Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Fungitoxic effect and phytochemical characteristics of Brazilian Cerrado weeds against *Rhizoctonia solani* and *Macrophomina phaseolina* fungi

Efeito fungitóxico e características fitoquímicas de plantas daninhas do Cerrado brasileiro frente aos fungos *Rhizoctonia solani* e *Macrophomina phaseolina*

E. R. S. Dilkin[a, b*], R. Matias[a], A. K. M. Oliveira[a] and B. O. Corrêa[a]

[a]Programa de Pós-graduação em Meio Ambiente e Desenvolvimento Regional, Universidade Anhanguera – Uniderp, Campo Grande, MS, Brasil
[b]Instituto Federal de Educação, Ciência e Tecnologia de Mato Grosso do Sul – IFMS, Campo Grande, MS, Brasil

Abstract

The use of natural products obtained from plants, for example, invasive plants, offers a variety of allelochemicals with fungicidal potential. With this in perspective, the objective was to evaluate the fungicidal potential of ethanolic extracts of Cerrado plants on *Rhizoctonia solani* and *Macrophomina phaseolina*. The ethanolic hydroalcoholic extract of the 12 plants identified as invaders in the Brazilian Cerrado was prepared (*Anacardium humile* Saint Hill; *Baccharis dracunculifolia* DC.; *Cenchrus echinatus* L; *Commelina erecta* L.; *Erigeron bonariensis* L.; *Digitaria horizontalis* Willd.; *Digitaria insularis* L.; *Porophyllum ruderale* Jacq. Cass; *Richardia brasiliensis* Gomes; *Sida rhombifolia* L.; *Turnera ulmifolia* L.; *Smilax fluminensis* Steud)) and phytochemical screening and determination of total phenols and flavonoids were performed. To evaluate the in vitro antifungal activity, the hydroalcoholic solutions at concentrations of 800, 1200, 1600, 2000, and 2400 µL 100 mL^{-1} were separately incorporated into BDA agar and poured into Petri dishes, followed by the mycelium disk of the fungus. As a control, two solutions were prepared, one ethanolic solution added to the BDA medium (2400 µg 100 mL^{-1}) and the other with BDA medium only. They were poured into Petri dishes, followed by a 0.5 cm diameter disk of mycelium of the fungus, incubated (23±2 ºC), with a 24-hour photoperiod. Among the constituents found in the plants, 75% are phenolic compounds, 58.3% are cardiotonic heterosides, 50% are steroids, 33.3% are flavonoids, 16.7% are anthraquinones, and 8.3% are alkaloids, saponins, and reducing sugars. Out of the 12 species, only the extracts of *C. erecta* and *R. brasiliensis* were active for *M. phaseolina* and *R. solani*. Thus, it is concluded that the ethanolic extract of *C. erecta* has the fungicidal potential to control diseases caused by fungi that are soil inhabitants. Of the other species, *A. humille, B. dracuncufolia, D. insulares, C. erecta, D. insulares, P. ruderale*, and *R. brasiliensis* have natural fungitoxic potential because they stand out in the content of polyphenols efficient in reducing the mycelial growth of *M. phaseolina* and *R. solani.*

Keywords: plant disease control, phytopathogens, fungitoxic action, secondary plants substances, fungicidal plants.

Resumo

O uso de produtos naturais obtidos de plantas, por exemplo, as plantas invasoras, oferece uma variedade de aleloquímicos com potencial fungicida. Tendo isso em vista, objetivou-se avaliar o potencial fungicida de extratos etanólicos de plantas do Cerrado sobre *Rhizoctonia solani* e *Macrophomina phaseolina.* Foi preparado o extrato hidroalcoólicos etanólico das 12 plantas apontadas como invasoras no Cerrado brasileiro (*Anacardium humile* Saint Hill; *Baccharis dracunculifolia* DC.; *Cenchrus echinatus* L; *Commelina erecta* L.; *Erigeron bonariensis* L.; *Digitaria horizontalis* Willd.; *Digitaria insularis* L.; *Porophyllum ruderale* Jacq. Cass; *Richardia brasiliensis* Gomes; *Sida rhombifolia* L.; *Turnera ulmifolia* L.; *Smilax fluminensis* Steud) e foi realizado o *screening* fitoquímico e a determinação de fenóis e flavonoides totais. Para avaliar a atividade antifúngica *in vitro*, as soluções hidroalcóolicas nas concentrações de 800, 1200, 1600, 2000 e 2400 µL 100 mL^{-1} foram incorporadas, separadamente, em ágar BDA, e vertidas em placa de Petri, seguido do disco de micélio do fungo. Como controle, foram preparadas duas soluções, uma solução etanólica adicionada ao meio BDA (2400 µg 100 mL^{-1}), e outra somente com meio BDA, a testemunha. Foram vertidas em placas de Petri, seguido um disco de 0,5 cm de diâmetro de micélio do fungo, incubados (23±2 ºC), com fotoperíodo de 24 horas. Dentre os constituintes encontrados nas plantas, 75% estão os compostos fenólicos, 58,3% estão os heterosídeos cardiotônicos, 50% estão os esteroides, 33,3% estão os flavonoides, 16,7% estão as antraquinonas e 8,3% estão os alcaloides, saponinas e açúcares redutores. Das 12 espécies, apenas os extratos de *C. erecta* e *R. brasiliensis* foram ativos para *M. phaseolina* e *R. solani*. Desse modo, conclui-se que o extrato etanólico de *C. erecta* apresenta potencial fungicida para controle de doenças causadas por fungos habitantes do solo. Das demais espécies, a *A. humille*, *B. dracuncufolia, D. insulares, C. erecta*, *D. insulares, P. ruderale* e *R. brasiliensis* possuem

*e-mail: eliane.dilkin@ifms.edu.br
Received: April 14, 2022 – Accepted: May 21, 2022

potencial fungitóxicos naturais por destacarem nos teores de polifenóis eficientes na redução do crescimento micelial de *M. phaseolina* e *R. solani*.

