas em especial para *Sclerotinia sclerotiorum*, seguido de *Colletotrichum gloeosporioides* e *Colletotrichum acutatum*.

Palavras-chave: gênero *Bauhinia*, *Colleotrichum*, *Sclerotinia*, atividade antifúngica, concentração de inibição do extrato floral.

1. Introduction

The pantropical botanical genus *Bauhinia* Plum. ex. L., with about 300 species, belongs to the Leguminosae family, Caesalpinioideae subfamily, Cercideae tribe (Vaz; Tozzi, 2003; Sobrinho et al., 2008), being popularly known as “*pata-de-vaca* in portuguese”, “cow's paw in English” or “*unha-de-boi* in Portuguese” and “ox's nail in English”. In Brazil, 98 species of *Bauhinia* are described being inserted in six sections: *Amaria* (S. Mutis) Endl.,

Bauhinia, *Benthamia* Fortunato & Wunderlin, *Caulotretus* DC., *Pauletia* (Cav.) DC. and *Schnella* (Raddi) Benth., corresponding to three of the four subgenera proposed by Wunderlin et al. (1987).

Bauhinia is known for its great use in pharmacology due to its anti-diabetic, analgesic, hypoglycemic, anti-inflammatory, anti-microbial properties as well as the effects on digestive disorders, rheumatism, sedation, among others (Silva; Filho, 2002). There are several phytochemical groups described for *Bauhinia* extracted from the special metabolism such as lactones, alcohols, polyalcohols, benzenoids, glycosides, stilbenoids, amino acids, chromans, fatty acids, flavonoids, terpenoids, steroids, triterpenes, tannins and quinones.

Several species of this genus are defended in scientific monographs which depicts important biological effects and activities, especially considering *B. guianensis*, *B. manca*, *B. candicans*, *B. pentandra*, *B. longifolia*, *B. divaricata*, *B. cheilantha*, *B. uruguayensis*, *B. bauhinioides*, *B. purpurea*, *B. forficata*, *B. monandra*, *B. holophylla*, *B. rufa* and *B. splendens* (Fuentes et al., 2004; Neuhoef et al., 2005; Menezes et al., 2007; dos Santos et al., 2014; Silva et al., 2015; Pinheiro et al., 2017). Among these exuberant amount of floristic species that are responsible for varied biological activities, we opted for *B. variegata*, *B. forficata* and *B. purpurea*.

Bauhinia variegata Linn. presents profuse branching with deciduous leaves measuring around 10 to 15 cm long that are rigidly subcoriaceous and deeply corded. The plant produces attractive bisexual, irregular flowers of light magenta color and the pods are long, hard, flat, dehiscent containing between 10 to 15 seeds, being widely found in India (Mali et al., 2007; Nunes et al., 2023). This species has a hepatoprotective, wound healing, anti-cancer, anti-diabetic, anthelmintic, insecticidal, anti-trogenic, antimicrobial, insecticidal, haemagglutination, antitumor, anti-arthritis, anti-ulcer, anti-trogenic, nephroprotective, anti-oxidant, anti-inflammatory, anti-tubercular (Mali et al., 2007; Dhale, 2011. Singh et al., 2019).

Bauhinia forficata Link is found in the Atlantic Forest biome, mixed rainforest, dense rainforest and seasonal deciduous forest as a medium-sized tree, reaching up to 8 m in height, deciduous, perennial, uncinatate leaves (bilobed), alternate, measuring between 8 and 9 cm in length, oval or lanceolate, glabrous, thorny, with white flowers (edible) *in natura*, being fleshy and sweet, and fruits of the linear pod type (Pizzolati et al., 2003). In particular, this species has a diuretic, hypoglycemic action, in the reduction of glycosuria, as a tonic, depurative and with activity in the control and treatment of elephantiasis. With fungicidal action, dos Santos et al. (2022) evaluated for the essential oil of *B. forficata* flowers an inhibiting action on the growth of fungi *Rhizopus microsporus*, *C. gloeosporioides* and *S. sclerotiorum*. In this species, the presence of free and glycosylated flavonoids, β -sitosterol and hanferol-3,7-diramnoside is reported (López; Santos, 2015).

