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Biological Potential of Extracts of Caatinga Plants in the Control of *Alternaria alternata* f. sp. *citri* in Citrus

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Abstract

In Brazil the Caatinga biome is formed by endemic species with numerous applications in medicine, cosmetics and agrochemicals. However, only 8% of the country's plant species are chemically evaluated annually. Thus, the work aimed to determine the free amino acid composition and phenolic substances of Caatinga plant species extracts, their antioxidant activity and the potential use of these compounds in the control of *A. alternata* in citrus. Leaf extracts from fourteen native plant species were evaluated for free amino acid contents; total and individual polyphenols; total flavonoids and antifungal susceptibility test. The relationship between the contents of the compounds detected in each plant extract with its antioxidant capacity and antifungal activity, has not yet been fully clarified. However, it is a fact that the extract of *Cleome hassleriana* constitutes a source rich in free amino acids. It is also verified that the extracts of *Mimosa tenuiflora* and *Caesalpinia ferrea* are efficient alternatives in the control of *Alternaria alternata* in citrus, because they present similar results for this purpose, and in the evaluation of their compositions in free amino acids, phenolic substances and antioxidant activity.

Keywords: antifungal agents, active compounds, flavonoids, amino acids

1. Introduction

The global trade in herbal medicines is around US $ 83 billion. Nearly 25% of the industrialized compounds are derived from plants (Palhares et al., 2015). In Brazil, it is estimated that this market generates approximately US$ 160 million per year (Rodrigues, 2016), although the relevant Brazillian flora holds 67% of the world's plants only 8% of the plant species are chemically evaluated yearly (Lima et al., 2016).

The Caatinga biome is recognized by botanical biodiversity. It is formed by endemic species with pharmacological potential that can revolutionize several industrial areas (Silva et al., 2015a). In recent years, studies aimed at the identification of total bioactive components and individual phenolic compositions derived from plants have provided numerous applications in medicine, cosmetics and agrochemicals (Uysal et al., 2017).

Flavonoids, terpenes, phenols and alkaloids are secondary plant metabolites derived from amino acids, the main constituents of proteins (Hildebrandt et al., 2015). These substances are excellent natural antioxidants capable of eliminating free radicals. Moreover, they have healing, anti-inflammatory and antimicrobial properties (Borges et al., 2017).

Research results on the use of natural compounds for phytopathogen management, such as those reported by Melo et al. (2016), and Fonseca et al. (2015), with extracts of *Caesalpinia ferrea* Mart. Ex Tul. and *Porophyllum ruderale* (Jacq.) Cass. in the control of *Colletotrichum* sp. and *Sclerotinia sclerotiorum*, respectively. Villalobos et al. (2016), demonstrated the antimicrobial activity of isoflavones and phenolic acids present in plant extracts in the control of *Cladosporium uredinicola* in fruits.

Citrus is an important economic activity in Brazil, with the country being the world's largest producer, with an estimated production of 17.54 million tons of fruits, with a large predominance of sweet orange [*Citrus sinensis* (L.) Osbeck.]. Few varieties make up the Brazilian citrus industry, making crops vulnerable to phytosanitary problems, such as those caused by the brown spot of alternaria, disease caused by *Alternaria alternata* f. sp. *citri* present in all producing regions of the country (Yang et al., 2016).

Thus, considering that the Caatinga a biome occurs exclusively in Brazil, and is composed of species that may constitute rich sources of secondary metabolites (Burrows, 2001), the objective of this research was to determine the composition in free amino acids and phenolic substances of Caatinga plant extracts, their antioxidant and antimicrobial activities against *Alternaria alternata* f. sp. *citri*.

2. Material and Methods

Fresh leaves of 14 plant species native to Caatinga (Table 1) were collected in Boa Vista, (7º15′28″ S and 36º14′7″ W) county of the State of Paraíba, northeastern Brazil. The collected material was packed in polyethylene bags and taken to the Phytopathology Laboratory of the Federal University of Paraíba (UFPB, Campus II, Areia, county of the State of Paraíba, Brazil).

