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Article

Phytochemical profile, evaluation of antimicrobial and antioxidant activity *in vitro* of the hydroalcoholic extract of two species of the genus Cyperus (Cyperaceae)

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Several factors contribute to the resistance of some pathogenic microorganisms and this fact requires the search for new therapeutic alternatives. The genus Cyperus (family Cyperaceae) groups species that present chemical compounds of pharmacological interest, mainly with antimicrobial action. Thus, the present work was carried out to investigate the antimicrobial activities, antioxidants and the phytochemical profile of *Cyperus articulatus* L. and *Cyperus iria* L. Hydroalcoholic extracts (1:1, v:v) of the aerial and underground parts of these species were used to analyze the total phenol content and to evaluate the *in vitro* antioxidant activity against the DPPH (2,2-diphenyl-1-picrylhydrazyl). The ethyl acetate and chloroform phases resulting from liquid-liquid partitioning of *C. articulatus* and *C. iria* extracts were evaluated in antimicrobial assays and subject to high performance liquid chromatography (HPLC-DAD) analysis. The chromatograms obtained by HPLC-DAD allowed us to identify four compounds: chlorogenic acid, catechin, quercetin, and quercitrin. The hydroalcoholic extracts of *C. articulatus* and *C. iria* showed a weak antioxidant activity with IC_{50} of 395.57 and 321.33 µg/mL (aerial parts), and 1,114.01 and 436.82 µg/mL (underground parts), respectively. Regarding antimicrobial activity, the chloroform phase of *C. iria* showed the best result at the concentration of only 31.2 µg/mL against the pathogens *Candida albicans* and *Staphylococcus aureus*. The ethyl acetate phases of the aerial parts of *C. articulatus* and *C. iria* did not show antimicrobial activity.

Keywords: Biological activities. Phytochemistry. Medicinal plants. *Cyperus articulatus*. *Cyperus iria*.

INTRODUCTION

Research using medicinal plants has significantly contributed to the development of new therapeutic strategies based on bioactive compounds (Firmo *et al.,* 2011). Plant drug use is common in developed and developing countries as homemade medicine, drugs and raw materials for the pharmaceutical industry, and represents a substantial proportion of products traded in the global market (Sivapalan, 2013). There is a growing interest in studies on medicinal plants used in traditional medicine that may be potential sources of new antimicrobial agents (Compean, Yalvez, 2014). This is because of the global spread of resistant microorganisms, which have caused a relevant biological risk, influencing our health and the health of the planet (Hernando-Amado *et al.,* 2019). Thus, the demand for natural antimicrobials has increased in recent decades, mainly for natural products extracted from plants that stand out for the potential of alternative treatments to bacterial control

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using less toxic and more efficient substances against bacterial resistance (Gomes *et al.,* 2018).

Species belonging to the Cyperaceae family represent an important source of active constituents with therapeutic properties (Gamal, Hani, Sabrin, 2015). In taxonomic terms, Cypereae is the second most diverse tribe and its largest genus, *Cyperus* (Linnaeus 1753:44) includes about 600 species, some of which are used in popular medicine (Larridon *et al.,* 2014; Reid, Carter, Urbatsch, 2014). The activities antioxidant and antimicrobial of some species of this genus have been widely studied and scientifically proven (Soumaya *et al.,* 2014; Essaidi *et al.*, 2014; Nassar *et al.,* 2015; Jing *et al.,* 2016). Such findings have raised the interest of the scientific community to continue the search for new substances for medical applications from the products obtained from *Cyperus* spp. The specie *Cyperus articulatus* L. popularly known as "priprioca" has been evaluated for its antibacterial (Oladosu *et al.,* 2011) and antimicrobial (Azzaz, El-Khateeb, Farag, 2014).

In addition to *C. articulatus*, some studies have reported that bioactive compounds identified in *C. rotundus* are extremely efficient when tested against pathogenic microorganisms (Sharma, Verma, Ramteke, 2014; Singh, Sharma, 2015). Regarding phytochemical findings, a wide spectrum of chemical compounds was isolated from the species *Cyperus rotundus* L. Some examples are: cyperotundol and methoxycyperotundol (Zhou, Yin, 2012), rotunduside A, B and C (Zhou, Zhang, 2013; Zhang *et al.,* 2014), cyperene-3,8-dione, cyperenol, cyperenoic acid, cyperusol C and D, α-rotunol (Xu *et al.,* 2015), cyperusphenol A, B, C and D (Ito *et al.,* 2012), α-cyperone (Jung *et al.,* 2013), cyperalin A (Ibrahim *et al.,* 2018) and 14-hydroxy-α-cyperone (Ahn *et al.,* 2015). In a wide bibliographic review, Peerzada *et al.* (2015) reported that the rhizomes and tubers of *C. rotundus* contain volatile oils, flavonoids, phenolic acids, coumarins, steroids and iridoid glycosides. Other studies were also conducted with *Cyperus esculentus* L. where identified the presence of well-known flavonoids such as quercetin and myricetin (Vega-Morales *et al.,* 2019).

As reported by some authors, pharmacological and chemical investigations of natural products obtained from plants are essential for the discovery of new therapeutic alternatives (Pereira, Cardoso, 2012; Silva *et al.,* 2014). To assist in this process, there are some types of specific approaches that guide the choice of potentially medicinal plants for the evaluation of their chemical constituents. The chemotaxonomic approach, for example, is characterized by the selection of species of a family or genus, for which there is some knowledge about the secondary metabolites of at least one species of the group (Albuquerque, Hanazaki, 2006; Silva *et al.,* 2013).