And finally *B. purpurea* L., a large tree that stands tall up to 17 meters high, deciduous having leaves 7.5 to 15 cm long, the flowers are conspicuous, pink and fragrant consisting five petals and fruits contained in pods with around 12-15 suborbicular seeds which is found native to the regions of Southern China and Southeast Asia and is also widely distributed in India (Kumar; Chandrashekar, 2011). In medicine, *B. purpurea* has antinociceptive, analgesic, antipyretic, anti-diabetic, antioxidant, nephroprotective, wound healing, anti-diarrheal, anti-rheumatic, anthelmintic, carminative, anti-asthmatic, anti-inflammatory and leucorrhoea activities (Kumar; Chandrashekar, 2011). In *B. purpurea*, groups of special glycosides, flavonoids, saponins, triterpenoids, phenolic compounds, oxepins, fatty acids and phytosterols are described (Chaudhari et al., 2013).

It has been observed that these three species present a considerable range of biological activities and actions including antifungal properties. Although, little is known about the antifungal activity; mainly phytopathological fungi such as *Sclerotinia sclerotiorum* (Lib.) de Bary (white mold). This plant pathogen exhibits cosmopolitan life by infecting more than 400 plant species worldwide, including agricultural crops and numerous weeds. In agriculture, *S. sclerotiorum* is a serious threat to dicotyledonous species such as sunflower, soybean, rapeseed, bean, chickpea, peanut, dry pea and lentils among other crops. It does also harms monocotyledonous species such as onion and tulip causing substantial losses in millions of dollars per year (Boland; Hall, 1994; Bolton et al., 2006).

Another important group of phytopathogens belongs to the genus *Coleotrichum* with representativeness for *C. gloeosporioides* (Penz.) and *C. acutatum* J. H. Simmonds (anthracnose) that attack several fruits of commercial interest such as citrus, strawberry, peach, blue-berries, coffee, avocado, mango, olive, almond, yam, stylosanthes, passion-fruit, tamarillo, cacao, *Hevea* sp., and papaya (Wharton; Diéguez-Uribeondo, 2004; Weir et al., 2012), flowers, leaves, and twigs (Gautam, 2014).

As noted, the floral organ of *Bauhinia* has scarce works, therefore, this study aimed to evaluate the floral hydroethanolic extract of *Bauhinia variegata*, *Bauhinia forficata* and *Bauhinia purpurea* regarding the fungicidal activity on *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* *in vitro*.

2. Material and Methods

2.1 Species collection and identification

Flowers of *Bauhinia*, *B. variegata* (17°43'09.1" S and 50°53'06.7"W), *B. forficata* (17°48'09.1"S and 50°55'48.4"W) and *B. purpurea* (17°43'25.9"S and 50°52'55.7"W) were collected from a rural and an urban area in the municipality of Rio Verde in 2021 and 2022. The *Bauhinia* identification key used was described in the study by Vaz & Tozzi (2003).

2.2 Extract production

300g of flowers were collected for each species of *Bauhinia* evaluated. The ethanolic extract was produced by static maceration for 48 h using an aliquot of 250 g of flowers in a 200 mL solution of 98% P.A. After this period, the extract was filtered through qualitative filter paper and reduced in a rotary evaporator. The extract was then dehydrated in a lyophilizer and kept under refrigeration at -12 °C until analysis.

2.3 Antifungal analysis

The agar diffusion method was used to determine the antifungal activity on *S. sclerotiorum*, *C. gloeosporioides* and *C. acutatum* as described by Toigo et al. (2023) modified. The strains used are: SS12-21, CG 16-21 and CA15-67 respectively. The fungal strains were cultured at 20 °C for 13 days for *S. sclerotiorum* and 28 °C for 7 days for the other strains.

A mycelium disc 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 floral extracts concentrations were used, dissolved in 0.5% dimethylsulfoxide (DMSO) to render doses between 50-500 $\mu\text{L mL}^{-1}$, in each plate 500 μL of the concentration was pipetted. The plates were transferred to an incubator at 20 °C (15 days) and 28 °C (8 days), respectively.