Table 1. Plant species used to obtain extracts, Areia, county of the State of Paraíba, Brazil 2018

Common name	Scientific name	Climate (BSh)*
Angico	*Anadenanthera macrocarpa* (Benth.)	
Catingueira	*Caesalpinia pyramidalis* Tul.	
Canafístula	*Peltophorum dubium* (Spreng) Taub.	
Jurema preta	*Mimosa tenuiflora* (Wild) Poir.	
Pau Ferro	*Caesalpinia ferrea* Mart. Ex Tul.	
Mulungú	*Erythrina velutina* Willd.	
Baraúna	*Schinopsis brasiliensis* Engl.	
Umbuzeiro	*Spondias tuberosa* L.	
Bom Nome	*Maytenus rigida* Mart.	
Feijão Bravo	*Capparis cynophallophora* L.	
Juazeiro	*Ziziphus joazeiro* Mart.	
Melão de São Caetano	*Momordica charantia* L.	
Mussambê	*Cleome hassleriana* L.	
Quixabeira	*Sideroxylum obtusifolium* (Roem. & Schult.) Penn.	

Note. *Climate classification according to Köppen and Geiger (1928). (http://pt.climate-data.org/country/114/)

BSh (a local steppe climate, with average rainfall less than 400 mm, average temperature in any month of the year exceeding 22.8 °C). (Source: Climate-data Org, 2015).

The leaves were washed in running water in the laboratory and disinfected in sodium hypochlorite solution (2%) for five minutes and put to dry at 25±2 °C for 24 hours. Thereafter, the leaves were placed in a forced circulation oven, at a temperature of 65±2 °C, until residual moisture was stabilized. Finally, they were ground in a knife mill in order to obtain powder, that is, Vegetable Raw Material (VRM), which was packed in plastic bags, identified and taken to the Phytochemicals Laboratory of the University of Trás os Montes and Alto Douro-UTAD, in Vila Real, county of the Portugal, to carry out the other experiments.

2.1 Determination of Free Amino Acid Content

The determination of free amino acid content was carried out in a HPLC-UV/VS detector, equipped with a C_{18} column (150 × 4.6 mm, 3 μm). Two solvents (A and B) were used as a mobile phase. They consisted of: (i) solvent A: mixture of 350 mM hydrogenphosphate disodium salt ($Na_2 \cdot HPO_4 \cdot 2H_2O$) p.a. (Riedel-de-Haen), 250 mM propionic acid (CH_3CH_2COOH, 79-09-4; Sigma-Aldrich) with the proportion of 1:1 ($^V/_V$), with acetonitrile (CH_3CN, gradient HPLC, Merck) and ultrapure water in the final ratio of 40:8:52 ($^V/_V$); (ii) solvent B: acetonitrile

mixture (CH_3CN, gradient HPLC, Merck), methanol (CH_3OH gradient HPLC, Merck) and ultrapure water in the ratio of 20:30:40 ($^{V}/_{V}$).

A binary gradient was used, with flow rate 1.3 ml/minute on a decreasing scale: at zero minutes 100% solvent A, at 9.5 minutes 89% solvent A, at 11 minutes 88% solvent A, at 13.6 minutes 80% solvent A, at 20.4 minutes 55% solvent A, at 23.4 minutes 50% solvent A, at 25.4 minutes 40% solvent A and at 32 minutes 0% solvent A and 100% solvent B, at 34 minutes 100% solvent A and at 37 minutes 100% solvent B. The quantification of the amino acid content was based on the external calibration, using standard (commercial) amino acid lines in different concentrations, with the results expressed in millimoles/100 grams dry weight.

2.2 Determination of Individual and Total Polyphenol and Total Flavonoid Contents

Leaf extracts were prepared with water extraction made from the VRM suspension in the ratio of 1:3, during 24 hours, with periodic agitation. The material was filtered on filter paper and the solution obtained was freeze-dried to obtain the crude powder extract, which was packed in plastic bags, identified and stored in a freezer at -20±2 °C until further analysis.

