

NOTA TÉCNICA

**Physicochemical evaluation and antioxidant and antifungal activities of essential oils from *Bauhinia forficata* Link. and *Bauhinia variegata* L. flowers**

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**Abstract:** The genus *Bauhinia* has about 300 identified species, with *Bauhinia forficata* and *Bauhinia variegata* introduced in Brazil in landscape projects, due to its lush aromatic flowers. The essential oil of the flower *Bauhinia forficata* and *Bauhinia variegata* showed low extraction yields equal to 0.0469 and 0.601%. In the gas chromatography analysis coupled to the mass spectrometer (GC-MS), 4 major compounds were found: germacrene 37.21 and 21.09%,  $\alpha$ -pinene 2.11 and 4.34%,  $\gamma$ -elemene 4.97 and 2.34% and silvestrene 8.93 and 9.54%, respectively for *Bauhinia forficata* and *Bauhinia variegata*. Two major compounds germacrene D 21.09% and limonene 8.35% were found only in the essential oil of the flower *Bauhinia variegata*. The percentage of mycelial inhibition *in vitro* demonstrated greater sensitivity for the strains of *S. sclerotiorum* and *C. gloeosporioides*, in contrast to that observed in *C. acutatum*, which showed to be more resistant to the concentrations of essential oil of the flower of *B. forficata*. The essential oil of the flower of *Bauhinia variegata*, presented low sensitivity for *Sclerotinia sclerotiorum* and *C. acutatum*, and high inhibition efficiency for *C. gloeosporioides*. Further work should be carried out evaluating the essential oils of the flowers of *Bauhinia forficata* and *Bauhinia variegata* in greenhouse and in the field by sprinkling or encapsulated micro and nano, to evaluate the behavior of both essential oils with the results *in vitro*.

**Keywords:** *Bauhinia forficata*, *Bauhinia variegata*, antifungal activity, *Sclerotinia*, *Colletotrichum*.

**Avaliação físico-química e atividades antioxidante e antifúngica dos óleos essenciais das flores de *Bauhinia forficata* Link. e *Bauhinia variegata* L.**

**Resumo:** O gênero *Bauhinia* apresenta cerca de 300 espécies identificadas, sendo a *Bauhinia forficata* e *Bauhinia variegata* introduzida no Brasil em projetos paisagísticos, devido as suas exuberantes flores aromáticas. O óleo essencial da flor de *Bauhinia forficata* e *Bauhinia variegata* apresentou baixo rendimento de extração igual a 0.0469 e 0,601%. Na análise por cromatografia gasosa acoplado ao espectrômetro de massas (GC-EM) foram encontrados 4 compostos majoritários germacreno 37,21 e 21,09%,  $\alpha$ -pineno 2,11 e 4,34%,  $\gamma$ -elemeno 4,97 e 2,34% e silvestreno 8,93 e 9,54%, respectivamente para *Bauhinia forficata* e *Bauhinia variegata*. Dois compostos majoritários germacreno D 21,09% e limoneno 8,35% foram encontrados apenas no óleo essencial da flor de *Bauhinia variegata*. A porcentagem de inibição micelial *in vitro* demonstrou maior sensibilidade para as cepas de *S. sclerotiorum* e *C. gloeosporioides*, ao contrário do observado em *C. acutatum* que apresentou ser mais resistente as concentrações de óleo essencial da flor de *B. forficata*. O óleo essencial da flor de *Bauhinia variegata*, apresentou baixa sensibilidade para *Sclerotinia sclerotiorum* e *C. acutatum*, e alta eficiência de inibição para *C. gloeosporioides*. Outros trabalhos deverão ser realizados avaliando os óleos essenciais das flores de *Bauhinia forficata* e *Bauhinia variegata* em casa-de-vegetação e no campo por aspersão ou micro e nano encapsulados, para avaliação do comportamento de ambos os óleos essenciais com os resultados *in vitro*.