Palavras-chave: controle de doenças de plantas, fitopatógenos, ação fungitóxica, substâncias secundárias de plantas, plantas fungicidas.

1. Introduction

In the global scenario, Brazil, besides being one of the leaders in the production and export of grains in the world, is at the forefront of soybean (*Glycine max,* Merrill) production technology. In tropical regions, as for position, it is in second place in the world (2017/18 crop), behind only the United States (Conab, 2018; Embrapa, 2021).

The measures to control pathogens that attack the soy crop, for example, are an issue that requires efficient management, including resistant varieties, synthetic chemical fungicides, and antagonist microorganisms that, applied separately or together, will make it possible to minimize the resistance of these pathogens, especially to agrochemicals that cause impacts to the environment and man.

Although, due to the increasing awareness of the risks involved in the constant use of pesticides, such as the contamination of the environment and food, researches aiming at the development of alternative methods in the control of pests are carried out in order to decrease agricultural and livestock losses and reduce the dependence on synthetic fungicides (Domene et al., 2016; Nascimento et al., 2016; Gonçalves-Trevisoli et al., 2017).

The active ingredients, found in plants, present an incomparable diversity of chemical groups. Therefore, these substances are promising for the search for new fungicides and the development of innovative products effective in the control of plant diseases, with less toxicity and environmental effects (Cushnie and Lamb, 2005; Alavijeh et al., 2012; Brito and Nascimento, 2015).

In this context, the Brazilian Cerrado is home to diverse plant species with significant heterogeneity of native and cosmopolitan plants. Many of them have been indicated as bioactive substances and are also investigated in the control of phytopathogens (Ramos et al., 2016). Soybean can be attacked by: *Aspergillus niger* Tiegh., 1867, *Aspergillus flavus* Link, 1809, *Aspergillus ochraceus* G. Wilh., 1877, *Fusarium incarnem* (Desm.) Sacc., 1886, *Cercospora sojina* Hara, 1915, *Rhizopus* sp., e *Penicillium* sp. (Bezerra et al., 2013). In corn, an important phytopathogen is *Fusarium graminearum*, capable of producing mycotoxins that contaminate grains and their derivatives (Oliveira et al., 2015).

In vitro studies on the activities of plant extracts are fast, safe, and accessible, through which it is possible to determine their toxic potential, thus being able to relate the bioactive activities of the plant to the inhibition of mycelial growth and sporulation of phytopathogenic fungi (Schwan-Estrada and Stangarlin, 2005; Ramos et al., 2016).

Research on the use of weed extracts has been little explored. However, for being resistant to different environmental conditions, this group can be dominant to the attack of some plant pathogen, soil inhabitant, for example, and control the development of diseases. Therefore, this study aimed to evaluate the fungicidal potential of 12 weed extracts from the Brazilian Cerrado in the control of phytopathogenic fungi.

2. Materials and Methods

2.1. Collection and preparation of the extracts

The botanical materials were collected from 20 matrices growing spontaneously in Cerrado areas at Anhanguera-Uniderp University (20°26'16.6" S 54°32'14.5 "W), and in a Cerrado fragment of the University's Três Barras School Farm (S20°26'20.64" W54°32'26.78") (see Table 1).

The samples of each specimen were cataloged in the University Herbarium, and, for collection and research purposes, authorization to access the genetic heritage was obtained from the Genetic Heritage Management Council (CGEN) (see Table 1).

2.2. Preparation of the extracts

The botanical material of each species (see Table 1), after drying in an aeration greenhouse at 45 ± 4 °C (MARCONI®, MA35) for 48 hours, was grinded in a knives mill (MARCONI®, MA048), sieved (25 mesh), and the powder was stored in a polyethylene bottle. Next, the powder of each sample (100 g) was exhaustively extracted with ethanol (99.8%) in an ultrasound bath (UNIQUE®, 1450) for 60 minutes, followed by 24 hours of static maceration until the drug's depletion. Then, the filtrate was concentrated and evaporated at a rotaevaporator (45 °C). The procedure was followed for 10 days, and the final drying occurred in a desiccator under reduced pressure.

2.3. Phytochemical prospection and profiling by UV-visible spectroscopy

The procedures described by Abreu-Matos (2009) and Simões et al. (2017) for the phytochemical prospection were followed, occurring by humid way, through precipitation reactions and/or color change. The analyses were performed in triplicate, and the results were compared and contrasted with the original extract, with readings based on Fontoura et al. (2015). The following were considered: negative reaction (-), discrete (turbidity) (±), weakly positive (+), partially positive (+±), positive (++), strongly positive (++±) and high intensity (+++), with frequency of 0, 5, 15, 25, 50, 75 and 100%, respectively.

Confirmations of the major chemical groups of each extract were obtained by scanning the spectrum in the UV-visible region (Femto®, 800XI), with the determination of the wavelength of maximum absorbance in the range of 200 to 800 nm, using ethanol as blank. The spectra were compared with ultraviolet spectra from the literature (Piironen et al., 2000; Jurasekova et al., 2006; Kasal et al., 2010; Silverstein et al., 2014; Lucas et al., 2015; Fouillaud et al., 2016; Leyva et al., 2017). All confirmatory

Table 1. List of collected species, location, time of collection (October 2017 to February 2018), record number and identification, Campo Grande, MS, 2018.