The total polyphenol content was determined by the Folin-Ciocalteau colorimetric method with modifications. Briefly, 0.5 ml of the solution (containing 1 mg/ml extract in methanol was added to 0.5 mL of the Folin-Ciocalteau 2N reagent and 1.0 mL of water. After 2 to 5 minutes, 0.5 mL of sodium carbonate (Na_2CO_3) was added to the tubes. After one hour incubation at room temperature, the resulting absorbance was measured at 760 nm using a spectrophotometer. Gallic acid in a concentration range of 2.5 to 12.5 µg/mL, dissolved in distilled water, was used to make the standard calibration curve. The total phenolic values were expressed as gallic acid equivalents (mg gallic acid/g dry weight).

The total flavonoid content was assessed by the colorimetric method with $AlCl_3$, based on the absorbance measured at 510 nm of the complex formed between the flavonoids existing in the samples and the aluminum present in the color reagent to form yellowish colored compounds. To this end, 1 mL of each previously prepared extract was added 4 mL of ultrapure water and 0.3 mL of 5% $NaNO_2$. The mixture was vortexed and allowed to stand for five minutes at room temperature. After this time, 0.3 mL of $AlCl_3$ was added. The mixture was allowed to stand at room temperature for 6 minutes. Thereafter, 2 mL of 1 M NaOH was added, the volume was made up to 10 mL with ultrapure water and the absorbances were read in a spectrophotometer at 510 nm. Simultaneously, a commercial catechin standard assay was prepared at different concentrations to find an equation that allowed quantification of the total flavonoid content. The equation found was $y = 2.5846 + 0.026x$ ($R^2 = 0.995$). The results were expressed as mg of catechin equivalents per gram of sample (mg of CAE/g dry weight).

2.3 Obtaining Fungal Inoculum

The inoculum of *A. alternata* was obtained by isolating citrus leaves with typical symptoms of MMA collected from sweet orange (*C. sinensis*) in the municipality of Tondela (40º31′22″ N and 08º04′11″ W), Portugal.

2.4 Antifungal Activity Test

The method of macrodilution for filamentous fungi, suggested by protocol M38-A of the National Committee for Clinical Laboratory Standards (NCCLS) was used to evaluate the the plant extract antifungal activity. The said method assessed the Minimum Inhibitory Concentration (MIC) and the Minimum Lethal Concentration (MLC) of the plant extracts under growth of *A. alternata*.

The RPMI 1640 (without bicarbonate, with glutamine and pH indicator) liquid culture medium was buffered with MOPS (3-N-morpholino-propanesulfonic acid), in the final concentration of 0.165 mol/l. The pH of the medium was adjusted with NaOH at 25±2 °C between 6.9 to 7.1, sterilized by filtration with milipore filter (with 0.25 µm membrane) and stored at 4±2 °C. For each assay the different dilutions of each vegetable extract were prepared and distributed in triplicate test tubes. Thereafter, the RPMI medium was added to give ten concentrations (163, 81.5, 40.7, 20.4, 10.2, 5.1, 2.55, 1.27, 0.64 and 0.32 µg/mL).

Fungal cultures, incubated for seven days of growth in Potato-Dextrose Agar (PDA) medium were used for the preparation of the cell suspensions. Convenient inoculum volumes were diluted in RPMI to obtain the final concentration of 2.5×105 spores/mL^{-1} and subsequently, 800 µl of the inoculum with culture medium was distributed in each of the test tubes that were already with the different plant extract dilutions. The médium growth and sterility were tested in the control treatments (Control: only with the culture medium and Control + with the fungus isolate). The tubes were incubated in a static oven at 25±2 °C for seven days. Thereafter the readings were taken to determine the Minimum Inhibitory Concentration (MIC) by visual comparison with the control tubes. 20 µL aliquots distributed in Petri dishes with SDA (Sabouraud Dextrose Agar) culture medium were withdrawn from each of the negative tubes to determine the Minimum Lethal Concentration (MLC), The

results were analyzed by checking the presence or absence of colonies of the fungus after seven days of incubation. All assays were performed in duplicate and repeated until concordant results were obtained.

