Chemical profile and antifungal activity of essential oil from the flower of *Bauhinia rufa* (Bong.) Steud.

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Abstract

Bauhinia rufa is one of the species found inhabiting the Cerrado domain. Annually this plant species presents flowering with a light sweet aroma. The objective of this work was to evaluate the chemical profile and antifungal activity of the essential oil of *B. rufa* flowers on *Sclerotinia sclerotiorum*, *Coletotrichum gloeosporioides* and *Aspergillus flavus*. Flowers were collected in the early hours of the morning, being sent to the laboratory for extraction and yield of essential oil by the Clevenger-type system. The chemical profile of the essential oil was obtained by gas chromatography with mass detector (GC-MS), and for antifungal activity, sequences of concentrations 100, 50, 25, 12.5; 6.25; 3.13 and 1.56 µL mL^{-1} on strains of *S. sclerotiorum*, *C. gloeosporioides* and *A. flavus*. The essential oil of *B. rufa* showed a yield of 0.05%, 28 compounds identified, the majority being Myrcene (8.27%), *O*-Cymene (31.14%), 1,2-dimethyl-4-ethyl-benzene (13.08%), *Trans*-Pinocarveol (12.55%) and Kryptone with (8.94%). The antifungal activity showed the best results for *S. sclerotiorum* and *C. gloeosporioides* where they exhibited fungistatic activity between 100 and 54.84% at all concentrations. For *A. flavus*, the best inhibition concentrations were observed between 100 to 25 μL^{-1} with 100%, and in the lowest concentrations of 12.5 and 6.25 μL^{-1} with 40.55 and 10.08%, no inhibition was observed at concentrations 3.13 and 1.56 μL^{-1}. The essential oil of *B. rufa* showed richness in chemical compounds and effective fungicidal action.

Keywords: *Sclerotinia sclerotiorum*, Antifungal activity, *Aspergillus flavus*, *Colletotrichum gloeosporioides*, *Bauhinia* genus

Resumo

Bauhinia rufa é uma das espécies encontradas habitando o domínio Cerrado. Anualmente esta espécie vegetal apresenta floração com aroma leve adocicado. O trabalho teve por objetivo avaliar o perfil químico e a atividade antifúngica do óleo essencial das flores de *B. rufa* sobre *Sclerotinia sclerotiorum*, *Coletotrichum gloeosporioides* e *Aspergillus flavus*. Flores foram coletadas nas primeiras horas da manhã, sendo encaminhadas ao laboratório para extração e rendimento do óleo essencial pelo sistema tipo Clevenger. O perfil químico do óleo essencial foi obtido por cromatografia gasosa com detector de massas (CG-MS), e para atividade antifúngica, foi realizado sequências de concentrações 100, 50, 25, 12,5; 6,25; 3,13 e 1,56 µL mL^{-1} sobre cepas de *S. sclerotiorum*, *C. gloeosporioides* e *A. flavus*. O óleo essencial de *B. rufa* apresentou rendimento de 0,05%, 28 compostos identificados, sendo os majoritários o mirceno (8,27%), *O*-cimeno (31,14%), 1,2-dimetil-4-etil-benzeno (13,08%), *Trans*-pinocarveol (12,55%) e criptona com (8,94%). A atividade antifúngica apresentou os melhores resultados para *S. sclerotiorum* e *C. gloeosporioides* onde exibiram em todas as concentrações atividade fungistática entre 100 a 54,84%. Para *A. flavus* foi observado as melhores concentrações de inibição entre as concentrações 100 a 25 μL^{-1} com 100%, e nas concentrações mais baixas de 12,5 e 6,25 μL^{-1} com 40,55 e 10,08%, não foi observado inibição nas concentrações 3,13 e 1,56 μL^{-1}. O óleo essencial de *B. rufa* apresentou exibiu riqueza em compostos químicos e efetiva ação fungicida.