**Palavras-chave:** *Bauhinia forficata*, *Bauhinia variegata*, atividade antifúngica, *Sclerotinia*, *Colletotrichum*.

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## INTRODUCTION

The genus *Bauhinia* has about 300 species. In Brazil, rich varieties of these vegetables are found in practically all biomes and Cerrado domain. The genus *Bauhinia* is included in herbal medicine, presenting species with biological actions, and especially in antifungal activity (De Lacerda et al., 2016; Vaz, 1979). The species *B. forficata* Link. subsp. *forficata* and *B. variegata* L., were introduced in Brazil at first to ornament avenues, streets, squares and parks, due to their arboreal size, presenting beautiful and aromatic white and lilac flowers arranged in axillary racemes (Nogueira; Sabino, 2012).

*B. forficata* is one of the species known as “*oxfoot, morotó, cow's hoof* or *unha de boi*” belongs to the Fabaceae family. The species is arboreal or shrubby, with beautiful and aromatic flowers (Farias et al., 2018). The leaves, barks and roots of *B. forficata* are used by the population as a phytotherapeutic species, being used to relieve coughs, colds, with calming and renal action in the treatment of urinary infections (Farias et al., 2018; Ostrosky et al., 2008). The leaf extracts showed antibacterial activity in studies for *A. baumannii*, *S. aureus* and *E. faecalis* (Farias et al., 2018), and candidacidal (De Oliveira; Lima, 2017). The compound kaempferitrin, the major flavonoid present in the *B. forficata* leaves, appears to be the main active principle, with different kind of medicinal properties, including antidiabetic potential (Filho, 2018).

*B. variegata* is a species also known as “*pata-de-vaca* or *unha-de-vaca*” of medium arboreal size, presenting aromatic flowers in lilac color. The bark of the tree is used as an astringent, tonic, in the treatment of ulcers, anti-inflammatory, antibacterial, antioxidant, anticancer and hepatoprotective activities. It also has antibacterial action proven in studies evaluating plant extracts against *B. cereus*, *S. aureus*, *E. coli* and *P. pseudoalcaligenes* (Parekh et al., 2006). Countless chemical classes of natural compounds have already been identified in *B. variegata*, as glycosides flavanones extracted from seeds and roots (5,7 dihydroxy and 5,7 dimethoxy flavanone-4-*O*- $\alpha$ -L rhamnopyrosyl- $\beta$ -D-glycopyranosides, Kaempferol-3-glucoside, lupeol, and betasitosterol. Seeds contain protein, fatty oil-containing oleic acid, linoleic acid, palmitic acid, and stearic acid. Flowers contain cyanidin, malvidin, peonidin, and kaempferol. Root contains flavanol glycosides) (Mishra et al., 2013; Rajani; Ashok, 2009; Bodakhe; Ram, 2007; Zhao et al., 2005; Yadava; Reddy, 2001).

Essential oils are produced by plants through secondary metabolism, presenting biological, agricultural, food and pharmaceutical actions. The synergism between the