2.5 Statistical Analysis

Data were analysed by the Analysis of Variance (NOVA) by the software SPPS v.17 (New Orchard Road, Armonk, New York, E.UA.), and the differences between means were separated by the Duncan test at 5% significance level.

3. Results and Discussion

C. hassleriana (extract 1), presented all the determined amino acids, with contents varying between 37.8 and 1433.9 mmol/100 g dry weight. Valine and Methionine, were detected exclusively in this species, whereas Tryptophan was observed only in *C. hassleriana* and *C. ferrea* (extracts 1 and 10 (Table 2). This is relevant because it indicates that the extract of *C. hassleriana* can constitute a rich source in amino acids, capable of acting as precursors in the biosynthetic compound synthesis. On one hand, Methionine is a precursor of ethylene, responsible for fruit maturation and plant senescence, on the other hand, Tryptophan, acts on the synthesis of indoleacetic acid, which promotes plant growth (Hildebrandt et al., 2015).

Table 2. Free content of amino acids in 14 plant species extracts, Areia, county of the State of Paraíba, Brazil, 2018

*Extracts	**Amino Acids Mmol/100 G Dry Weight							
	Tyr	Ala	Val	Gln	Leu	Met	Phe	Ile
1	1433.9 A	1312.8 A	1147.3 A	912.2 A	527.8 A	519.4 A	432.0 A	425.6 Ab
2	730.9 B	283.3 C	0 B	0.4 D	436.7 Ab	0 B	70.9 E	50.1 Ab
3	725.7 B	153.6 Cde	0 B	43.8 Bc	70.5 Bc	0 B	86.0 D	107.1 Ab
4	192.3 C	560.1 B	0 B	30.8 Bcd	179.7 Abc	0 B	160.9 C	108.9 Ab
5	160.8 Cd	152.3 Cde	0 B	10.1 Bcd	328.8 Abc	0 B	160.4 C	44.3 Ab
6	158.4 Cd	706.2 B	0 B	0 D	94.7 Bc	0 B	207.4 B	310.3 Ab
7	139.9 Cd	206.4 Cd	0 B	42.5 Bc	286.8 Abc	0 B	75.9 E	0 B
8	136.0 Cd	214.4 Cd	0 B	45.9 B	56.6 Bc	0 B	234.9 B	20.8 Ab
9	125.9 Cd	127.9 Cde	0 B	6.1 Cd	137.8 Abc	0 B	87.3 D	12.8 Ab
10	116.0 Cd	29.7 E	0 B	0 D	48.2 Bc	0 B	92.5 D	117.4 Ab
11	102.5 D	130.2 Cde	0 B	2.5 D	35.8 C	0 B	163.1 C	74.1 Ab
12	102.4 D	237.6 C	0 B	0 D	405.3 Abc	0 B	144.0 C	463.7 A
13	6.7 E	51.1 De	0 B	0 D	49.7 Bc	0 B	82.9 D	0 B
14	4.8 E	12.9 E	0 B	0 D	16.6 C	0 B	66.4 E	0 B
*Extracts	**Amino Acids Mmol/100 G Dry Weight							
	Gly	Asn	Arg	Thr	Ser	Glu	Asp	Trip
1	317.5 B	239.9 D	211.0 A	188.0 B	159.3 A	116.4 B	77.6 C	37.8 A
2	3.5 E	36.2 Fg	7.0 Bc	0 D	4.4 Cd	0 C	0 D	0 A
3	6.0 E	207.0 De	18.9 B	3.9 D	19.2 C	12.7 C	8.8 D	0 A
4	91.0 D	262.3 D	0 C	255.0 A	0 D	196.2 A	145.9 B	0 A
5	149.2 C	129.2 Fe	0.5 C	0 D	0.7 D	0 C	0 D	0 A
6	542.9 A	212.5 A	0 C	53.3c	81.0 B	84.1 B	274.6 A	0 A
7	22.1 E	237.4 D	0 C	0 D	0.1 D	6.3 C	0.1 D	0 A
8	18.5 E	777.2 B	4.7 C	0 D	5.8 Cd	6.1 C	5.8 D	0 A
9	10.3 E	93.3 Fg	0 C	0 D	10 Cd	0 C	0 D	0 A
10	38.8 E	260.5 D	0 C	0 D	0 D	1.7 C	14.5 D	40.5 A
11	20.3 E	490.1 C	1.2 C	0 D	0 D	0 C	0 D	0 A
12	16.0 E	426.6 C	4.3 C	3.4 D	10.3 Cd	4.8 C	9.9 D	0 A
13	0 E	259.5 D	0 C	0 D	0 D	0 C	0 D	0 A
14	0 E	0 G	0 C	0 D	0 D	0 C	0 D	0 A