Knowing that the genus *Cyperus* groups species that may be sources of chemical compounds of pharmaceutical interest, highlighting *Cyperus rotundus*, which has been widely studied, including with proven activity against pathogenic microorganisms (Aeganathan *et al.,* 2015; Dadook, Mehrabian, Irian, 2016; Zhang *et al.,* 2017), it is likely that other species of the same genus also produce compounds with bactericidal and fungicidal action. Thus, the present research aimed to analyze antimicrobial and antioxidant activities and investigate the phytochemicals of *Cyperus articulatus* L. and *Cyperus iria* L.

MATERIAL AND METHODS

Botanical material

Cyperus articulatus L. and *Cyperus iria* L. were collected at the Serra Dois Irmãos, Viçosa, Alagoas, on January, 2018. *Exsiccatae* of the botanical material were identified by specialist Ana Paula do Nascimento Prata and deposited in the Herbarium of the Institute of the Environment of Alagoas, under the numbers MAC-64297 (*C. articulatus*) and MAC-64296 (*C. iria*).

Obtaining and concentrating extracts

Both aerial and underground parts of *C. articulatus* and *C. iria* were oven dried at 45°C and pulverized in a knife mill. The extraction was carried out by maceration using 10 g of powder of the species for 200 mL of hydroalcoholic solution (1:1, v/v). The botanical material in the form of hydroalcoholic extract was concentrated on a rotary evaporator at a constant temperature of approximately 60°C until complete evaporation of the solvent (Bezerra *et al.,* 2019).

Partitioning of bioactive extracts

In order to carry out the liquid-liquid partitioning process of the crude hydroalcoholic extract of *C. articulatus* and *C. iria*, a separation funnel was used according to the methodology proposed by Bezerra *et al.* (2019). A total of 20 mL of ethyl acetate was used for 20 mL of crude extracts of the aerial parts and 20 mL of chloroform for 20 mL of crude extracts of the underground parts. The phases resulting from the partitioning were obtained separately, reserved for the analyses in High Performance Liquid Chromatography (HPLC-DAD) and tested against pathogenic microorganisms.

Total phenols

For the determination of total phenolics, the Folin-Ciocalteu method was used as described by Waterman and Mole (1994), with some adaptations. Aliquots of the extracts of the aerial parts (AP) and underground parts (UP) of *C. articulatus* and *C. iria* at five different concentrations (AP: 50, 70, 90, 110, 130 µg/mL and UP: 300, 500, 700, 900, 1100 µg/mL). Subsequently, 0.25 ml of the Folin-Ciocalteu reagent was added and after 2 minutes, 1 mL of sodium carbonate (Na_2CO_3). The volume of each flask was completed with distilled water. Each solution was left to stand at room temperature protected from light and, precisely after 2 hours, its reading was taken in a spectrophotometer at 760 nm and compared with the standard curve of gallic acid at six concentration points 2, 4, 5, 6, 8 and 10 µg/mL. The equation obtained from the gallic acid standard curve was: $y = 0.1024x - 0.0164$, where y is the absorbance and x is the concentration; ($R^2 = 0.9775$). The total phenolic content was expressed in µg equivalent of gallic acid (EGA/µg) per µg of the extracts of *C. articulatus* and *C. iria*, considering their dry extract content (Bezerra *et al.,* 2019).

High Performance Liquid Chromatography (HPLC)

The separation of the bioactive compounds was carried out in High Performance Liquid Chromatography (HPLC) with ultraviolet detector (UV) and diode array (DAD), where ethyl acetate and chloroformic phases of *C. articulates* and *C. iria* were injected at a flow rate of 0.6 mL/min for 72 minutes using a Jupiter 5u C18 300A reverse phase column as stationary phase and a mixture of methanol, water and 0.1% trifluoroacetic acid as mobile phase, as described by Bezerra *et al.* (2019). Chromatograms were recorded at wavelengths at 254 nm. To identify the substances, an analytical standard was used specifying the retention time obtained from the sample and its respective wavelengths (Table I).

TABLE I - Standard used for identification of bioactive compounds by high performance liquid chromatography

Retention time (min)	Compounds	λ1 (nm)	λ2 (nm)	λ3 (nm)
23,39	Catechin	233	279	-
27,25	Chlorogenic acid	-	246	325
45,03	Quercitrin	-	256	349
48,18	Quercetin		255	369

In vitro antioxidant activity

Free radical scavenging (FRS) by the DPPH method was evaluated following the methodology of Mensor *et al.* (2001) with adaptations. To measure the scavenging capacity of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl), 2.0 mL of DPPH solution was inserted into a 5 mL flask. Subsequently, aliquots of the extracts of the aerial parts (AP) and underground parts (UP) of *C. articulatus* and *C. iria* were added at six different concentration points (AP: 700, 500, 300, 100, 80, 60 µg/mL and UP: 1400, 1200, 1000, 800, 600,

400 µg/mL). The final volume of the flask was filled with ethanol and after 30 minutes the absorbance was measured at 518 nm. Trolox® was used as a standard reference compound at the following concentrations: 20, 15, 10, 7.5, 5 and 2.5 µg/mL.