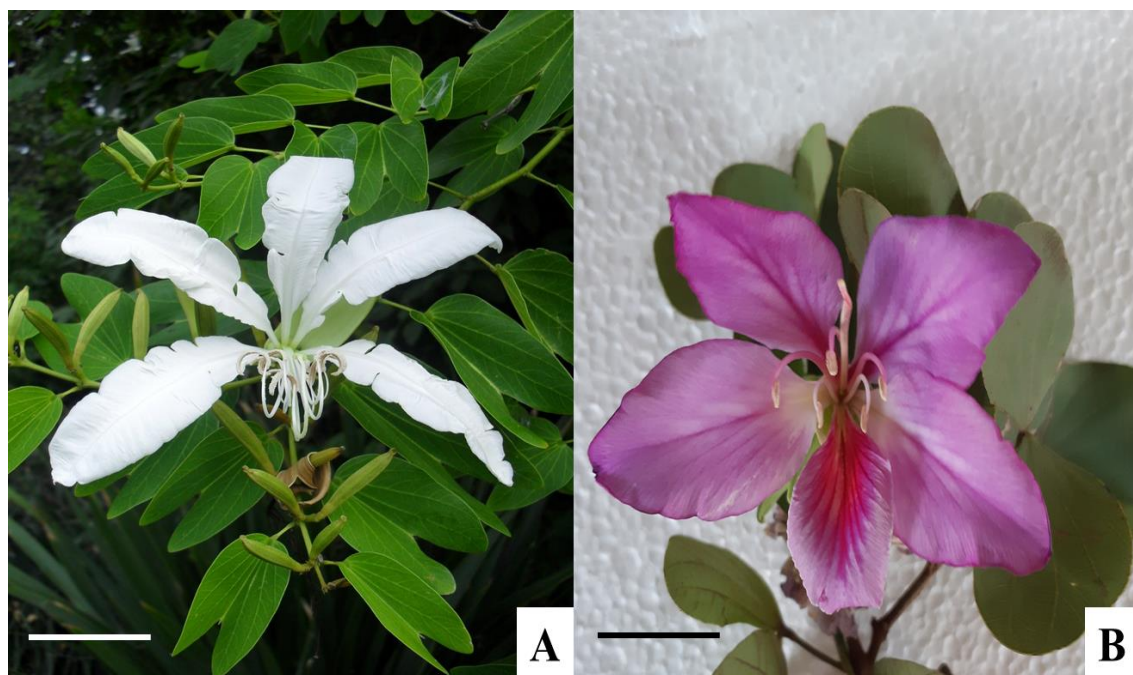
compounds that form the essential oil has high efficiency as a natural antifungal and antioxidant agent being used in different processes (Xavier et al., 2016). Free radicals induce oxidative damage to various biomolecules including proteins, lipids, lipoproteins, and DNA (Rajani; Ashok, 2009; Gopinathan et al., 2004; Shetgiri; D'Mello, 2003). Several fungi have phytopathological action, causing large losses in the production of grains, fruits and vegetables, such as *S. sclerotiorum* (Lib.) de Bary, *C. gloeosporioides* Penz and *C. acutatum*, which annually cause serious agricultural losses even in the harvest, transport and storage, generating major economic problems (Silva et al., 2018; Ramos et al., 2016; Wu et al., 2016).

In this context, the present investigation was carried out to evaluate the physicochemical and antioxidant and antifungal activities by the essential oil of the flower of *Bauhinia forficata* and *Bauhinia variegata* collected in two regions in the state of Goiás, Brazil.

## MATERIALS AND METHODS

### *Collection of flowers*

The flowers were collected from individuals of *Bauhinia forficata* and *Bauhinia variegata* at night in the months of October and November 2019 in municipalities of Iporá and Jataí, GO, Brazil, with the following geographical coordinates: 16°25'48.8''S 51°06'45.3''W and 17°53'40.7''S 51°43'41.9''W, respectively. In Figure 1, images of *Bauhinia forficata* and *Bauhinia variegata* flowers evaluated in this study are presented.



**Figure 1.** Flowers of *Bauhinia forficata* and *Bauhinia variegata*. In (A) *B. forficata* (Source: by Peter Symes, 2018), and in (B) *B. variegata* (Source: author, 2019). Bars: 15 cm.

### ***Extraction of essential oil***

A 500 g aliquot of flowers was processed with 500 mL of distilled water. The solution was transferred to Clevenger type reflux apparatus for 3 hours, as described by (Bezerra-Silva et al., 2014). The hydrolate was collected and washed 3 times with 30 mL dichloromethane (P.A – ACS) in a separatory funnel. The organic fraction was collected and dried with anhydrous sodium sulfate (P.A – ACS). The essential oil was weighed on an analytical balance, and the percentage yield determined, the according to equation 1, in analytical balance.

$$\% \text{ Yield} = [\text{Mass essential oil}/\text{Fresh vegetable mass (g)}]*100 \text{ Eq. [1]}$$

### ***Chemical profile of essential oil***

The identification of the main components of *B. forficata* and *B. variegata* flowers essential oil was obtained by gas chromatography with coupled mass detector (GC-MS) Thermo Scientific (Mod. Trace GC/MS Polaris). Individual components were identified by their mass spectra with the database, as well as comparing standard fragmentation with the literature (Adams, 2007) and Nist (11).