Note. *Plant species: *C. hassleriana* (extract 1); *S. tuberosa* (extract 2); *M. tenuiflora* (extracto); *M. charantia* (extract 4); *S. brasiliensis* (extract 5); *P. dubium* (extract 6); *A. macrocarpa* (extract 7); *E. velutina* (extract 8); *C. cynophallophora* (extract 9); *C. ferrea* (extract 10); *C. pyramidalis* (extract 11); *Z. joazeiro* (extract 12); *S. obtusifolium* (extract 13); *M. rígida* (extract 14). **Amino acids: Asp: Aspartate; Glu: Glutamic acid; Asn: Asparagine; Ser: Serine; Gln: Glutamine; Gly: Glycine; Thr: Threonine; Arg: Arginine; Ala: Alanine; Tyr: Tyrosine; Val: Valine; Met: Methionine; Trip: Tryptophan; Phe: Phenylalanine; Ile: Isoleucine; Leu: Leucine. *** Means in a column followed by the same lower case letter are not significantly different at $P \leq 0.05$, by the Duncan's test.

The aromatic amino acids phenylalanine, tyrosine and tryptophan are produced in the shikimate pathway. They are directly involved in the formation of phenolics responsible for plant defense (Maeda & Dudareva, 2012). In this study, we found significant concentrations of tyrosine in extracts of *C. hassleriana*, *M. tenuiflora* and *S. tuberosa* with 1433.4, 730.9, 725.7 mmol/100 g of dry weight, respectively (Table 2). This molecule is a precursor of compounds, such as L-3,4-dihydroxyphenylalanine (L-DOPA), a substance found in leaf extracts of *Mucuna pruriens* (L.) var. and *Vicia faba* L., used in the synthesis of natural phytochemicals, destined to the control of pathogens and weeds (Soares et al., 2014).

The Caatinga plant species present physiological variations due to the severe climatic and environmental conditions, which together with other factors can influence the primary and secondary metabolites production quality and quantity (Silva et al., 2015b).

There is evidence that some genes encode anabolic amino acid enzymes in response to different abiotic stresses in plants (Maeda & Dudareva, 2012). Taiz and Zeiger (2013) emphasize that periods of high biosynthetic activity, caused by high temperatures, can induce the synthesis of amino acids, such as aspartate and glutamate, from organic acids originating from the Krebs Cycle.

Silva et al. (2009), reported an increase in the content of amino acids such as proline and glycine in response to water stress in the *S. tuberosa* leaves. These authors related this accumulation to drought tolerance and elimination of free radicals. However, in the literature there is a lack of information regarding the use of amino acids in crops, as well as research that relates the presence of these substances in the composition of native plants in Brazil, perhaps due to the small number of published works.

Another way to evaluate the bioactive components in plant extracts is the presence of phenolic compounds and the antioxidant activity of these substances. Thus, the results of this study show that the highest phenolic and total flavonoid contents were determined in *M. tenuiflora* and *C. ferrea* (extracts 1 and 2) and in *M. rigida* (extract 4), respectively (Table 3). Similarly, the extracts of *M. tenuiflora* and *C. ferrea* showed more efficient antioxidant activity, since the mean free radical sequestration was higher than 80%, when minimum inhibitory concentrations (IC 50) were used in values between 21 and 24 μg/ml of these compounds (Table 3).

Research results suggests positive correlation between antioxidant activity and total phenolic content (Camargo et al., 2016), due to its representativeness among plant bioactive substances, since 40% of the organic carbon present in the biosphere is in the form of phenolic compounds (Böttcher et al., 2008).