Palavras-chave: *Sclerotinia sclerotiorum*, Atividade antifúngica, *Aspergillus flavus*, *Colletotrichum gloeosporioides*, Gênero *Bauhinia*

Resumen

Bauhinia rufa es una de las especies encontradas habitando el dominio Cerrado. Anualmente esta especie vegetal presenta floración con un ligero aroma dulce. El objetivo de este trabajo, fue evaluar el perfil químico y la actividad antifúngica del aceite esencial de flores de *B. rufa* sobre *Sclerotinia sclerotiorum*, *Coletotrichum gloeosporioides* y *Aspergillus flavus*. Las flores fueron colectadas en las primeras horas por la mañana, siendo enviadas al laboratorio para extracción y rendimiento de aceite esencial por el sistema tipo Clevenger. El perfil químico del aceite esencial se obtuvo por cromatografía de gases con detector de masas (CG-MS), y para actividad antifúngica, secuencias de concentraciones 100, 50, 25, 12.5; 6,25; 3.13 y 1.56 µL mL^{-1} en cepas de *S. sclerotiorum*, *C. gloeosporioides* y *A. flavus*. El aceite esencial de *B. rufa* presentó un rendimiento de 0,05%, identificándose 28 compuestos, siendo los mayoritarios mirceno (8,27%), *O*-cimeno (31,14%), 1,2 dimetil-4-etil-benceno (13,08%), *Trans*-pinocarveol (12,55%) y kriptona con (8,94%). La actividad antifúngica mostró los mejores resultados para *S. sclerotiorum* y *C. gloeosporioides* donde exhibieron actividad fungistática entre 100 y 54.84% en todas las concentraciones. Para *A. flavus* las mejores concentraciones de inhibición se observaron entre las concentraciones de 100 a 25 μL^{-1} con 100%, y en las concentraciones más bajas de 12.5 y 6.25 μL^{-1} con 40.55 y 10.08%, no se observó inhibición a las concentraciones de 3.13 y 1,56 μL^{-1}. El aceite esencial de *B. rufa* mostró riqueza en compuestos químicos y eficaz acción fungicida.

Palabras clave: *Sclerotinia sclerotiorum*, Actividad antifúngica, *Aspergillus flavus*, *Colletotrichum gloeosporioides*, Género *Bauhinia*

1. Introduction

The Cerrado domain is the second largest in terms of area, fauna and flora in Brazil. Currently, about 12,000 plant species are described, many of which are endemic and present a significant degree of threat of extinction (Bordino et al., 2018; Santos et al., 2017). Among this voluminous number of species, the genus *Bauhinia* stands out, which is widely found in tropical and pantropical areas, being a very diverse genus, comprising approximately 300 species (Wunderlin et al., 1987). In Brazil, 61 species are described for the genus *Bauhinia* (Silva et al., 2016).

The genus *Bauhinia* L. was established by Carl von Linnaeus in 1753, belonging to the tribe *Cercideae* Bronn, being considered the most basal of the family (Silva et al., 2016; Lewis; Forest, 2005). The genus *Bauhinia* has often been presented as a taxon with wide circumscription including *Phanera* (Lour.) Wunderlin, K. Larsen & S. Larsen as a subgenus, however, the subgenus has been re-established with generalist status with molecular and morphological bases being represented by lianas. with pegs, flowers with a single differentiated petal and samaroid vegetables (Mackinder; Clark, 2014). In Brazil, the genus *Bauhinia* is popularly known as "pata-de-vaca, casco-de-vaca, casco-de-boi and orchid tree", some species being used in ornamentation and afforestation of urban roads, in the reforestation of degraded areas, some herbal medicines and also used as stakes and posts (Fernandes et al., 2015; Lorenzi, 2009).

In Brazil, the following species of *Bauhinia* are described, *B. acreana* Harms., *B. bauhinioides* (Mart.) JFMacbr., *B. brevipes* Vogel, *B. caloneura* Malme, *B. campestres* Malme, *B. cheilantha* (Bong.) Steud ., *B. conwayi* Rusby., *B. corniculata* Benth., *B. cupulata* Benth., *B. curvula* Benth., *B. goyazensis* Harms., *B. holophylla* (Bong.) Steud., *B. longicuspis* Benth., *B. longifolia* (Bong.) Steud., *B. longipedicellata* Ducke., *B. marginata* (Bong.) Steud., *B. mollis* (Bong.) Steud., *B. pentandra* (Bong.) D. Dietr., *B. platypetala* Burch. ex Benth., *B. acuruana* Moric., *B. pulchella* Benth., *B. rufa* (Bong.) Steud., *B. ungulata* L., *B. vespertilio* S. Moore, *B. aculeata*, *B. alata*, *B. forficata*, *B. outimouta*, *B. pentandra* and *B. variegata* found throughout the Brazilian territory (Silva et al., 2016; Duarte-Almeida et al., 2003).