### ***Relative density of the essential oil***

The relative density of the essential oil was determined according to Alarcón et al. (2019). A 1 mL capacity pycnometer was used. The dry pycnometer was initially weighed on an analytical balance to obtain a constant weight at 25 °C. Then, the essential oil was added and weight was determined, according to equation 2.

$$\text{g mL}^{-1} = [(\text{Mass}_{\text{pycnometer}} + \text{sample}) - (\text{Mass}_{\text{pycnometer}})](\text{g})/\text{Essential oil volume (mL)} \text{ Eq. [2]}$$

### ***Antioxidant test***

The antioxidant activity was determined by the reduction of the free radical DPPH. Sample-hexane solutions (2 mL) prepared at 0.1 and 50 mg mL were added to 2 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution in hexane. After 60 min, the absorbance was measured at 517 nm. The blank was hexane and the control solution was prepared with 2 mL DPPH solution and 2 mL hexane. The antioxidant activity was performed in a 96 well microplate reader (Polaris, Mod. EE). The reduction percentage was calculated according to the equation 3.

$$((AC-AS)/AC)*100 \text{ Eq. [3]}$$

Where AS is the absorbance of the sample solution containing antioxidant and AC is the absorbance of control solution. IC<sub>50</sub> was defined as the amount of sample (μL mL) that produced a 50% decrease in the initial DPPH concentration. Lower IC<sub>50</sub> values indicate higher reducer activity, as described by Mezza et al. (2018).

### ***Antifungal test***

For antifungal activity, isolates of *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Colletotrichum acutatum* were used. Cultures were maintained in watery solution of PDA (Potato, Dextrose, Agar), belonging to the technological chemistry lab. The evaluation of the essential oil of *B. forficata* and *B. variegata* flowers started from concentrations of 100 (pure oil); 50; 25; 12.5; 6.25; 3.13 e 1.56 μL mL<sup>-1</sup> of essential oil diluted in dimethylsulfoxide (DMSO) (P.A – ACS). As a negative control, was used the control sample (absence of essential oil) and DMSO, and as a positive control the fungicide *Frownicide*<sup>®</sup> at a concentration of 10 μL mL<sup>-1</sup>. The concentrations of the essential oils were added to the PDA culture medium, as well as to the commercial fungicide and DMSO treatments with the *Drigalski* handle. The experiment was performed in quadruplicate, for each concentration, as well positive and negative controls.

The concentrations of the essential oil were added to the PDA culture medium as well as to the treatments with commercial fungicide *Frownicide*<sup>®</sup> and DMSO using a *Drigalski* handle. After addition of the concentrations and treatments, 1 mycelium disc of *S. sclerotiorum*, *C. gloeosporioides* and *C. acutatum* with 7 mm of diameter, was deposited separately in the center of the *Petri* dish. They were then incubated at the following temperatures 20, 23 and 25 °C respectively, as described by Garcia *et al.* (2012), Celoto *et al.* (2008) and Adaskaveg and Hartin (1997) adapted.

The evaluation consisted of daily colony diameter measurements by means of a digital caliper, 24 hours after the start of incubation and ended when the control treatment fungal colonies completely reached the internal area of the plate. The percentage of mycelial growth inhibition was determined according to the equation 3, proposed by Garcia *et al.* (2012).

$$\text{PIG\%} = (\text{CTD} - \text{CTD}^*)/\text{CTD}^*100 \text{ Eq. [3]}$$

Where PGI = Percentage Growth Inhibition, CTD = Control Treatment Diameter, CTD\* = Chemical Treatment Diameter.

### Statistical analysis

Statistical analysis consisted of triplicates for essential oil yield, and quadruplicate for antifungal assay, followed by ( $\pm$ ) standard deviation. Data were evaluated by *Student* and *Tukey* tests with significance level ( $p \leq 0.05$ ). The statistical software used was *PAST3* (free version).