The DPPH radical scavenging capacity was calculated according to the equation: Radical scavenging capacity DPPH (%) = 100 - ((ABS sample - ABS white)*100)/ABS control)). Where: *ABS Sample = Absorbance of the sample solution in DPPH; ABS Control = Absorbance of reference solution in DPPH and ABS white = Absorbance of sample solution without DPPH.* The results concerning the antioxidant activity were expressed by means of the calculation of IC_{50} (inhibitory concentration), where the equation of the line referring to the absorbance values of the extracts was used, replacing the value of y with 50 to obtain the concentration of the sample with the capacity to reduce 50% of the DPPH radical. Assays were performed in triplicate and the value was expressed as mean ± standard deviation (SD).

In vitro antimicrobial activity

The efficiency of the ethyl acetate and chloroformic phases obtained from *C. articulatus* and *C. iria* were tested against the following pathogenic microorganisms: *Staphylococcus aureus* (Gram-positive bacterium); *Pseudomonas aeruginosa* (Gram-negative bacterium), and *Candida albicans* (fungus) (Bezerra *et al.,* 2019).

Minimum Inhibitory Concentration (MIC)

The serial microdilution technique was performed in triplicate, following the methodologies described by Sampaio *et al.* (2009), CLSI (2012), and Arendrup *et al.* (2012), with modifications. The ethyl acetate and chloroformic phases of the aerial and underground portions of *C. articulates* and *C. iria* were diluted in DMSO at 1% in H_2O at 1000 µg/mL. Subsequently, the phases were diluted into 96-well microplates containing 80 µL of Brain Heart Infusion (BHI) medium per well. Inoculations of *S. aureus* cells ATCC 27664, *P. aeruginosa* ATCC 25619 or *C. albicans* ATCC 36802 were standardized using the colony suspension method and the MacFarland 0.5 scale, as described in the protocols of CLSI (2012) and Arendrup *et al.* (2012). At each phase dilution, 20 µl of a microbial suspension containing 106 CFUm-1 of *S. aureus* ATCC 27664 or *P. aeruginosa* ATCC 25619 or 105 CFUmL-1 of *C. albicans* ATCC 36802 were added, thus obtaining the serial dilution, 15.62, 31.25, 62.50, 125, 250, 500, and 1000 µg/mL of the phases, in the final volume of 100 µL per well. As a negative control, the same microbial inocula were used in BHI broth without antimicrobials. The Minimum Inhibitory Concentration (MIC) was determined by spectrophotometry in an Elisa reader at 560-630 nm after 24 hours of incubation in aerophily at 37°C. The MIC was defined as the lowest concentration range of antimicrobial capable of inhibiting 100% of the microbial growth, in relation to the negative control.

Minimum Bactericidal and Fungicidal Concentration (MBC and MFC)

To determine the minimum bactericidal and fungicidal concentration (MBC and MFC), the methodology described by Lubian *et al.* (2010), with modifications. MBC and MFC were determined from the results obtained from MIC. A 50 µl aliquot of the wells that showed inhibition of the microorganisms was seeded onto the surface of petri dishes containing bromocresol green agar (BCG), mannitol salt agar and cetrimide selective agar for the development of *C. albicans*, *S aureus* and *P. aeruginosa*, respectively. The petri dishes were incubated at 37ºC for 24h. MBC and MFC were determined by the lower concentration of *C. articulatus* and *C. iria* phases that did not show bacterial and fungal growth. All tests were performed in triplicate.

Statistical analyzes

Statistical analyzes were performed using GraphPad Prism 5.0 software and Microsoft Excel® 2010 software. A means test was applied to differentiate the phenol content of *C. articulatus* and *C. iria* extracts. The comparison between the groups was performed through analysis of variance (ANOVA), considering all results with p below 0.05 using the Tukey test at 5% probability.

RESULTS AND DISCUSSION

Total phenols

The analyses regarding the quantification of the total phenol content of the hydroalcoholic extracts of the *C. articulatus* and *C. iria* aerial parts presented phenolic compound concentrations equivalent to 4.830 µg EAG/µg and 5.027 µg EAG/µg, respectively. Extracts of the underground parts of both species studied presented much lower levels, indicating a significant difference at the 5% probability level by Tukey test when compared with the aerial parts (Figure 1).

From data found in the literature, it was evidenced that the highest levels of phenolic compounds in *Cyperus rotundus* were identified in the extracts obtained from its leaves 136.0 mg 100 g^{-1}, while concentrations of this species below of 60 mg 100 g^{-1} were found in the tuber extracts (Quayyum *et al.,* 2000). Veber *et al.* (2015) also reported in their work that the 50% hydro-ethanolic extract of *Syzygium cumini* L. leaves presented the highest results of phenolic compounds at a concentration of 221.03 mg 100 g^{-1} in relation to the other types of hydro-ethanolic extracts at 25%, 75% and 95%. According to Storck *et al.* (2013), broccoli leaves have a total polyphenol content higher than 137.15 mg 100 g^{-1} compared with their stalk 41.40 mg 100 g^{-1}.