Bauhinia are characterized as trees, shrubs or sub-shrubs; do not have tendrils; the leaves are alternate, spiraled or ditic, bilobed, bipartite or bifoliolate, deciduous or persistent stipules, intra-stipular nectary which may or may not be present, type of palminervia nervation, between 3 to 13 ribs; the inflorescences are racemose, paniculate or pseudo-racemous, terminal or axillary, the flowers are pentamerous, zygomorphic or actinomorphic, dialysepalous or gamosepalous calyx, dialipetalous corolla, white, pinkish or reddish, androecium that to heterodynamo, dialystemone, 10 stamens, ovary uniovulated to pluriovulated; the fruits are samaroid vegetables or legumes (Silva et al., 2016; Vaz; Tozzi, 2003).

The species *B. rufa*, has a shrubby phytophysiognomy, the leaf organ has bilobed, cartaceous to leathery leaves, the base of the blade is rounded to truncated, has between 11 and 15 veins, the marginal vein is inconspicuous, the apex is acute to obtuse, the divaricated wolves; the adaxial face is of the glabrous type; the abaxial face is opaque, the leaves have glandular trichomes, the primary, secondary and tertiary veins are very prominent; it has a robust, villous-tomentose petiole; the inflorescence is 7-16 cm long, tomentosa, the flowers have a pedicel between 0.4-2 cm long and bracts 0.2-0.4 cm long, this species of *Bauhinia* has aroma flowers; do not have fruits (Silva et al., 2016).

Flowers have been enchanting with their colors, beauty and aromas for thousands of years, where some of them are also used in ornamentation and as ingredients in confectionery such as *Bauhinia variegata* (L.) (Heleno et al., 2017). Flowers are vegetative organs of numerous species, presenting in the background, several secondary compounds, where many of these have biological activity. Among the compounds we can find nutrients, proteins, carbohydrates, carbohydrates, organic acids, terpenoids, carotenoids, flavonol compounds, vitamins and essential oils that configure varied aromas for different plant species (Mlcek; Rop, 2011).

The inflorescences present essential oils rich in chemical compounds of great importance for the agricultural, pharmaceutical and food industries. Essential oils are one of the classes of secondary metabolites that have antioxidant, antifungal, antibacterial, leishmanicidal, insecticidal, antitumor, hypertensive and trypanosomide activities, and are also used in the perfumery industry (Tariq et al., 2019; Ammar et al., 2015; Sagdic et al., 2013; Góis et al., 2013; Xie; Zhang, 2012; Mao et al., 2006).

Studies on the chemical constitution and possible biological actions of essential oils are still almost non-existent for *Bauhinia rufa*. Study developed by Duarte-Almeida et al. (2004) evaluated the essential oil of the leaf of *B. rufa* where they found a large amount of compounds, the major ones being viridiflorol and spathulenol. Other species of the genus *Bauhinia* have already been studied, presenting a great diversity of chemical compounds in essential oils such as β-Bourbonene, β-Elemene, β-Caryophyllene, *Allo*-Aromadendrene, Germacrene D, γ-Elemene, Bicyclogermacrene, δ-Cadiene, α-Cadinol, *Iso*-Spatulenol, α-Elemene, Lepidozenol, α-Humulene, and Copaene (Duarte-Almeida et al., 2004).

The objective of this work was to characterize the chemical profile, and antifungal activity of the essential oil of the *Bauhinia rufa* flower against *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Aspergillus flavus*.

2. Materials and Methods

B. rufa flowers were collected in June 2019, in a reserve area located in the Rio Verde, Goiás, Brazil, with the following geographic coordinates: 17°43'04.5"S and 50°53'10.4"W. One exsiccate was herborized and deposited in the Herbarium of IF Goiano, Campus Rio Verde with the following number. HRV 1116.