Table 3. Total phenolic average content (mg GAE/dry weight), total flavonoids (mg CAE/g dry weight) and plant extract antioxidant activity, Areia, county of the State of Paraíba, Brazil, 2018

*Extracts	Total Phenolic	Total Flavonoid	DPPH (%)	IC50 (μg/mL)
1	75.86 a	12.77 d	80.35 a	24.02 bc
2	73.70 a	15.34 c	80.26 a	21.11 abc
3	66.12 b	8.13 e	71.17 c	34.06 de
4	59.31 c	33.39 a	56.60 e	17.22 ab
5	57.44 c	17.34 b	58.51 d	12.04 a
6	39.52 d	1.91 h	38.90 f	118.33 g
7	31.16 e	13.21 d	72.84 c	42.34 e
8	23.05 f	6.42 f	75.09 b	76.23 f
9	15.54 g	6.53 f	60.06 d	24.17 bc
10	12.34 h	0.35 i	20.48 h	268.09 j
11	11.67 hi	3.11 g	55.76 e	76.21 f
12	11.15 jk	2.18 h	24.50 g	251.11 i
13	10.65 jk	0 i	24.50 g	170.24 h
14	4.71 l	0 i	13.08 i	NA**

Note. *Plant species: *M. tenuiflora* (extract 1); *C. ferrea* (extract 2); *S. brasiliensis* (extract 3); *M. rígida* (extract 4); *S. obtusifolium* (extract 5); *Z. joazeiro* (extract 6); *A. macrocarpa* (extract 7); *C. pyramidalis* (extract 8); *S. tuberosa* (extract 9); *P. dubium* (extract 10); *C. cynophallophora* (extract 11); *E. velutina* (extract 12); *C. hassleriana* (extract 13); *M. charantia* (extract 14). **NA: Extracts that did not present a percentage of inhibition superior to 50%. *** Means in a column followed by the same lower case letter are not significantly different at $P \leq 0.05$, by the Duncan's test.

The relevant levels among the individual polyphenols determined in *S. brasiliensis* (extract 1) were quercetin, catechin and petunidine (Table 4). The highest amounts of Isorhamnetin and hydroxycinnamic, ellagic and gallic acids were detected in *C. pyramidalis* (extract 2), and the latter compound was also highlighted in the samples of *S. tuberosa* and *C. ferrea* (extracts 4 and 6). It is also worth mentioning the higher content of other flavonoids, such as Kaemferol and ferulic acid (extracts 3 and 5, respectively) and malvidin, anthocyanin present in extracts of *M. tenuiflora* and *M. rigida* (extracts 3 and 7) (Table 4).

Table 4. Individual polyphenol avrage content in mg/100 g/dry weight, Areia, county of the State of Paraíba, Brazil, 2018

*Extracts	Polyphenol				
	Quercetin	Kaemferol	Gallic acid	Isorhamnetin	Catequin
1	148.09 a	0.92 d	1.08 d	-	46.67 a
2	25.76 b	-	9.32 a	32.96 a	22.09 b
3	15.52 c	49.87 a	4.08 b	8.59 c	-
4	20.31 bc	0.48 d	9.92 a	2.52 e	-
5	15.37 c	-	4.76 b	4.56 d	-
6	5.59 e	1.59 c	9.09 a	-	-
7	3.80 f	2.92 c	-	2.38 e	-
8	7.02 d	5.94 b	0.62 e	-	3.72 d
9	1.25 g	1.19 c	-	-	3.19 d
10	4.19 f	-	-	2.31 e	-
11	5.92 e	1.22 c	-	11.29 b	-
12	16.64 c	-	1.24 d	-	6.73 c
13	0.59 i	0.29 e	-	-	1.54 e
14	4.13 f	-	-	-	-