The diameter of the zone of inhibition was measured and recorded as an indicator of antifungal activity and expressed in percentage (%). The commercial reference fungicide Frownicide 500 SC (Ishihara Sangyi Kaisha, LTD, Japan) (Registry in *Ministério da Agricultura, Pecuária e Abastecimento* (MAPA) the Brazil, n°. 07695) was used as a positive control (dose of 10 $\mu\text{L mL}^{-1}$). The agar diffusion assays applied against the three fungi were performed in quadruplicate. Mycelial growth was obtained daily until complete fungal growth separately on control plates. For measurement, a digital caliper was used.

2.4 Statistical analysis

The results were presented through the mean. *Tukey's* test was applied to assess significant differences between samples, with a significance level of 5%. The statistical program used was SISVAR.

3. Results and Discussion

It is known that medicinal and aromatic plants have molecules capable of reacting and inhibiting the growth of various forms of microorganisms including fungi and this ability comes from the phytochemicals present in them (Chopra et al., 1992; Gunalan et al., 2011).

Concentrations between 300-500 $\mu\text{L mL}^{-1}$ proved to be more efficient in mycelial inhibition among the three fungal species evaluated (Table 1). Noted, that *C. gloeosporioides* is sensitive to the phytochemical composition of the floral extract of *B. variegata*. We suggest that in future studies the extract should be analyzed to verify its composition and quantification so that we can better understand this biological activity on this phytopathogen. The fungi *S. sclerotiorum* and *C. acutatum* proved to be resistant where there was inhibition only between concentrations 300-500 $\mu\text{L mL}^{-1}$.

Studies show positive responses with important inhibition rates in several fungal groups and among these in phytopathogens. Gunalan et al. (2011) found that the *in vitro* ethanolic extract of the leaf of *B. variegata* expressed potential fungal inhibition activity for *T. rubrum* 25%, *T. mentagrophytes* 20%, *Aspergillus niger* 40.90%, *F. oxysporum* 20%, *Candida albicans* 20% and *Mucor hiemalis* 9.83%.

Table 1. Antifungal activity against *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* by the floral extract of *Bauhinia variegata*.

Strains	<i>B. variegata</i> floral extract – Concentrations in $\mu\text{L mL}^{-1}$ (%)					
	50	100	200	300	400	500
<i>S. sclerotiorum</i>	0.00 ^e	0.00 ^e	0.00 ^e	11.54 ^{cd}	14.68 ^c	20.01 ^b
<i>C. gloeosporioides</i>	0.00 ^g	13.41 ^f	21.11 ^e	27.03 ^d	39.88 ^{cb}	41.94 ^b
<i>C. acutatum</i>	0.00 ^e	0.00 ^e	0.00 ^e	5.10 ^d	14.15 ^c	19.66 ^b

Note: Frowncide Fungicide 500 SC (100%)^a inhibition positive Control. Negative control DMSO (0%) inhibition. The same letter on the same line, does not present a significant difference according to the statistical test applied (Tukey's test with significance level 5%). Source: Authors, 2023.

Bauhinia forficata floral extract showed mild antifungal activity against *C. gloeosporioides*. The other fungal strains do not demonstrate growth inhibition (Table 2). In the study by dos Anjos et al. (2022), researchers obtained potential antifungal activity on *S. sclerotiorum*, *R. microsporus* and *C. gloeosporioides* by evaluating the essential oil of *B. forficata* flowers. As for the phytochemistry of *B. forficata*, Simões & Almeida (2015) evaluated the extract obtained from the stem of *B. forficata* regarding its phytochemical constitution, where they obtained positive results for flavonoids, phenols, tannins, depsides and depsidones, reducing sugars and anthraquinones, some of these groups being responsible for antifungal effects in several groups involved in pathologies in humans and animals, and phytopathologies in plants of commercial interest.

Comparing with *Bauhinia* species, Pawar & Nasreen (2016) evaluated the aqueous leaf extract of *B. racemosa* where they obtained for *Alternaria alternata* an average of 19%, for *Colletotrichum capsici* an average of 18%, and for *Phytophthora infestans* an average of 32% of mycelial inhibition. These authors also evaluated the phytochemistry of *B. racemosa* where they described the positive presence of tannins, alkaloids, saponins, terpenoids, glycosides, flavonoids and steroids.

Table 2. Antifungal activity against *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* by the floral extract of *Bauhinia forficata*.