## RESULTS AND DISCUSSION

Table 1 shows the physicochemical and bioactive results of the essential oils of the flowers of *B. forficata* and *B. variegata*.

**Table 1.** Physicochemical and bioactive parameters of the essential oils flowers of *Bauhinia forficata* and *Bauhinia variegata*.

Parameters	<i>Bauhinia forficata</i>	<i>Bauhinia porpurea</i>
Yield (%)	0.0469 $\pm$ 0.04 <sup>b</sup>	0.0601 $\pm$ 0.08 <sup>a</sup>
Density (g mL) 25 °C	0.903 $\pm$ 0.04	0.907 $\pm$ 0.09
Solubility EtOH 70% (v/v)	Positive	Positive
DPPH IC <sub>50</sub> ( $\mu$ L mL <sup>-1</sup> )	1.84 $\pm$ 0,09 <sup>b</sup>	1.91 $\pm$ 0,12 <sup>a</sup>

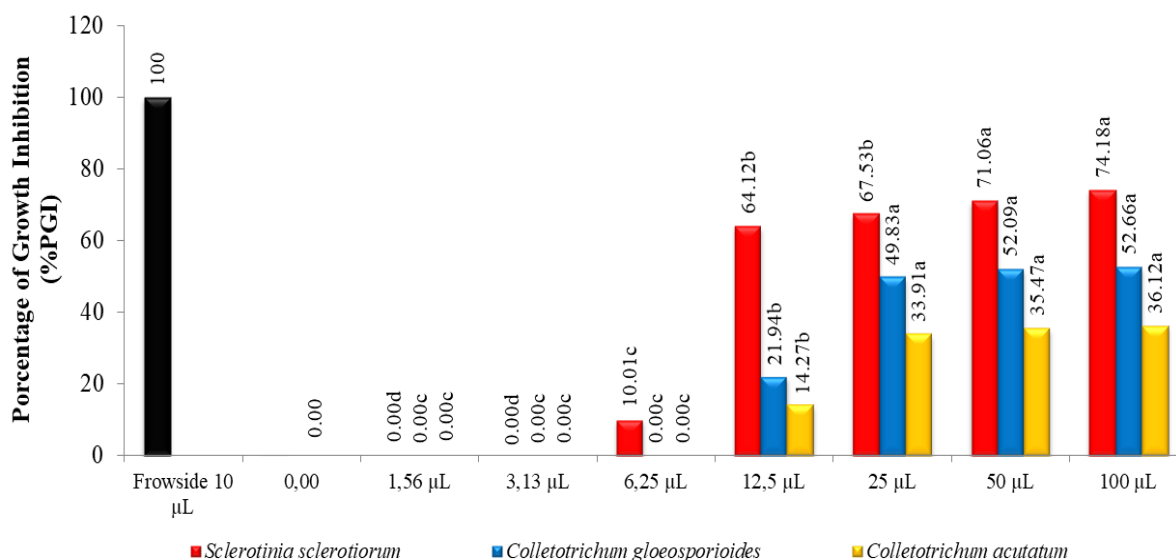
Values in average of triplicates followed by ( $\pm$ ) standard deviation. Different letters show significant difference by the Student test ( $p \leq 0.05$ ).

The essential oil yield of *B. forficata* and *B. variegata* flowers was 0.0469<sup>b</sup> and 0,0601<sup>a</sup>, the relative density of the oils was 0.903 and 0.907 g mL<sup>-1</sup>, respectively (Table 1). The solubility in 70% (v/v) aqueous ethanol solution was positive for both oils. Antioxidant activity was higher for *B. forficata* flower oil of IC<sub>50</sub> 1.84  $\mu$ L mL<sup>-1</sup>, followed by *B. variegata* flower oil of IC<sub>50</sub> 1.91  $\mu$ L mL<sup>-1</sup> (Table 1). Alarcón et al. (2019) evaluated the essential oil of *Eucalyptus globulus*, where they obtained results close to that of this study for solubility and relative density.