*Extracts	Polyphenol				
	Petunidin	Hydroxycinnamic Acid	Elagic Acid	Malvidine	Ferulic Acid
1	23.79 a	-	2.40 c	0.57 c	-
2	-	16.70 a	5.81 a	-	-
3	0.44 c	6.63 c	-	6.13 a	6.72 b
4	-	-	-	-	-
5	0.37 c	8.15 b	1.55 d	0.68 c	9.19 a
6	0.38 c	-	4.37 b	-	-
7	-	-	-	6.46 a	-
8	-	-	-	-	1.09 d
9	-	6.58 c	-	-	2.94 c
10	3.39 b	-	-	1.70 b	-
11	-	-	-	-	-
12	-	-	-	-	-
13	-	2.24 d	-	-	-
14	-	-	0.19 e	-	-

Note. *Plant extracts: *S. brasiliensis* (extract 1); *C. pyramidalis* (extract 2); *M. tenuiflora* (extract 3); *S. tuberosa* (extract 4); *P. dubium* (extract 5); *C. ferrea* (extract 6); *M. rigida* (extract 7); *Z. joazeiro* (extract 8); *C. hassleriana* (extract 9); *S. obtusifolium* (extract 10); *C. cynophallophora* (extract 11); *A. macrocarpa* (extract 12); *E. velutina* (extract 14); *M. charantia* (extract 14). ** Means in a column followed by the same lower case letter are not significantly different at $P \leq 0.05$, by the Duncan's test.

Phenolic compounds originate from secondary metabolism. The more importante are tannins, lignans, coumarins, lignins and flavonoids (Gomes et al., 2015). Among the various functions of these metabolites, it is worth noting their antimicrobial and antioxidant action, due to the absorption and neutralization of free radicals, chelating the triplet and singlet oxygen or decomposing peroxides (Olivoto et al., 2017).

Huber and Rodriguez-Amaya (2008), evaluating the extract of green tea leaves extracted from *Camellia sinensis* (L.) Kuntze, found that the antioxidant power of this species has been correlated to flavonoids such as quercetin, catechins, kaempferol, gallic acid and petunidine. These compounds were detected in the plant species evaluated

in this study, therefore, it is presumed that the antioxidant activity of their extracts is related to these substances. However, it can not be ruled out that flavonoids can act by reducing the oxidation rate in synergism with other phenolic compounds and not only attribute this property to this constituent, as verified by Zapata-Vahos et al. (2015).

In Table 5, the ethanolic extract of *C. ferrea* showed a greater fungitoxic capacity to the isolate of *A. alternata*, inhibiting its mycelial growth from the concentration of 5.1 µg/mL, being also lethal to the fungus in this same concentration. It was also verified that *S. brasiliensis* extract was efficient, inhibiting the sporulation of this fungus in the minimum dosage of 40.8 µg/mL, however, its lethal potential was only observed when the maximum concentration of 163 µg/mL. The extract of *M. tenuiflora* also showed promising results, with inhibitory and lethal potential starting from the concentration of 81.5 µg/mL.

The extracts of *C. cynophallophora*, *Z. joazeiro*, *S. obtusifolium* and *S. tuberosa* did not show inhibitory or fungicidal action against *A. alternata* (Table 5). Similar results were observed by Melo et al. (2016) when evaluating ethanolic extracts of *C. ferrea* in the control of *Colletotrichum* sp. in native fruits. Guimarães et al. (2015) studying the management of phytopathogens in agriculture, found that ethanolic extracts of *S. brasiliensis* were efficient as fungicides for *Fusarium solani* and bacteria of the genus *Xanthomonas, Pectobacterium* and *Ralstonia*.

Table 5. Minimum Inhibitory Concentration (MIC) and Minimum Lethal Concentration (MLC) in µg/mL of ethanolic plant extracts on *Alternaria alternata*, Areia, county of the State of paraíba, Brazil, 2018

Plant species	Ethanolic extract	
	MIC	MLC
C. ferrea	5.1	5.1
S. brasiliensis	40.8	163.0
M. tenuiflora	81.5	81.5
P. dubium	81.5	163.0
A. macrocarpa	81.5	163.0
E. velutina	163.0	163.0
C. hassleriana	163.0	-
M. charantia	163.0	-
M. rígida	163.0	-
C. pyramidalis	163.0	-
C. cynophallophora	-	-
Z. joazeiro	-	-
S. obtusifolium	-	-
S. tuberosa	-	-
*Positive Control	+	+
**Negative Control	-	-

Note. * Tube containing the culture medium with the fungus isolate. ** Tube containing only the culture medium.