Strains	<i>B. forficata</i> floral extract – Concentrations in $\mu\text{L mL}^{-1}$ (%)					
	50	100	200	300	400	500
<i>S. sclerotiorum</i>	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b
<i>C. gloeosporioides</i>	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c	16.11 ^b
<i>C. acutatum</i>	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b	0.00 ^b

Note: Frowncide Fungicide 500 SC (100%)^a inhibition positive Control. Negative control DMSO (0%) inhibition. The same letter on the same line, does not present a significant difference according to the statistical test applied (Tukey's test with significance level 5%). Source: Authors, 2023.

The different concentrations of *B. purpurea* floral extract do not demonstrate antifungal action against *C. gloeosporioides* in any of the concentrations. Slight activity was observed between concentrations 300-500 $\mu\text{L mL}^{-1}$ for *C. acutatum* and between 400-500 $\mu\text{L mL}^{-1}$ for *S. sclerotiorum* (Table 3). *Bauhinia purpurea* also demonstrated in the study developed by Boonphong et al. (2007) antifungal activity. *Bauhinia purpurea* also demonstrated antifungal activity in the study by Derbalah et al. (2012), these researchers verified that the leaf extract showed a strong inhibition action on *Sclerotium rolfsii* in concentrations varying between 50-200 ppm with inhibition activity between 5-75%.

Although our results were positive for most of the fungal strains tested, the *Bauhinia* floral extracts showed inferior results to the synthetic commercial fungicide Frowncide 500 SC. This same parameter is discussed by Pawar & Nasreen (2016) where the *B. racemosa* extract, even with promising results, showed a lower inhibition rate than the commercial fungicide Propiconazole. Although, little is known about the activities of *B. purpurea*, Negi et al. (2012) verified promising antibacterial activity for extracts obtained by different organic solvents. In addition, *B. purpurea* appears to be a species rich in phytochemicals such as lupeol, stigmaterol, lanosterol,

ergosterol, beta-tocopherol, phytol, hexadeconic acids, hexadeconic acids methyl esters, octadecadienoic acids and octadecatrienoic acid.

Table 3. Antifungal activity against *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* by the floral extract of *Bauhinia purpurea*.

Strains	<i>B. purpurea</i> floral extract – Concentrations in $\mu\text{L mL}^{-1}$ (%)					
	50	100	200	300	400	500
<i>S. sclerotiorum</i>	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c	5.3 ^c	11.57 ^b
<i>C. gloeosporioides</i>	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^b
<i>C. acutatum</i>	0.00 ^d	0.00 ^d	0.00 ^d	9.22 ^c	14.57 ^b	16.09 ^b

Note: Frownicide Fungicide 500 SC (100%)a inhibition positive Control. Negative control DMSO (0%) inhibition. The same letter on the same line, does not present a significant difference according to the statistical test applied (*Tukey's* test with significance level 5%). Source: Authors, 2023.

Our data show for *B. variegata*, *B. forficata* and *B. purpurea* new directions on the knowledge of the biological activities in which the floral extracts present for the *Bauhinia* group in particular for fungicidal activity on three important phytopathological fungi that cause great agricultural losses every years.

4. Conclusions

The floral extracts of *Bauhinia variegata*, *Bauhinia forficata* and *Bauhinia purpurea* prove to be good options for replacing synthetic agrochemicals on the phytopathological fungi *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* *in vitro*. New studies should be carried out evaluating the antifungal activity in the greenhouse and in the field for a better comparison between these three variables.

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6. Author contributions

Charles Henrique de Marques Lima: study design, collection of fungi, production of floral extracts, antifungal analysis, writing of the study and publication. *Porshia Sharma*: guest researcher, translation corrections and scientific analysis. *Matheus Vinícius Abadia Ventura*: technical and scientific coordination. *Antonio Carlos Pereira de Menezes Filho*: identification of plant species, production of floral extract, antifungal analysis, scientific writing, translation and submission. *Carlos Frederico de Souza Castro*: funds for the acquisition of equipment, glassware, reagents and laboratory analysis. *Elizabeth Nunes da Rocha*: advisor and scientific corrections.

7. Conflicts of interest

There are no conflicts of interest.

8. Ethical approval

Not applicable.

9. References

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