Species	Organs	Location	Herb. Rec. No	CGEN Rec. No
Anacardium humile A. St. Hil.	L.	Area 1	8542	ACD32C5
Baccharis dracunculifolia DC.	L.	Area 2	8540	
Cenchrus echinatus L	L. and S.	Area 1	8544	
Commelina erecta L.;	L.		8541	
Erigeron bonariensis L.	A.P.		8543	
Digitaria horizontalis Willd.	A. P.		8546	
Digitaria insularis L.;	L.	Area 2	8545	
Porophyllum ruderale Jacq. Cass	L. and S.	Area 1	8538	
Richardia brasiliensis Gomes	L.		8547	
Sida rhombifolia L.	L.		8537	
Smilax fluminensis Steud.	L.	Area 2	8539	
Turnera ulmifolia L.	L.	Area 1	8536	

Identification: authors. L. = Leaves; L. and S. = Leaves and Stems; A. P. = Aerial Parts (Leaves, Flowers and Stems); Rec. No. = Record Number; Herb. = Herbarium; CGEN = National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen).

analyses were developed with three repetitions and compared with literature data.

When necessary, the reactions were monitored by thin layer chromatography (TLC) using aluminum chromatoplates covered with Merck silica gel 60G $F_{254+354.}$ The eluent system, band visualization (irradiation with ultraviolet light: 254 and 365 nm), and developers were based on Wagner and Bladt (2009).

2.4. Determination of phenolic compounds and flavonoids

To quantify total phenols, the Folin-Ciocalteu method was applied with gallic acid (Vetec®) (10 to 350 mg mL^{-1}) as standard ($Y = 0.7182 x + 0.0927$, $R^2 = 0.982$) (Sousa et al., 2007). Flavonoids were evaluated by the aluminum chloride method and quercetin (Sigma®) as standard ($Y = 0.1114 x - 0.0030$), $R^2 = 0.999$) (Peixoto-Sobrinho et al., 2008).

Quartz cuvettes were used to read the samples and standards, and the results of the analyses performed in triplicates were reported as average and standard deviation.

2.5. Antifungal activity

The isolated *R. solani* and *M. phaseolina* fungi belong to the phytopathogenic fungi collection of the Phytopathology Laboratory (Anhanguera-Uniderp), located in Campo Grande, MS (latitude 20°26'16.6" S) (longitude 54°32'14.5" W).

For the antifungal activity of each plant, a stock solution of 0.2 g of the ethanolic extract in 100 mL^{-1} of ethanol (99.8%) was used. From this solution, different aliquots were used and poured into a melting BDA culture medium with the volume of 100 mL^{-1} at the concentrations of 800, 1200, 1600, 2000, and 2400 µL 100 mL^{-1}. For the control, two solutions were prepared: one containing ethanol (99.5%) added to the BDA medium (2400 µg 100 mL^{-1}), called ethanolic solution, and another with only BDA medium (without plant extract), called control solution.

Then, 10 mL^{-1} of culture medium was poured in different concentrations, and a 0.5 cm diameter mycelium disk of *R. solani* was deposited in the center of each plate. The same procedure was done for *M. phaseolina*. After sealing, they were kept in a growth chamber (23±2 ºC), with a 24-hour photoperiod.

Evaluations were performed daily from the growth of the control for three days, measuring the mycelial growth diameter of two orthogonal axes. Based on the data obtained, the Growth Inhibition Percentage (GIP) was calculated for each plant extract. Thus, the GIP was calculated using the GIP formula = [(control diameter – treatment diameter)/control diameter] x 100, for each extract in relation to the control.

3. Results and Discussion

The 12 plant extracts showed the diversity of secondary metabolite classes and are demonstrated in Table 2. From the class of the constituents investigated, phenolic compounds were the predominant ones in 75% of the plants under study, followed by steroids and cardiotonic heterosides (50.0%), flavonoids (33.3%), anthraquinones (16.7%), alkaloids, reducing sugars, saponins, and triterpenes (8.3%).

Among the classes with the highest frequency (+++= 100%), with polarity characteristics, are the cardiotonic heterosides, saponins, and reducing sugars, followed by phenolic compounds and derivatives (flavonoids and anthraquinones) with standard polarity characteristics, and with lower polarity are steroids and triterpenes.

The predominance of the majoritarian constituents (+++= 100%) was confirmed by scanning the absorption spectrum in the UV-visible region. In the spectra, it was possible to observe characteristic bands for similar maximum absorption at 280 nm, corresponding to phenolic

Table 2. Results of the phytochemical analysis of total contents, flavonoids, mass spectroscopy (VU) and GIP of the ethanolic extract of the 12 Cerrado weeds, in Campo Grande, MS.