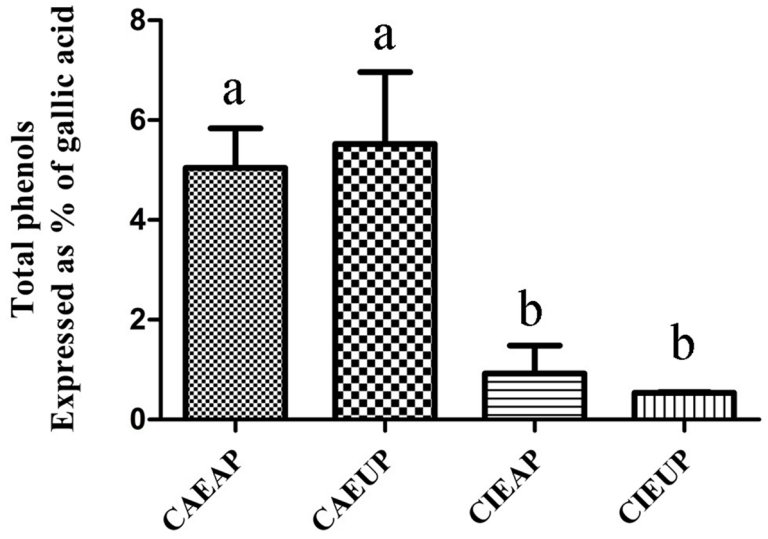

FIGURE 1 - Total phenol content of the hydroalcoholic extracts of *Cyperus articulatus* L. and *Cyperus iria* L.

CAEAP: *C. articulatus* extract - Aerial Part; CAEUP: *C. articulatus* extract – Underground part; CIEAP: *C. iria* extract - Aerial part; CIEUP: *C. iria* extract – Underground part.

Equal letters indicate that there is no significant difference and different letters indicate that there is significant difference between the groups according to the Tukey test. p value considered significant below 0.05.

High Performance Liquid Chromatography (HPLC)

From the standards used by the HPLC technique, it was not possible to identify the compounds that occur in the ethyl acetate phase of *C. articulatus* aerial parts (Figure 2). In order to identify unknown substances that occur in the highest intensity peaks of EAFCAAP, further analysis by means of infrared and nuclear magnetic resonance (NMR) techniques is necessary to elucidate the chemical structure of these compounds. The spectrometer-coupled HPLC can also be used to assist in this identification process.

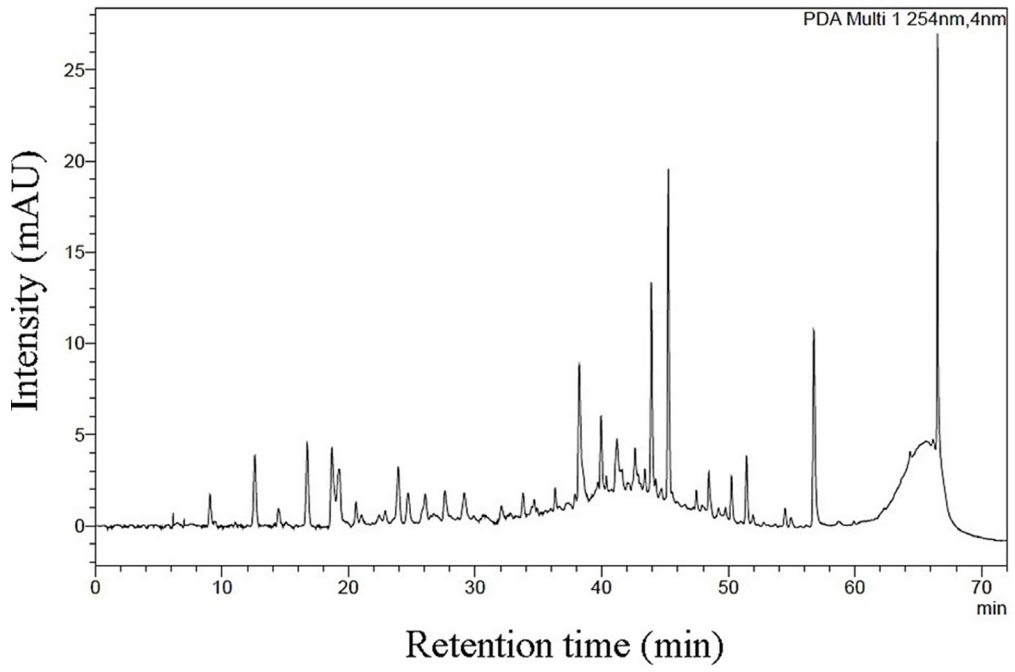

FIGURE 2 - Chromatographic profile of the ethyl acetate phase of the aerial parts of *Cyperus articulatus* L. (EAFCAAP) at 254 nm wavelength.

Through the chromatographic profile of the ethyl acetate phase of *C. iria* parts, it was possible to separate a polyphenol of pharmacological interest known as catechin (Figure 3). Importantly, chlorogenic acid was another substance separated in the EAFCIAP chromatogram by the techniques used.