The essential oil from the flowers of *B. rufa* was extracted by hydrodistillation in a Clevenger-type equipment. In triplicate, about 250 g of floral material were weighed. The material was then ground in a food processor with 250 mL of distilled water. The system was refluxed for 2 h. Then, the fractions were collected and washed three times with 10 mL of dichloromethane in a separatory funnel. The fraction containing dichloromethane was collected and dried with anhydrous sodium sulfate. Soon after, it was filtered and the supernatant stored in a place protected from light and heat for complete evaporation of the solvent. The yield was calculated according to the equation 1.

$$\text{Yield (\%)} = (\text{mass of essential oil/mass of plant material})*100 \quad \text{Eq. [1]}$$

To determine the chemical profile, the essential oil was evaluated in a gas chromatograph system (Shimadzu GC Mod. QP 5000), with a fused silica capillary column (Optima®, 5-0.25 µm (30 m x 0.25 mm)), with electronic impact ionization (II) mass detector (70 eV). The identification of the chemical compounds of the essential oil was based on the Kovats Index, using a series of *n*-alkanes (C-1 to C-40). Retention time comparison was performed using the Nist-11 spectrophotometer and Adams literature (2007).

The antifungal test was performed using isolates of *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Aspergillus flavus*, maintained in potato, dextrose and agar (PDA) medium. The strains were donated by the Natural Products laboratory and Technological Chemistry laboratory at IF Goiano, Rio Verde Campus, Goiás, Brazil. The antifungal activity of the essential oil on the mycelial growth of *S. sclerotiorum*, *C. gloeosporioides* and *A. flavus*, was evaluated through different concentrations, starting from 100 (pure oil); 50; 25; 12.5; 6.25;

3.13 and 1.56 µL mL^{-1}. As a negative control, we used the control (absence of essential oil), fungicide Frowncide 500 SC®, at a concentration of 10 µL mL^{-1} and dimethylsulfoxide (DMSO) as a positive control.

Essential oil concentrations were added to the PDA culture medium after sterilization and cooling, as well as for treatments with commercial fungicide and DMSO. After solidification of the medium, in a laminar flow chamber, 1 mycelium disc for each strain of *S. sclerotiorum*, *C. gloeosporioides* and *A. flavus* with 7 mm in diameter, was deposited in the center of the *Petri* dish of 10 cm in diameter. Then, they were incubated at a temperature between 20, 23 and 25 °C, respectively, as described by Garcia et al. (2012) with adaptations.

The evaluation consisted of daily measurements of colony diameter, using a manual caliper, started 24 h after the start of incubation and ended when the fungal colonies from the control treatment completely reached the inner area of the plate. The determination of the growth inhibition percentage (PGI) was performed as proposed by Garcia et al. (2012). The equation used was PGI = (DCT – DCTr)/DTT x 100. Where: PGI = percentage of growth inhibition, DCT = diameter in the control treatment, DCTr = diameter in the chemical treatment.

The analysis consisted of an evaluation in triplicate followed by ± SD. To determine the statistical difference, the Tukey's test ($p < 0.05$) was used. The statistical software used was PAST 3 (free version).

3. Results and Discussion

The essential oil yield was 0.05% ± 0.03 CV. The volatile compound was colorless, with a slight sweet aroma. Table 1 shows the chemical profile of the essential oil of the *B. rufa* flower. It was not possible to compare the yield of essential oil from the inflorescence of *B. rufa* with other species of the genus, due to the lack of studies. With this, the result obtained was compared with other studies of inflorescences where they evaluated the yield obtained for other groups of vegetables. Değirmenci and Erkurt (2019) found a yield of 0.57% for oil from *Citrus aurantium* flowers. Sharma and Kumar (2016) evaluated the yield of *Rosa* x *damascena* after storage periods at -20 °C, 4 °C and *in natura* with yields of 0.0049; 0.0040 and 0.0051% respectively.