Families and species (popular name)	Produce (g)	Phytochemical	Dosage		VU (mm)	*Rhizoctonia solani* (GIP)	*Macrophomina phaseolina* (GIP)
			P.C.	Flav.			
Anacardeaceae.							
Anacardium humile A. Saint Hill. (Cajuzinho-do-cerrado)	4,11	P.C.; Flav.; Anthr. (100%). Card. Het.; Ster.; R.S. (50%). Tan.; Cum; Trit. (25%).	311,77a	112,94c	260 280 340 480 500	5,0% in 1600 µL 100 mL^{-1}	10,4% in 1600 µL 100 mL^{-1}
Asteraceae							
Baccharis dracunculifolia DC. (Alecrim-do-campo)	16,33	Card. Het.; Ster. (100%). P.C.; Flav. (50%). Tan.; Cum.; Trit. (25%).	270,17b	145,09a	280 290 360 400	13,5% in 2000 µL 100 mL^{-1}	Inactive
Erigeron bonariensis (L.) Cronquist. (Buva)	2,75	P.C.; Trit.; Ster.; Card. Het. (100%). Flav.; R.S. (50%). Tan.; Cum. (25%).	122,15e	94,91d	280 360 400	Inactive	4,2% in 2400 µL 100 mL^{-1}
Porophyllum ruderale (Jacq.) (Arnica-brasileira)	7,11	P.C.; Flav. (100%). R.S. (50%). Tan.; Cum.; Ster. (25%).	160,4d	125,33b	260 280 360	4,2% in 800 µL 100 mL^{-1}	Inactive
Poaceae							
Cenchrus echinatus L. (Capim-carrapicho)	2,11	P.C.; Sap. (100%). R.S.; Flav. (50%). Tan.; Cum.; Card. Het.; Trit.; Ster (25%).	57,49f	19,22g	280 294 360	Inactive	Inactive
Digitaria horizontalis Willd. (Capim-colchão)	1,05	P.C.; Ster. (100%). R.S.; Trit.; Flav. (50%). Tan.; Cum.; Card. Het. (25%).	152,48d	72,48e	280 400	13,85% in 1600 µL 100 mL^{-1}	Inactive
Digitaria insularis L. Fedde (Capim-amargoso)	6,78	P.C. (100%). Card. Het.; Flav. (50%). Tan.; Cum. (25%).	268,67b	142,34a	280 360	10,4% in 2000 µL 100 mL^{-1}	Inactive
Commelinaceae							
Commelina erecta (L.) (Erva-de-santa-luzia)	5,99	Flav.; Anthr.; Ster. (100%). Tan.; Trit.; Sap. (75%). P.C.; Cum.; Alk.; Card. Het.; R.S. (25%).	276,20b	129,00b	280 339 350 420 439	33,20% in 1600 µL 100 mL^{-1}	37,4% in 1200 µL 100mL^{-1}

VU = Visible Ultraviolet; P.C. = Phenolic Compounds; Tan. = Tannins; Flav. = Flavonoids; Cum. = Cumarins; R.S. = Reducing Sugars; Sap. = Saponins; Card. Het. = Cardiotonic Heterosides; Anthr. = Anthraquinones; Trit. = Triterpenes; Ster. = Steroids; Alk. = Alkaloids. GIP = Growth Inhibition Percentage. Averages followed by the same lower case letter in the column do not differ statistically in the Tukey test ($p \geq 0.05$). VC = Variation Coefficient. significant at 5%.

Table 2. Continued...

Families and species (popular name)	Produce (g)	Phytochemical	Dosage		VU (mm)	*Rhizoctonia solani* (GIP)	*Macrophomina phaseolina* (GIP)
			P.C.	Flav.			
Malvaceae							
Sida rhombifolia L. (Guanxuma)	3,95	Card. Het.; Ster. (100%). P.C.; Trit.; R.S. (50%). Tan.; Cum.; Flav. (25%).	52,34f	32,61f	284 400	6,0% in 2400 µL 100 mL^{-1}	8,0% in 800 µL 100 mL^{-1}
Rubiaceae							
Richardia brasiliensis Gomes. (Poaia)	5,12	P.C.; Flav. (100%). Tan. (75%). Sap.; Card. Het.; R.S. (50%). Flavonas; Alk.; Cum.; Ster.; Trit. (25%).	239,03c	126,66b	280 300 340 350	25,40% in 800 µL 100 mL^{-1}	28,5% in 800 µL 100 mL^{-1}
Smilacaceae							
Smilax fluminensis Steud. (Salsaparrilha)	3,70	P.C.; Card. Het.; Ster. (100%). R.S.; Trit. (50%). Tan.; Cum.; Flav. (25%).	158,86d	86,83d	280 360 400	1,25% in 1600 µL/100 mL.	Inactive
Turneraceae							
Turnera ulmifolia L. (Chanana)	7,62	P.C.; Flav.; R.S. (100%). Card. Het. (50%). Cum.; Trit.; Ster. (25%).	116,03e	75,80e	280 290 360 560	7,50% in 2000 µL 100 mL^{-1}	17,5% in 2400 µL 100 mL^{-1}

VU = Visible Ultraviolet; P.C. = Phenolic Compounds; Tan. = Tannins; Flav. = Flavonoids; Cum. = Cumarins; R.S. = Reducing Sugars; Sap. = Saponins; Card. Het. = Cardiotonic Heterosides; Anthr. = Anthraquinones; Trit. = Triterpenes; Ster. = Steroids; Alk. = Alkaloids. GIP = Growth Inhibition Percentage. Averages followed by the same lower case letter in the column do not differ statistically in the Tukey test ($p \geq 0.05$). VC = Variation Coefficient. significant at 5%.

compounds (Li et al., 2008; Zuanazzi et al., 2017), which were found with a frequency of 100% in nine extracts (75%).

Phenolic compounds are the phytochemical class with the largest distribution in the plant kingdom, but in a heterogeneous manner (Lin et al., 2016). In general, they are related to plant defense responses, such as coloring agents for camouflage against herbivores and natural predators. Among the properties attributed to this class is the antibacterial and antifungal potential (Edreva et al., 2008).

Regarding the constitution of the flavonoids, depending on the number of hydroxyl groups present and their location (Jurasekova et al., 2006), there are two absorption bands, between 240-285 nm (band II), referring to the absorption of the ring A (hemiacetal), and a second band between 300-400 nm (band I) representing the B ring of the flavonoid. For the extracts investigated, the characteristic flavonoid bands are between 260-290 nm (band II) and 300-400 nm (band I).