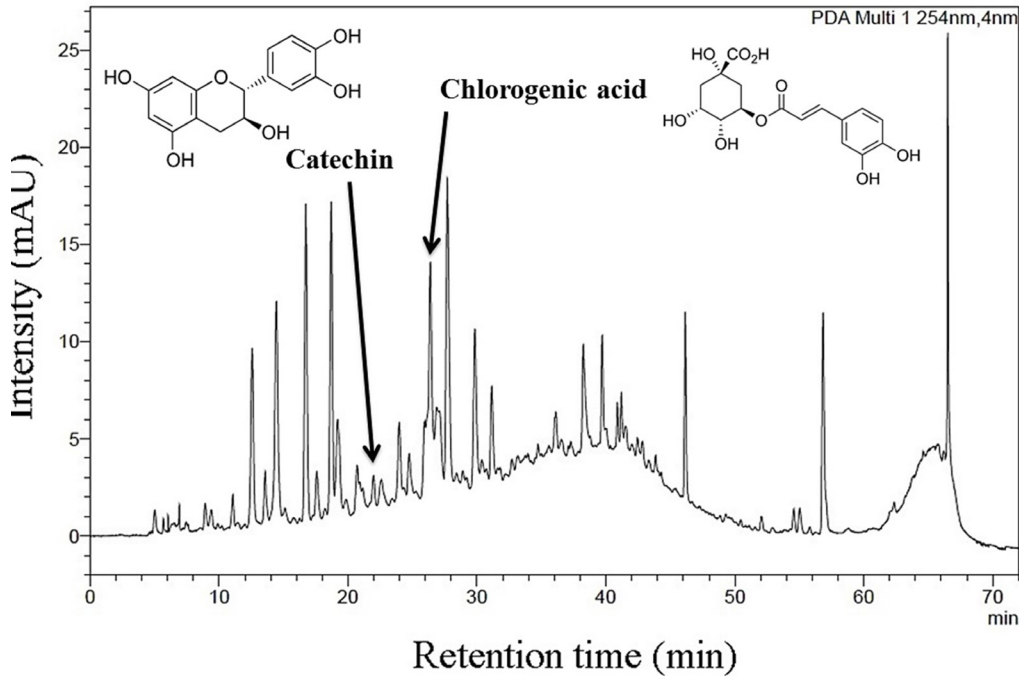

FIGURE 3 - Chromatographic profile of the ethyl acetate phase of the aerial parts of *Cyperus iria* L. (EAFCIAP) at 254 nm wavelength.

Regarding catechin, Senger, Schwanke, Gottlieb (2010) point out that the antioxidant activity associated with this substance can prevent oxidative stress-induced cytotoxicity in different tissues. Chlorogenic acid was another separate compound in EAFCIAP, and although its action is not lasting when isolated, it also contributes to the antioxidant activity of plant extracts (Sartori, Costa, Ribeiro, 2014). These reports found in the literature regarding the antioxidant potential of catechin and chlorogenic acid reinforce that extracts of *C. iria* aerial parts present bioactive compounds with high free radical scavenging capacity.

According to the chromatographic profile obtained from the chloroform phase of the underground parts of *C. articulatus*, it was not possible to identify the substances present in the highest intensity peaks (Figure 4). However, in the lowest intensity peaks of the analyzed sample, the compound known as chlorogenic acid was separated.