The major compounds for *B. forficata* and *B. variegata* were germacrene B (37.21; 21.09%),  $\alpha$ -pinene (2.11; 4.34%),  $\gamma$ -elemene (4.97; 2.34 %) and silvestrene (8.93; 9.54%), respectively. The major compounds germacrene D (21.09%) and limonene (8.35%) were only observed in the essential oil of *B. variegata*. Bezerra-Silva et al. (2014) evaluated the essential oil of *B. forficata* in three Headspace, Clevenger and Supercritical systems, where they found the following major compounds  $\alpha$ -pinene (36.56%) Headspace,  $\beta$ -pinene (11.38%) Hadspace,

*E*- $\beta$ -ocimene (9.03%) Headspace, germacrene B (15.20; 56.85 and 65.75%) Headspace, Clevenger and Supercritical, respectively.

Figure 2 shows the percentage of mycelial inhibition activity in *S. sclerotiorum*, *C. gloeosporioides* and *C. acutatum* by essential flower oil of *B. forficata*.



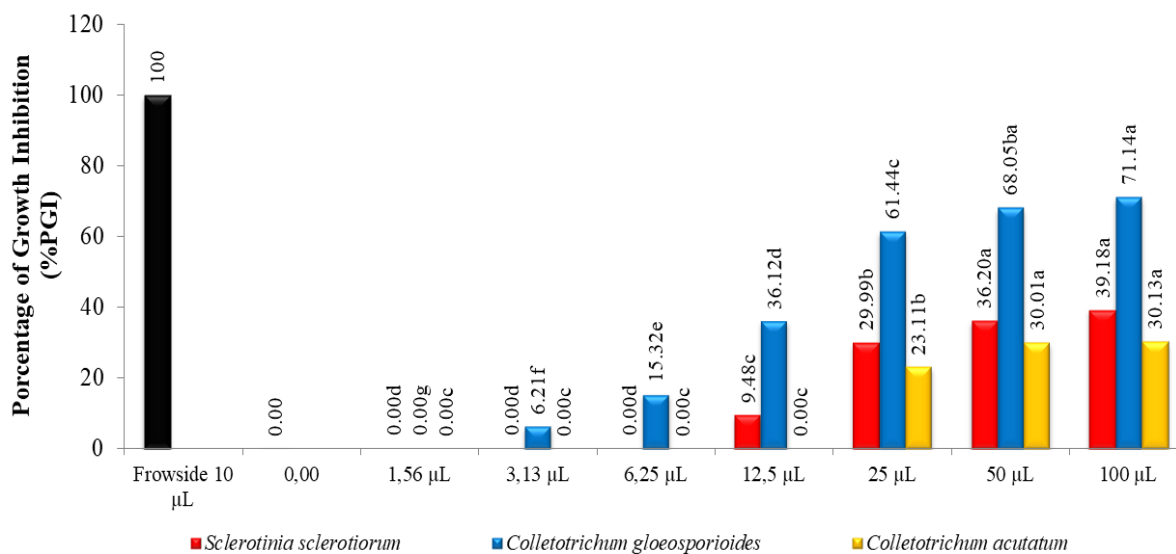
**Figure 2.** Growth Inhibition Percentage (%GIP) by *B. forficata* flower essential oil against *S. sclerotiorum*, *C. gloeosporioides* and *C. acutatum*. Different letters show statistical difference by the *Tukey* test ( $p \leq 0.05$ ).

For *S. sclerotiorum*, the formation of 4 groups with statistical difference by *Tukey* test is observed, with inhibition percentages between 64.12 to 74.18% in the highest concentrations 12.5; 25; 50 and 100  $\mu\text{L mL}^{-1}$ . For *C. gloeosporioides*, 3 groups with statistical difference by *Tukey* test, with higher inhibition rates between 21.94 and 52.66% at 12.5; 25; 50 and 100  $\mu\text{L mL}^{-1}$ . However, only in the highest concentrations were growth inhibitions observed. For *C. acutatum* there was the formation of 3 groups, with inhibition percentage between 33.91 to 36.12% in the highest concentrations of 25; 50 and 100  $\mu\text{L mL}^{-1}$ . However, concentrations 25; 50 and 100  $\mu\text{L mL}^{-1}$  showed no significant difference between concentrations by *Tukey* test (Figure 2).