Anthraquinones have an important characteristic in their electronic absorption spectra. A strong absorption in the ultraviolet region refers to the presence of chromophore formed by the system, with a high degree of conjugation between the condensed aromatic system (C=C conjugates). These transitions are responsible for the $\pi \rightarrow \pi^*$ absorption with the carboxyl or carboxylic group and have maximum absorption with a band between 405 and 508 nm. Benzenoid bands appear regularly within the 240-260 nm range with intense absorption, with average absorption at 320-330 nm (Lucas et al., 2015; Fouillaud et al., 2016; Leyva et al., 2017) and were detected in the extracts of *A. humile* and *C. erecta* as one of the majority constituents. It is noted that the wavelengths showed the same profile. However, the absorption bands were at different positions (see Table 2).

Anthraquinones were also found in *A. humile* leaves by Andrade-Filho et al. (2010), who evaluated the insecticidal potential against *Bemisia tuberculata* (Bondar, 1923) (Hem.: Aleyrodidae). On the other hand, in the species *C. erecta*, no anthraquinones were registered (Ekeke and Ogazie, 2018), only for *Commelina diffusa* (Khan et al., 2011).

Meanwhile, the steroids detected in the extracts of *B. dracunculifolia, E. bonariensis, D. horizontalis, C. erecta, S. rhombifolia,* and *S. fluminensis* have a maximum absorption between 206 nm-220 nm.

Phytosterols are a class of hormones, with a standard chemical structure called cyclopentane-perhydrophenanthrene, with A, B, and C rings attached to a cyclopentane ring (D ring) (Pérez and Escandar, 2013). However, depending on the diene conjugation system (homo or heteroannular), with the presence of the enone system, there are hexocyclic characters of double bond and other additional conjugations, among others in the sterol molecule. Therefore, its spectrum can show a maximum absorption, for example, between 220-350 nm (Kasal et al., 2010).

The triterpenes were predominantly found in the extract of *E. bonariensis* and present maximum absorption with band between 200-520 nm. A variety of biological applications are described for this class, among them are anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory, and tonic. As an antimicrobial, a likely mechanism of action is the incorporation of triterpenes into the lipid bilayer, promoting the modification of the erythrocyte membrane (Dzubak et al., 2006; Boulogne et al., 2012).

Among the 12 extracts evaluated, the *A. humile* showed significant percentages of total phenols 311.77±2.36 mg of gallic acid $100g^{-1}$, higher and statistically different from the other species under study, followed by the species *B. dracuncufolia, D. insulares,* and *C. erecta* with contents equal and higher than the other plants.

The flavonoids followed a similar profile for *B. dracuncufolia* and *D. insulares*, with equal and higher contents than the other species, followed by *P. ruderale*, *C. erecta*, and *R. brasiliensis* (see Table 2). These results are in agreement with the phytochemical screening.

Among the species with expressive contents of total phenols and flavonoids, besides the presence of anthraquinones, steroids, and cardiotonic heterosides, *C. erecta* stood out, reducing 33.20% of the mycelial growth of *M. phaseolina* at the concentration of 1600 µL 100 mL^{-1} and 37.4% at 1200 µL 100 mL^{-1} against *R. solani* (see Table 2).

Based on these results, it is possible to relate the inhibitory effect of this extract to its majoritarian constituents, which were higher than those detected in the ethanolic extract of *R. brasiliensis* (phenolic compounds and cardiotonic heterosides), with lower rates of mycelial growth against *C. erecta*, with 25.40% (800 µL 100 mL^{-1}) reduction of mycelial growth of *M. phaseolina*, and for *R. solani* the reduction was 28.5% in 800 µL 100 mL^{-1}. However, *R. brasiliensis* showed a lower percentage of inhibition compared to the two extracts at a lower concentration.

The fungicidal potential of phenolic compounds and flavonoids is mainly related to hydroxyl (OH) in their structures. Their position in methylated quantities and the number of substitutes in the aromatic ring formation (their esterified forms) make them more toxic, interfering in the fungus membrane (Falcão et al., 2013). Although information about the mechanism of action of the flavonoids is not well defined, Cushnie and Lamb (2005) pointed out that flavonoids have the ability to favor metabolic modifications in pathogens, acting in the inhibition of nucleic acid synthesis, cytoplasmic membrane function, and energy metabolism.

In this same line of activity are the anthraquinones, which constitute a large group of quinoid compounds. Based on a structure composed of three benzene rings, there are condensed aromatic groups (lipophilic groups) and carboxyl and carboxylic groups, as well as the presence of –OH, $–CH_3$, $–OCH_3$, $–CH_2OH$, –CHO, –COOH, or more complex groups. Anthraquinones are attributed the ability to act on membrane disruption and metabolic inhibition by irreversibly complexing with nucleophilic amino acids of the proteins, thus resulting in protein deactivation and loss of cellular function (Lucas et al., 2015; Fouillaud et al., 2016; Leyva et al., 2017). In addition, the oxidative potential of this class favors the triggering of cell apoptosis, an effect that occurs by intercalation between vicinal nucleotides of the DNA strand via ionic and van der Waals interactions with anthraquinones, blocking the polymerases and interfering with protein synthesis (Jampilek, 2016).

Another class found in the ethanolic extract of *C. erecta* refers to steroids, which have a hormonal function in plants. Traditionally, they are called phytosteroids and are related to increased plant resistance to pathogen infestation. Due to their hydrophobic characteristics, the probable mechanism of action is associated with their capacity to interact with the lipidic membrane wall and the cell membrane of the fungus, thus interfering in its permeability and causing structural changes. With this, it can affect both lipid synthesis and fungal cell wall formation (Tobouti et al., 2017; Diefenbach et al., 2018).