On the other hand, by the results of Table 6, for the fungicidal potential of the natural compounds diluted in water, it is noted that unlike ethanol extraction (Tables 5), the antifungal action of *C. pyramidalis* extract was efficient inhibiting the growth of *A. alternata* at a concentration of 20.4 µg/mL water. It was also verified that the use of this extract diluted in the proportion of 40.8 µg/mL presented lethal activity to this pathogen. Again, the compounds from *C. cynophallophora*, *Z. joazeiro*, *S. obtusifolium* and *S. tuberosa*, were inefficient in fungal control, as well as the aqueous extracts of *E. velutina*, *C. hassleriana, M. charantia, M. rigida* and *A. macrocarpa* (Table 6).

Probably the toxicological and fungicidal activity of the extract of *C. pyramidalis* (Table 6), is related to its extraction in aqueous medium, since some flavonoids with antimicrobial action, such as quercetin, catechin, Isorhamnetina and other phenolics such as tannin present in their composition, are all soluble in water. Similarly, Barbosa Junior et al. (2015) evaluating Brazilian semiarid plants, verified that the aqueous extract of the leaves of *C. pyramidalis*, inhibited the growth of *Cryptococcus neoformans*, a microorganism that affects wild animals and fruit plant species.

Table 6. Minimum inhibitory Concentration Inibitória (MIC) and Minimum Lethal Concentration (MLC) in µg/mL of aqueous plant extracts on *Alternaria alternata*, Areia, state of Paraíba, Brazil, 2018

Plant species	Aqueous extracts	
	MIC	MLC
C. pyramidalis	20.4	40.8
S. brasiliensis	81.5	81.5
M. tenuiflora	81.5	81.5
P. dubium	81.5	-
C. ferrea	81.5	-
E. velutina	-	-
C. hassleriana	-	-
M. charantia	-	-
M. rígida	-	-
A. macrocarpa	-	-
C. cynophallophora	-	-
Z. joazeiro	-	-
S. obtusifolium	-	-
S. tuberosa	-	-
*positive Control	+	+
**Negative Control	-	-

Note. * Tube containing the culture medium with the fungus isolate. ** Tube containing only the culture medium.

Although the extracts of some plant species evaluated in this study did not present any inhibitory or lethal activity to *A. alternata*, there are reports of these products in the control of phytopathogens in agriculture. Lazzeri and Manici (2001) demonstrated the antimicrobial potential of leaves of *C. hassleriana* in the incidence of *Phytium* sp. in the soil. The efficiency of natural fungicides in fruit management was reported by Demartelaere et al. (2015), and Borges et al. (2013), in the control of *Colletotrichum gloesporioides* in papaya and *Alternaria cucumerina* in watermelon, using extracts of *M. charantia* and *M. tenuiflora*, respectively.

The extract properties may probably be attributed to the activity of the tannins, which complex proteins and metal ions and the inhibition of enzymes, providing antimicrobial effect (Borges et al., 2017). The flavonoids mechanism of action is probably their ability to inhibit the development of phytopathogens. These compounds form complexes with fungal cell wall proteins, degrading and/or preventing their synthesis, increasing the vulnerability of protoplasts by exposure to adverse environmental conditions, increasing membrane permeability, thus leading to cell lysis (Arif et al., 2009).

4. Conclusion

The relationship between the contents of the compounds detected in each plant extract with its antioxidant capacity and antifungal activity, has not yet been fully clarified. However, it is a fact that the extract of *Cleome hassleriana* constitutes a source rich in free amino acids. It is also verified that the extracts of *Mimosa tenuiflora* and *Caesalpinia ferrea* are efficient alternatives in the control of *Alternaria alternata* in citrus, because they present similar results for this purpose, and for evaluation of their compositions in free amino acids, phenolic substances and antioxidant activity. However, in spite of the promising results obtained in vitro tests, field studies are essential to confirm their potential for use in the control of this phytopathogen.

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