The essential oil of *B. rufa* flowers presented a total of 28 chemical compounds, with myrcene (8%), *O*-Cymene (31%), 1,2-dimethyl-4-ethyl-benzene (13%), *Trans*-Pinocarveol (12%) and Kryptone with (8%) being the major compounds, 0.98% of the total chemical compounds were not identified. Değirmenci; Erkurt (2019) found 77 compounds in the essential oil of *Citrus aurantium* flowers, the majority compound being Linalool (14.12%). Study carried out by Marinho et al. (2018) evaluated three species of Fabaceae, where they did not find essential oil in *B. rufa* inflorescences in the year 2018. Sharma and Kumar (2016) found 35 compounds in the essential oil of *Rosa* x *damascena*, the major compounds being Citronellol + Nerol with an average between three treatments of (38.10%) and Nonadecane with (17.45%). Biasi et al. (2009) found 11 compounds in the essential oil of the flowers of *Ocimum gratissimum*, the major compounds being eugenol (80.83%) and α-*Trans*-Farnesene (8.56%).

Table 1. Chemical profile by GC-MS of the essential oil of the *Bauhinia rufa* flower.

Compound	**RI**	**RA (%)**
2-Pentanone	786	1.23
α-Pinene	941	3.35
Verbene	964	0.12
β-Pinene	985	0.28
Myrcene	997	**8.27**
O-Cymene	1018	**31.14**
Limonene	1025	0.07
Sylvestrene	1035	0.18
1,2 dimethyl-4-ethyl-benzene	1081	**13.08**
Trans-Sabinol	1135	3.26
Trans-Pinocarveol	1143	**12.55**
Iso-Pinacarveol	1147	4.37
Cryptone	1169	**8.94**
β-Fenchol	1193	1.13
Eugenol	1359	0.42
α-Copaene	1376	0.08
β-Cubebene	1386	3.09
α-Cedrene	1413	0.11
β-Caryophyllene	1418	0.12
γ-Elemene	1434	2.00
α-Humulene	1454	1.38
Allo-Aromadendrene	1460	1.12
Germacrene D	1480	0.74
Acifilene	1497	0.09
Bicyclogermacrene	1500	0.12
δ-Cadidene	1521	0.03
β-Acorenol	1637	0.31
α-*Epi*-Muurolol	1641	1.34
Total identified		99.92
Not identified		0.98

Note: RI = Retention Index. RA (%) = Retention Area in percentage (%). Source: Authors, 2019.

It can be seen in Table 2, that the highest concentrations of essential oil showed the greatest inhibition of mycelial growth in *S. sclerotiorum*, *C. gloeosporioides* and *A. flavus*. The concentrations of 100, 50 and 25 μL^{-1} obtained 100% inhibition, except for *C. gloeosporioides*. However, statistically there was no difference between the three concentrations. The same was observed for the concentration of 6.25 μL^{-1} for *S. sclerotiorum* that there was no statistical difference between the first group.

For *S. sclerotiorum*, two groups were observed that differed statistically between the concentrations evaluated by the *Tukey's* test ($p < 0.05$). For *C. gloeosporioides* and *A. flavus*, 4 groups were observed for both fungi with statistical difference. For *A. flavus*, it was the fungus that showed the highest resistance at low concentrations

between 1.56 and 3.13 μL^{-1}. But it is worth mentioning that the concentration of 6.25 μL^{-1} had a low antifungal activity, possibly it is necessary that there is a need to incorporate other essential oils to accentuate the fungistatic action.

In this study, all PGI results were compared with the positive control, the commercial fungicide Frowncide at a concentration of 10 μL^{-1}, which showed 100% mycelial inhibition for the three fungi. The essential oil obtained from the flowers of *B. rufa* showed exceptional antifungal activity against the three fungi that annually cause considerable economic losses in grains and fruits.