On the other hand, cardiotonic heterosides contain in their chemical structure a steroidal core and an unsaturated lactonic pentagonal ring at the C17 position (aglycone or genin portion), with hydrophobic characteristics. In addition to one or more sugar units attached to the hydrophilic C3, they act specifically on sodium and potassium channels (Simões et al., 2017).

Similarly, cardiotonic heterosides with hydrophilic characteristics, by containing one or more sugar units attached to the C3 of the steroidal core, this one attached to an unsaturated lactonic pentagonal ring at the C17 position (aglycone or genin portion), the hydrophobic part, act particularly on sodium and potassium channels (Rates and Bridi, 2017).

Furthermore, there are indications of the direct action of heterosides on the membrane, acting as a detergent, when the lipophilic portion of the heterosides forms a complex with cholesterol, the lipophilic portion of the membrane, and the hydrophilic portion outside the cell (Holm-Freiesleben and Jäger, 2014). It enables the formation of ion channels that destroy the osmotic integrity of the pathogen's cell membrane, leading to the loss of intracellular contents, such as intracellular K^+, and cell death (Lewis, 2011).

Therefore, it is possible to infer an antagonistic effect of constituents in the face of the complexity of a variety of metabolites with fungicidal potential and also the possibility of fungistatic activities. It demonstrates a tendency of competition among the phytoconstituents for the receptor sites of the target pathogens, thus preventing

the mycelial growth of the fungi at the five concentrations evaluated.

It is concluded that, of the investigated plants, the species *A. humille, B. dracuncufolia, C. erecta, D. insularis, P. ruderale*, and R. *brasiliensis* are potential sources of fungitoxic substances and, therefore, could be useful in tests with other phytopathogens.

The ethanolic extracts of *C. erecta* and *R. brasiliensis* are rich in polyphenols, efficient in reducing the mycelial growth of *M. phaseolina* and *R. solani*, and could be an alternative control agent.

Acknowledgements

To the Coordination of Superior Level Staff Improvement (CAPES), for granting the scholarship, and the financial support from the National Council for Scientific and Technological Development (CNPq), the Center for Research in the Pantanal (CPP), the Support Foundation for the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDETEC), the National Institute of Wetlands (INAU) and Universidade AnhangueraUniderp, for funding the Interdisciplinary Research Group (GIP) and Natural Products (PN). Thanks to the Federal Institute of Mato Grosso do Sul (IFMS).

References

ABREU-MATOS, F.J.A., 2009. *Introdução à fitoquímica experimental*. 3rd ed. Fortaleza: Edições UFC, 150 p.

ALAVIJEH, P.K., ALAVIJEH, P.K. and SHARMA, D., 2012. A study of antimicrobial activity of few medicinal herbs. *Asian Journal of Plant Science Research*, vol. 2, no. 4, pp. 496-502.

ANDRADE FILHO, N.N., ROEL, A.R., PORTO, K.R.A., SOUZA, R.O., COELHO, R.M. and PORTELA, A., 2010. Toxidade do extrato aquoso das folhas de *Anacardium humile* para *Bemisia turbeculata. Ciência Rural*, vol. 40, no. 8, pp. 1689-1694. http://dx.doi.org/10.1590/S0103-84782010005000125.

BOULOGNE, I., PETIT, P., OZIER-LAFONTAINE, H., DESFONTAINES, L. and LORANGER-MERCIRIS, G., 2012. Insecticidal and antifungal chemicals produced by plants: a review. *Environmental Chemistry Letters*, vol. 10, no. 4, pp. 325-347. http://dx.doi.org/10.1007/s10311-012-0359-1.

BRITO, N.M. and NASCIMENTO, L.C., 2015. Potencial fungitóxico de extratos vegetais sobre Curvularia eragrostidis (P. Henn.) Meyer in vitro. *Revista Brasileira de Plantas Medicinais*, vol. 17, no. 2, pp. 230-238. http://dx.doi.org/10.1590/1983-084X/10_057.

COMPANHIA NACIONAL DE ABASTECIMENTO – CONAB, 2018 [viewed 24 January 2019]. *Trabalho realizado pela Conab mostra tendências de mercado para a próxima safra* [online]. Available from: https://www.conab.gov.br/ultimas-noticias/2488-trabalho-realizado-pela-conab-mostra-tendencias-de-mercado-para-a-proxima-safra.html

CUSHNIE, T.P. and LAMB, A.J., 2005. Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, vol. 26, no. 5, pp. 343-356. http://dx.doi.org/10.1016/j.ijantimicag.2005.09.002. PMid:16323269.

DIEFENBACH, A.L., MUNIZ, F.W.M.G., OBALLE, H.J.R. and RÖSING, C.K., 2018. Antimicrobial activity of copaiba oil (*Copaifera* ssp.) on oral pathogens: systematic review. *Phytotherapy Research*, vol. 32, no. 4, pp. 586-596. http://dx.doi.org/10.1002/ptr.5992. PMid:29193389.

DOMENE, M.P., GLÓRIA, E.M., BIAGI, J.D., BENEDETTI, B.C. and MARTINS, L., 2016. Efeito do tratamento com óleos essenciais sobre a qualidade fisiológica e sanitária das sementes de milho (*Zea mays*). *Arquivos do Instituto Biológico*, vol. 83, no. 0, pp. 1-6. http://dx.doi.org/10.1590/1808-1657000072014.