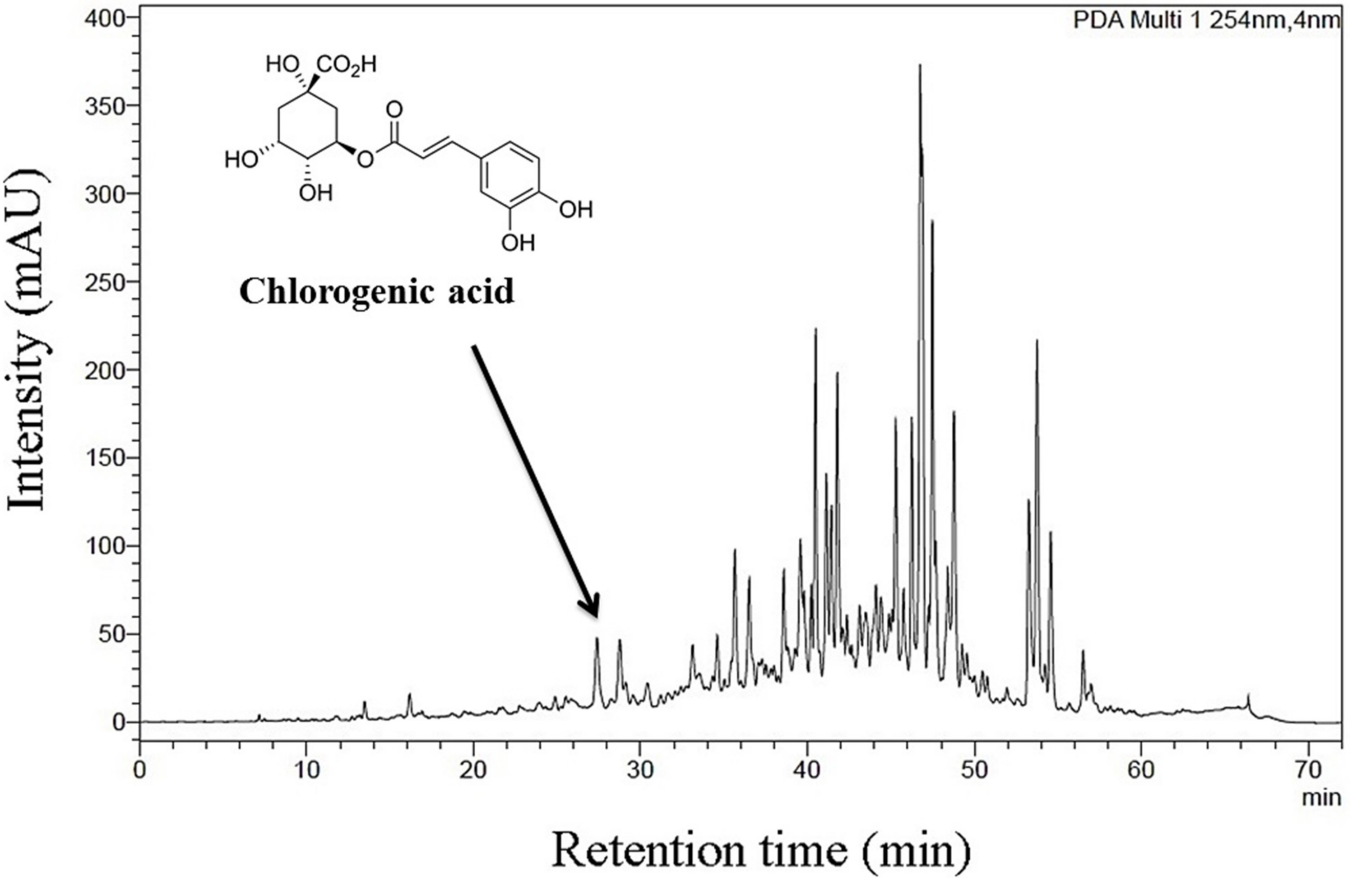

FIGURE 4 - Chromatographic profile of the chloroformic phase of the underground parts of *Cyperus articulatus* L. (CFCAUP) at 254 nm wavelength.

In addition to the chlorogenic acid separated on the CFCAUP chromatogram, Metuge *et al.* (2014) identified more than 80 compounds in the essential oil of *C. articulatus* rhizomes through analyses performed in gas chromatography coupled with mass spectrometer. The main chemical constituents reported by these authors were: monoterpenes, sesquiterpenes, hydrocarbons, fatty acids and fatty acid derivatives. Isolated secondary metabolites of *Cyperus rotundus* are widely used for therapeutic purposes, which is one of the most studied

species of the genus *Cyperus* (Peerzada *et al.,* 2015; Al-Snafi, 2016). According to Oliveira and Melo (2018), extracts of this plant have flavonoids, tannins, alkaloids, saponins, triterpenes and sterols, except leucoanthocyanidins.

The major compound separated in the chromatogram obtained from the underground chloroform phase of *C. iria* is quercitrin. This substance is characterized by being a glycoside formed from the flavonoid quercetin that also appeared in the analyzed sample (Figure 5).

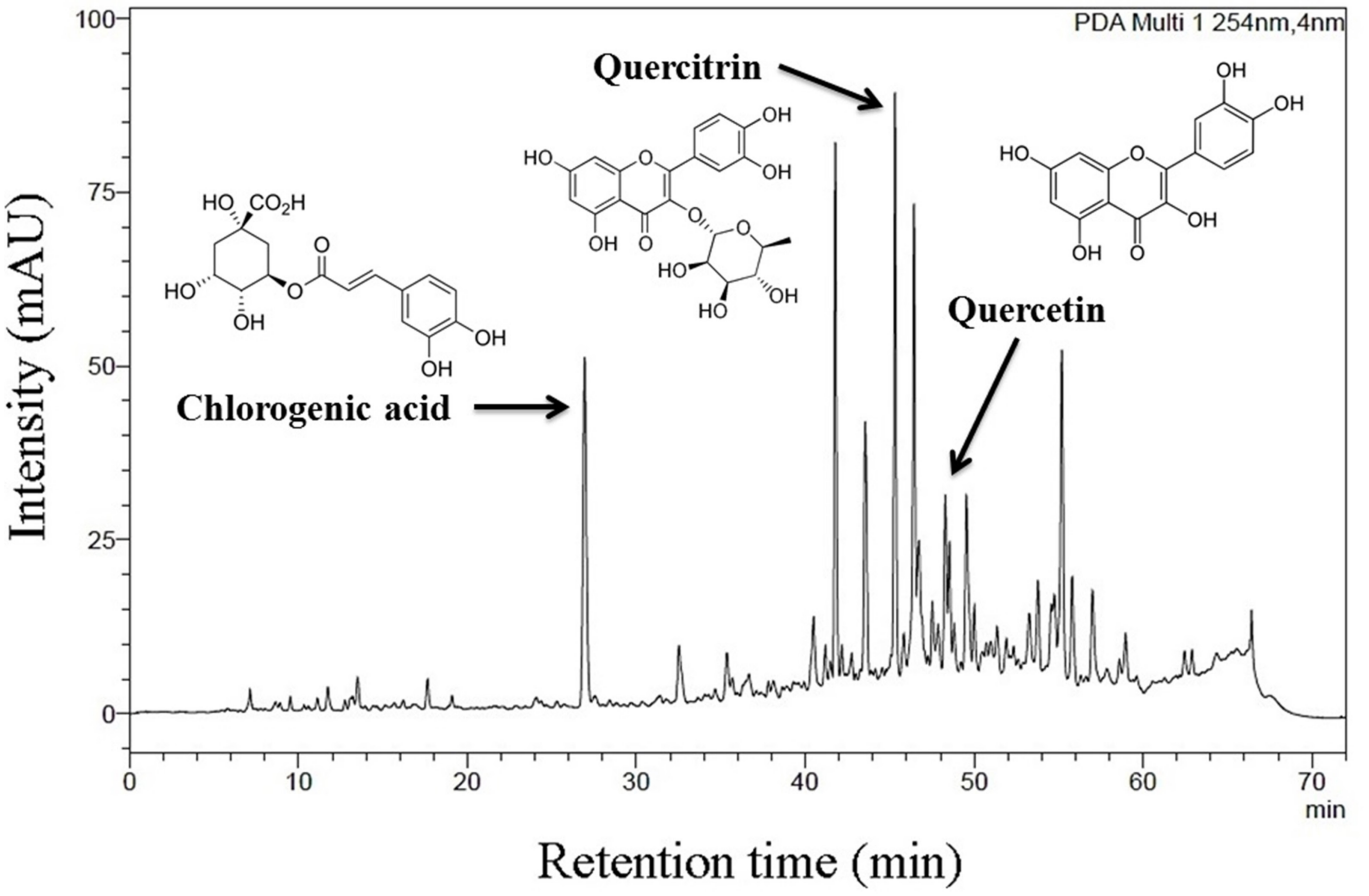

FIGURE 5 - Chromatographic profile of the chloroformic phase of the underground parts of *Cyperus iria* L. (CFCIUP) at 254 nm wavelength.