The *S. sclerotiorum* and *C. gloeosporioides* presented higher sensitivity to essential oil concentrations of *B. forficata* flower. The same was not observed in *C. acutatum*, which presented higher mycelial resistance at usual concentrations of essential oil.

Figure 3 shows the percentage of mycelial inhibition activities in *S. sclerotiorum*, *C. gloeosporioides* and *C. acutatum* by essential flower oil of *B. variegata*.





**Figure 3.** Growth Inhibition Percentage (%GIP) by *B. variegata* flower essential oil against *S. sclerotiorum*, *C. gloeosporioides* and *C. acutatum*. Different letters show statistical difference by the Tukey test ( $p \leq 0.05$ ).

For *S. sclerotiorum*, the formation of 4 groups with statistical difference by Tukey test is observed, with inhibition percentages between 29.99 to 39.18% in the highest concentrations 25; 50 and 100  $\mu\text{L mL}^{-1}$ . For *C. gloeosporioides*, the formation of 6 groups with statistical difference by Tukey test, with higher inhibition rates between 36.12 to 71.14% at 12.5; 25; 50 and 100  $\mu\text{L mL}^{-1}$  (Figure 3). For *C. acutatum* there was the formation of 3 groups, with inhibition percentage between 30.01 to 30.13% in the highest concentrations of 50 and 100  $\mu\text{L mL}^{-1}$ . However, concentrations 50 and 100  $\mu\text{L mL}^{-1}$  showed no significant difference between concentrations by Tukey test.

This fungus showed less sensitivity to the essential oil the flower of *B. variegata*. The experiment with the three strains and the two essential oils was compared with the commercial fungicide of *Frowcide* at a concentration of 10  $\mu\text{L mL}^{-1}$ .

Some works in the literature mention good efficiency of essential oils in inhibiting mycelial growth of fungi for *S. sclerotiorum*, *C. gloeosporioides* and *C. acutatum*. Silva et al. (2018) evaluated the essential oil of *P. guajava* leaves in two harvest periods, where they obtained mycelial inhibition efficiency against *S. sclerotiorum*, in the highest concentrations 300 and 200  $\mu\text{L mL}^{-1}$ , with inhibition percentages between 94.9 to 90.0% and at the lowest concentration of 100  $\mu\text{L mL}^{-1}$  from 80.0 to 77.5%. Ramos et al. (2016) found an effective fungal inhibition capacity for *C. gloeosporioides* in essential oils of *melaleuca*, eucalyptus,



lemon, lemongrass, cloves, cinnamon, *nim*, mint, citronella, copaiba, coconut and ginger. Dias-Arieira et al. (2010), found mycelial growth inhibition activity for *C. acutatum* between 74.4 to 84.4% *nim* essential oil (*A. indica*), and between 35.6 to 91.1%, growth inhibition for the essential oil of *eucalyptus* (*E. citriodora*), both for concentrations of 0.25 to 1.50%.

## CONCLUSION

The essential oil of the flower of *Bauhinia forficata* and *B. variegata* showed low extraction yield. Both oils showed physicochemical results close to those observed in the literature for other essential oils, in particular, good results of antioxidant activity by the DPPH free radical reduction test were obtained. The chemical profile showed a total of four major compounds for the oil *B. forficata* and *B. variegata*. However, the *B. variegata* oil presented the compounds germacrene D and limonene. Essential oils have demonstrated good effectiveness as natural fungicidal agents, however, only in the highest concentrations. New experiments should be carried out to evaluate the antifungal activity in greenhouses and in the field, comparing the fungicidal action in vitro.

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