DZUBAK, P., HAJDUCH, M., VYDRA, D., HUSTOVA, A., KVASNICA, M., BIEDERMANN, D., MARKOVA, L., URBAN, M. and SAREK, J., 2006. Pharmacological activities of natural triterpenoids and their therapeutic implications. *Natural Product Reports*, vol. 23, no. 3, pp. 394-411. http://dx.doi.org/10.1039/b515312n. PMid:16741586.

EDREVA, A., VELIKOVA, V., TSONEV, T., DAGNON, S., GÜREL, A., AKTAŞ, L. and GESHEVA, E., 2008. Stress-protective role of secondary metabolites: diversity of functions and mechanisms. *General and Applied Plant Physiology*, vol. 34, no. 1-2, pp. 67-78.

EKEKE, C. and OGAZIE, C.A., 2018. Phytochemical study on *Commelina diffusa* Burn. F. Subsp. Diffusa J. K. Morton and *Commelina erecta* L. (Commelinaceae). *Nigerian Journal of Life Sciences*, vol. 8, no. 1, pp. 74-85.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA – EMBRAPA, 2018 [viewed 14 August 2018]. *Soja em números (safra 2017/2018)* [online]. Available from: https://www.embrapa.br/soja/cultivos/soja1/dados-economicos

FALCÃO, S.I., TOMÁS, T., VALE, N., GOMES, P., FREIRE, C. and VILAS-BOAS, M., 2013. Phenolic quantification and botanical origin of Portuguese propolis. *Industrial Crops and Products*, vol. 49, pp. 805-812. http://dx.doi.org/10.1016/j.indcrop.2013.07.021.

FONTOURA, F.M., MATIAS, R., LUDWIG, J., OLIVEIRA, A.K.M., BONO, J.A.M., MARTINS, P.F.R.B., CORSINO, J. and GUEDES, N.M.R., 2015. Seasonal effects and antifungal activity from bark chemical constituents of *Sterculia apetala* (Malvaceae) at Pantanal of Miranda, Mato Grosso do Sul, Brazil. *Acta Amazonica*, vol. 45, no. 3, pp. 283-292. http://dx.doi.org/10.1590/1809-4392201500011.

FOUILLAUD, M., VENKATACHALAM, M., GIRARD-VALENCIENNES, E., CARO, Y. and DUFOSSÉ, L., 2016. Anthraquinones and derivatives from marine-derived fungi: structural diversity and selected biological activities. *Marine Drugs*, vol. 14, no. 4, p. 64. http://dx.doi.org/10.3390/md14040064. PMid:27023571.

GONÇALVES-TREVISOLI, E.D.V., FORLIN-DILDEY, O.D., BROTI-RISSATO, B., COLTRO-RONCATO, S., BROETTO, L., BARRIENTOS-WEBLER, T.F., KUHN, O.J. and STANGARLIN, J.R., 2017. Produtos fitossanitários não biológicos disponíveis e potenciais para agricultura. In: M. ALAVARSE-ZAMBOM, O.J. KUHN, N.L. SOARES-SILVA, J.R. STANGARLIN, R. VIANNA-NUNES, V.M. FÜLBER and C. EYNG, eds. Ética do cuidado, legislação e tecnologia na agropecuária. Marechal Cândido Rondon: Ciências Agrárias, pp. 155-177.

HOLM-FREIESLEBEN, S. and JÄGER, A.K., 2014. Correlation between plant secondary metabolites and their antifungal mechanisms-a review. *Medicinal & Aromatic Plants*, vol. 3, no. 2, p. 1000154.

JAMPILEK, J., 2016. Potential of agricultural fungicides for antifungal drug discovery. *Expert Opinion on Drug Discovery*, vol. 11, no. 1, pp. 1-9. http://dx.doi.org/10.1517/17460441.2016.1110142. PMid:26549424.

JURASEKOVA, Z., GARCIA-RAMOS, J.V., DOMINGO, C. and SANCHEZ-CORTES, S., 2006. Surface-enhanced Raman scattering of flavonoids. *Journal of Raman Spectroscopy*, vol. 37, no. 11, pp. 1239-1241. http://dx.doi.org/10.1002/jrs.1634.

KASAL, A., BUDESINSKY, M. and GRIFFITHS, W.J., 2010. Spectroscopic methods of steroid analysis. In: H.L.J. MAKIN and D.B. GOWER,

eds. *Steroid analysis*. Dordrecht: Springer, pp. 27-161. http://dx.doi.org/10.1023/b135931_2.

LEWIS, R.E., 2011. Current concepts in antifungal phasmacology. *Mayo Clinic Proceedings*, vol. 86, no. 8, pp. 805-817. http://dx.doi.org/10.4065/mcp.2011.0247. PMid:21803962.

LEYVA, E., LOREDO-CARRILLO, S.E., LÓPEZ-LÓPEZ, L.I., ESCOBEDO-AVELLANEDA, E.G. and NAVARRO-TOVAR, G., 2017. Importancia química y biológica de naftoquinonas. Revisión bibliográfica. *Afinidad*, vol. 74, no. 577, pp. 37-50.

LI, H.-B., WONG, C.-C., CHENG, K.-W. and CHEN, F., 2008. Antioxidant properties in vitro and total phenolic contents in methanol extracts from medicinal plants. *Lebensmittel-Wissenschaft + Technologie*, vol. 41, no. 3, pp. 385-390. http://dx.doi.org/10.1016/j.lwt.2007.03.011.