In studies by Vechia *et al.* (2016), quercitrin was found to be a major substance identified in hydroalcoholic extracts of aerial parts of the species *Solidago chilensis* (Asteraceae), and this constituent is identified by chromatographic and spectroscopic methods. Quercitrin-related therapeutic properties have been described by Ballester *et al.* (2006), demonstrating that this substance was able to completely restore intestinal hydroelectrolytic transport, which resulted in a reduction in the incidence of diarrhea, one of the symptoms that characterizes intestinal inflammation.

According to Jiménez, Martínez, Fonseca (2009), quercetin is a flavonoid that meets the ideal requirements for effective antioxidant function because its potential is five times greater than vitamins C and E. Antimicrobial activity is another benefit associated with quercetin. Camargo and Raddi (2008) reported that 120 µg/mL of this flavonoid demonstrated 70% inhibition of *S. aureus* growth compared with control.

In vitro antioxidant activity

Based on the IC_{50} values obtained from antioxidant activity, which expresses the amount of drug needed to reduce the initial DPPH concentration by 50% (Lôbo *et al.*, 2010), it was evidenced that Trolox® had the best results compared with the samples of the analyzed extracts, requiring a concentration of only 1.8 µg/mL for the scavenging activity of DPPH radicals to occur. These results were expected as Trolox® is widely used as a reference standard for *in vitro* antioxidant testing (Chaudhuri *et al.,* 2012; Felhi *et al.,* 2017; Jaradat *et al.,* 2017).

Regarding the evaluation of samples of hydroalcoholic extracts of the aerial parts of *C. articulatus* and C. *iria*, the antioxidant activity was verified at concentrations of 395.57 µg/mL and 321.33 µg/mL, respectively. The extracts of the underground parts of the two species studied presented values above 400 µg/mL for the same reduction of the DPPH radical and, therefore, showed an antioxidant activity lower than the aerial parts (Table II). Forero-Doria *et al.* (2014) also elucidated antioxidant properties in *Cyperus digitatus* Roxb. indicating that the extracts of this plant may be useful in preventing the progress of various oxidative stress related disorders.

TABLE II - *In vitro* antioxidant activity of the hydroalcoholic extracts *of Cyperus articulatus* L. and *Cyperus iria* L.

Hydroalcoholic extracts – AP	**IC_{50} – µg/mL**	**AA (% ± SD)[1]**
C. articulatus	395.57	52.32 ± 0.03
C. iria	321.33	48.74 ± 0.06
Hydroalcoholic extracts – UP	**IC_{50} – µg/mL**	**AA (% ± SD)[1]**
C. articulatus	1,114.01	24.51 ± 0.18
C. iria	436.82	42.95 ± 0.10
Reference Standard	**IC_{50} – µg/mL**	**AA (% ± SD)[1]**
Trolox®	1.8	92.45 ± 0.10

AP: Aerial part; UP: Underground part; AA: Antioxidant activity; [1]Average ± standard deviation

It is likely that the antioxidant activity of *C. articulatus* and C. *iria* extracts head-on the DPPH radical is related to the high phenol content identified in the aerial parts of these species, as previously observed in Figure 1. According to Abrahão *et al.* (2010), the free radical scavenging capacity associated with phenolic compounds is mainly due to their reducing properties and chemical structure. Regarding flavonoids, the most studied group among phenolic compounds, Almeida *et al.* (2010) explain that the antioxidant effects of these substances are attributed to the ability to capture and neutralize oxidizing species such as superoxide anion (O_2), hydroxyl radical or peroxide radical, acting by synergism with other antioxidants such as vitamins C and E (Simões, Almeida, 2015).

Lee and Park (2019) evidenced that quercetin was more effective than quercitrin and avicularin in terms of DPPH free radical scavenging capacity, reactive oxygen species (ROS) scavenging capacity, and constant rate of $^1O_2$ extinction. According to Li *et al.* (2016), a DPPH elimination assay confirmed that quercitrin and isoquercitrin could efficiently eliminate DPPH radicals. This result implies that quercitrin and isoquercitrin exert antioxidant activities when subjected to direct reactions for the elimination of radicals. Chlorogenic acid, a compound also identified in the aerial and underground parts of *C. articulatus* and *C. iria*, has promising antioxidant properties. Santana-Gálvez, Cisneros-Zevallos, Jacobo-Velázquez (2017) reported that this compound can be a basis substance

for food preservation, particularly for the inhibition of lipid oxidation.