Xavier et al. (2016) obtained a percentage of mycelial inhibition in *S. sclerotiorum* between 87.63 and 28.27% for concentrations between 300 and 50 μL^{-1} with essential oil from the leaves of *Cardiopetalum calophyllum*. Oliveira et al. (2019) found a percentage of inhibition against the strain of *Colletotrichum theobromicola* of 68.80% at the highest concentration, and at the lowest concentration of 17% of the essential oil of *Zingiber officinale*. Martinazzo et al. (2019) evaluated the essential oil of *Cymbopogon citratus* at different concentrations against *A. flavus*. The best concentrations obtained by the authors were 1.0; 0.8 and 0.6 μL^{-1} with antifungal activity between 100 and 34%, and in the lowest concentrations of 0.4 and 0.2 μL^{-1} with inhibition between 100 and 11%.

Valente et al. (2018) evaluated the inhibition activity against the strains of *Cladosporium herbarium*, *Aspergillus niger* and *Penicilium expansum* at different concentrations of essential oil from the leaves of *Callistemon viminalis*, the researchers obtained the best results at the highest concentration of essential oil of 0.2% with inhibition for *C. herbarium* of 43.7%, and for *P. expansum* with maximum inhibition of 36.4%. However, the applied concentrations did not show fungistatic activity for *A. niger*. The researchers cite that the usual concentrations partially inhibited the sporulation of *A. niger*, but their growth was induced.

Essential oils present not only fungistatic efficiency for agricultural fungal strains, but also for strains that cause pathologies in humans and animals. As in the study against strains of *Candida* spp., where antifungal action was observed in different concentrations of essential oil from *Lippia sidoides* leaves proposed by Brito et al. (2015); in *Candida glabrata* by the essential oil of *Pogostemon cablin* evaluated by Pimenta et al. (2019). Several oils also have bacteriostatic action against strains of *Streptococcus mutans*, *S. mitis*, *S. sanguinis*, *S. sobrinus* and *Bacteroides fragilis* in minimal inhibitory concentration with essential oil from the leaves of *C. calophyllum* evaluated by Xavier et al. (2016); and by Silvestri et al. (2010), evaluating the essential oil of clove (*Eugenia caryophyllata*) against *Bacillus subtilis*, *Enterococcus faecalis*, *Micrococcus luteus*, *Sarcina* sp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Acinetobacter* sp., *Aeromonas* sp., *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella choleraesuis*, *Serratia* sp., *Shigella flexneri* and *Xanthomonas campestris*.

As antiviral agents, essential oils play an important role in controlling and fighting viral agents as in many RNA and DNA viruses. There are important results obtained evaluating types 1 and 2 of the Herpes simplex virus (HSV-1 and HSV-2), dengue virus type DEN-2, adeno influenza virus type 3, polyvirus, Junin virus and Coxsackie virus B1 using essential oils in the treatment (Tariq et al., 2019; Allahverdiyev et al., 2004).

Table 1. Percentage of growth inhibition of *Sclerotinia sclerotiorum*, *Colletotrichum gloeosporioides* and *Aspergillus flavus* in different concentrations of essential oil from *Bauhinia rufa* flower.

Microrganism	**Essential oil concentration ($\mu L\ mL^{-1}$)**						
	Inhibition Zone (mm)						
	100	**50**	**25**	**12.5**	**6.25**	**3.13**	**1.56**
S. sclerotiorum	100a	100a	100a	98.14a	76.18b	70.36b	66.50cb
C. gloeosporioides	100a	100a	93.12a	87.04b	80.11cb	56.33d	50.84d
A. flavus	100a	100a	100a	40.55b	10.08c	0d	0d

Note: Commercial antifungal Frowncide 500 SC = 100a of mycelial inhibition. Equal letters on the same line do not differ by *Tukey's* test ($p < 0.05$). Source: Authors, 2019.

4. Conclusions

The essential oil of the *Bauhinia rufa* flower has a low yield, although it has a great richness of identified volatile

compounds. As for the antifungal activity, the best results were observed for *Sclerotinia sclerotiorum* and *Colletotricum gloeosporioides*, which showed great inhibition efficiency at all essential oil concentrations, the same was not observed for *Aspergillus flavus*, which showed growth inhibition until low concentration.

It is worth mentioning that this study only evaluated the percentage of inhibition of mycelial growth *in vitro*, requiring further studies that can be evaluated at the same concentrations in greenhouses and fields, so that the results can be compared and thus propose new methods of prevention or remediation in the control of these three agricultural pests.

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