LIN, Y., LIN, H., LIN, Y., ZHANG, S., CHEN, Y. and JIANG, X., 2016. The roles of metabolism of membrane lipids and phenolics in hydrogen peroxide-induced pericarp browning of harvested longan fruit. *Postharvest Biology and Technology*, vol. 111, pp. 53-61. http://dx.doi.org/10.1016/j.postharvbio.2015.07.030.

LUCAS, N.C., FERREIRA, A.B.B. and NETTO-FERREIRA, J.C., 2015. Fotoquímica de naftoquinonas. *Revista Virtual de Química*, vol. 7, no. 1, pp. 403-463.

NASCIMENTO, L.S.N., RABELO, S.A.C., SILVA, G.R., NASCIMENTO, F.C. and SANTOS, R.C., 2016. Atividade biológica de *Davilla kunthii* A. St. –Hil. (Dilleniaceae). *Revista Brasileira de Plantas Medicinais*, vol. 18, no. 1, pp. 172-179. http://dx.doi.org/10.1590/1983-084X/15_051.

OLIVEIRA, A.J., GIMENES, D.C., HIROZOAWA, M., MACAGNAN, R. and ONO, E.Y.S., 2015. Potencial antagonista de leveduras frente a Fusarium graminearum. In: *Anais do V Simpósio de Bioquímica e Biotecnologia*, 5-7 August 2019, Londrina, Brazil. São Paulo: Blucher, vol. 1, no. 2, p. 382. http://dx.doi.org/10.5151/biochem-vsimbbtec-22196.

PEIXOTO SOBRINHO, T.J.S., SILVA, C.H.T.P., NASCIMENTO, J.E., MONTEIRO, J.M., ALBUQUERQUE, U.P. and AMORIM, E.L.C., 2008. Validação de metodologia espectrofotométrica para quantificação dos flavonoides de *Bauhinia cheilantha* (Bongard) Steudel. *Revista Brasileira de Ciências Farmacêuticas*, vol. 44, no. 4, pp. 683-689. http://dx.doi.org/10.1590/S1516-93322008000400015.

PÉREZ, R.L. and ESCANDAR, G.M., 2013. Spectrofluorimetric study of estrogen–cyclodextrin inclusion complexes in aqueous systems. *Analyst*, vol. 138, no. 4, pp. 1239-1248. http://dx.doi.org/10.1039/c2an36395j. PMid:23314132.

PIIRONEN, V., TOIVO, J. and LAMPI, A.-M., 2000. Natural sources of dietary plant sterols. *Journal of Food Composition and Analysis*, vol. 13, no. 4, pp. 619-624. http://dx.doi.org/10.1006/jfca.2000.0898.

RAMOS, K., ANDREANI JUNIOR, R. and KOZUSNY-ANDREANI, D.I., 2016. Óleos essenciais e vegetais no controle in vitro de Colletotrichum gloeosporioides. *Revista Brasileira de Plantas Medicinais*, vol. 18, no. 2, suppl. 1, pp. 605-612. http://dx.doi.org/10.1590/1983-084x/15_192.

RATES, S.M.K. and BRIDI, R., 2017. Heterosídeos cardioativos. In: C.M.O. SIMÕES, E.P. SCHENKEL, J.C.P. MELLO, L.A. MENTZ and P.R. PETROVICK, eds. *Farmacognosia: do produto natural ao medicamento*. Porto Alegre: Artmed, pp. 272-284.

SCHWAN-ESTRADA, K.R.F. and STANGARLIN, J.R., 2005. Extratos e óleos essenciais de plantas medicinais na indução de resistência. In: L.S. CAVALCANTI, R.M. PIERO, P. CIA, S.F. PASCHOLATI, M.L. RESENDE and R.S. ROMEIRO, eds. *Indução de resistência em plantas a patógenos e insetos*. Piracicaba: FEALQ, pp. 125-132.

SILVERSTEIN, R.M., WEBSTER, F.X., KIEMLE, D. and BRYCE, D., 2014. *Spectrometric identification of organic compounds*. 8th ed. New Jersey: John Wiley e Sons, 464 p.

SIMÕES, C.M.O., SCHENKEL, E.P., MELLO, J.C.P., MENTZ, L.A. and PETROVICK, P.R., 2017. *Farmacognosia: do produto natural ao medicamento*. Porto Alegre: Artmed, 502 p.

SOUSA, C.M.M., SILVA, H.R., VIEIRA-JUNIOR, G.M., AYRES, M.C.C., COSTA, C.L.S., ARAÚJO, D.S., CAVALCANTE, L.C.D., BARROS, E.D.S., ARAÚJO, P.B.M., BRANDÃO, M.S. and CHAVES, M.H., 2007. Fenóis totais e atividade antioxidante de cinco plantas medicinais. *Química Nova*, vol. 30, no. 2, pp. 351-355. http://dx.doi.org/10.1590/S0100-40422007000200021.

TOBOUTI, P.L., MARTINS, T.C.A., PEREIRA, T.J. and MUSSI, M.C.M., 2017. Antimicrobial activity of copaiba oil: A review and a call for further research. *Biomedicine e Pharmacotherapy*, vol. 94, pp. 93-99. http://dx.doi.org/10.1016/j.biopha.2017.07.092.

WAGNER, H. and BLADT, S., 2009. *Plant drug analysis: a thin layer chromatography atlas*. 2nd ed. New York: Springer, 384 p.

ZUANAZZI, J.A.S., MONTANHA, J.A. and ZUCOLOTTO, S.A., 2017. Flanonoides. In: C.M.O. SIMÕES, E.P. SCHENKEL, J.C.P. MELLO, L.A. MENTZ and P.R. PETROVICK, eds. *Farmacognosia: do produto natural ao medicamento*. Porto Alegre: Artmed, pp. 209-235.