In vitro antimicrobial activity

Comparing the results regarding microdilution of the ethyl acetate phases of the aerial parts of the studied species, it was found that only *C. articulatus* showed inhibitory activity against *Staphylococcus aureus* at MIC of 125 µg/mL (Table III). Oladosu *et al.* (2011) suggested in their study that *C. articulatus* essential oils can be used in formulations for the development of antimicrobial drugs. According to these authors, the determination of the minimum inhibitory concentration using the agar oil dilution method presented MIC of 14 µg/mL for *S. aureus* and 10 µg/mL for *P. aeruginosa*. In a preliminary antibacterial screening performed by Kilani-Jaziri *et al.* (2011), ethyl acetate extract from *Cyperus rotundus* was shown to be effective against *Staphylococcus aureus*, *Escherichia coli, Salmonella typhimurium, Salmonella enteritidis* and *Enterococcus faecalis* at MIC of 2.5 mg/mL. Antibacterial activity regarding *C. rotundus* was also highlighted by Singh and Sharma (2015).

TABLE III - Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal and Fungicidal Concentration (MBC and MFC) of the ethyl acetate and chloroform phases of the aerial and underground parts of *Cyperus articulatus* L. and *Cyperus iria* L.

Strains	**EAFCAAP (µg/mL)**	**CFCAUP (µg/mL)**	**EAFCIAP (µg/mL)**	**CFCIUP (µg/mL)**
	MIC	**MIC**	**MIC**	**MIC**
Candida albicans	-	62.5	-	31.2
Staphylococcus aureus	125	125	-	31.2
Pseudomonas aeruginosa	-	250	-	250
	MFC	**MFC**	**MFC**	**MFC**
Candida albicans	-	125	-	31.2
	MBC	**MBC**	**MBC**	**MBC**
Staphylococcus aureus	-	500	-	250
Pseudomonas aeruginosa	-	250	-	250

EAFCAAP: Ethyl Acetate Phase of *C. articulates*; CFCAUP: Chloroformic Phase of *C. articulatus* - Underground part; EAFCIAP: Ethyl Acetate Phase of *C. iria* - Aerial part; CFCIUP: Chloroformic Phase of *C. iria* – Underground part; MIC: Minimum Inhibitory Concentration; MFC: Minimum Fungicidal Concentration; MBC: Minimum Bactericidal Concentration

The best MIC and MFC results were evidenced in CFCIUP. This chloroform phase showed bacteriostatic and fungistatic effects against *Candida albicans* and *Staphylococcus aureus* pathogens, inhibiting the growth of these microorganisms in the MIC of 31.2 µg/mL. For MFC, a concentration of only 31.2 µg/mL was also required to induce the fungicidal effect on *C. albicans* yeast. All these data indicate that *C. iria* underground parts can be an important source of natural products, such as quercetin, a compound identified in high performance liquid chromatography assays (Figure 5), which has a broad spectrum of activity against pathogenic microorganisms (Camargo, Raddi, 2008). In a phytochemical study by Kishore and Alluraiah (2013), it was possible to evidence the presence of bioactive compounds such as anthraquinones, flavonoids, steroids and phenols in *C. iria*, which have efficient properties in the treatment of microbial diseases. Antifungal activity

evaluations of alkaloids extracted from *C. iria* were performed by Zhou *et al.* (2010), the authors pointed out that these compounds have been shown to be effective against the tested microorganisms.

Gram-negative bacterium *Pseudomonas aeruginosa* showed greater resistance to the chloroform phases of the underground parts of *C. articulatus* and *C. iria*, requiring MIC and MBC of 250 µg/mL for the bacteriostatic and bactericidal effect of this pathogen to occur. In a study by Bezerra *et al.* (2019), the chloroform phases of the underground parts of *Kyllinga odorata* Vahl and *Oxycaryum cubensis* Poepp. & Kunth were responsible for inhibiting this Gram-negative bacterium at the minimum inhibitory concentrations of 500 µg/mL and 62.5 µg/mL, respectively. According to Kobayashi, Sadoyama, Vieira (2009), resistance conferred by *P. aeruginosa* presumes the difficulty of establishing which antimicrobial combination options for the treatment of severe infections caused by this pathogen. Figueiredo *et al.* (2007) also state that *P. aeruginosa* is a multi-drug resistant Gram-negative bacterium.

Chlorogenic acid, a substance identified in the aerial and underground parts of *C. articulatus* and *C. iria*, has been reported in the literature as having activity against a wide range of microorganisms, including bacteria, yeasts, fungi, viruses, and amoebas (Santana-Gálvez, Cisneros-Zevallos, Jacobo-Velázquez, 2017). These antimicrobial properties can be useful for the food industry in its constant search for new, natural molecules for the preservation of food products (Santana-Gálvez, Cisneros-Zevallos, Jacobo-Velázquez, 2017). Su *et al.* (2019) reported that chlorogenic acid increased the permeability of the intracellular membrane and induced the exfoliation of the outer membrane in *P. aeruginosa*. Thus, damages to intracellular and external membranes, as well as disruption of cellular metabolism, resulted in death. In addition to chlorogenic acid, a study on quercetin showed a broad-spectrum antibacterial activity against bacterial pathogens. According to Gopikrishnan *et al.* (2017), quercetin inhibited gram-positive bacteria at a concentration of 6.3 µg/mL, considering that the MIC for gram-negative bacteria varies between 12.5 and 50.0 µg/mL.

CONCLUSION

Cyperus articulatus and *Cyperus iria* presented chemical compounds of great pharmacological importance, such as: chlorogenic acid, catechin, quercetin and quercitrin. The hydroalcoholic extracts of the aerial and underground parts of both species showed low antioxidant activity by the DPPH method. The chloroform phases of the underground parts of *C. articulatus* and *C. iria* showed inhibitory activity when tested against *C. albicans, S. aureus* and *P. aeruginosa*, indicating that the studied species are sources of antibacterial and antifungal compounds.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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