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Phytochemical screening, chromatographic profile and evaluation of antimicrobial and antioxidant activities of three species of the Cyperaceae Juss. Family

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Some species of the family Cyperaceae have been studied extensively for presenting bioactive compounds of pharmacological interest. Thus, the present research aimed to investigate the antimicrobial, antioxidant and chemical constituents of *Kyllinga odorata* Vahl, *Oxycaryum cubensis* Poepp. & Kunth and *Rhynchospora nervosa* Boeck. Hydroalcoholic extracts (1:1, *v:v*) of aerial and underground parts. These species were used for analysis of phytochemical prospection, quantification of total flavonoid and phenol content, and for evaluation of *in vitro* antioxidant activity against DPPH radical (2,2-diphenyl-1-picrylhydrazyl). The ethyl acetate and chloroform phase resulting from the liquid-liquid partitioning of the extracts of *K. odorata*, *O. cubensis* and *R. nervosa* were evaluated in *in vitro* antimicrobial assays and analyzed by high performance liquid chromatography (HPLC-DAD) identification of chemical substances. From the chromatograms obtained by HPLC-DAD, substances of great pharmacological importance were identified, such as: chlorogenic acid, myricitrin, catechin, apigenin, quercetin, luteolin, chrysin and rutin. The hydroalcoholic extract of the aerial parts of *R. nervosa* had the highest content of flavonoids (1.521 µg EQ/µg) and total phenols (5.020 EGA/µg), suggesting a direct relation with the excellent antioxidant activity of this species (IC_{50} = 122.11 µg/ml). It was evidenced that the chloroform phase of *O. cubensis* showed the best result, inhibiting the growth of *Candida albicans, Staphylococcus aureus* and *Pseudomonas aeruginosa* with the minimum inhibitory concentration of 15.6, 31.2 and 62 µg/ml, respectively.

Key words: Cyperaceae, biological activities, natural products, medicinal plants.

INTRODUCTION

The Cyperaceae family has a cosmopolitan distribution and plays a dominant role in wetland vegetation (Larridon et al., 2013). This family is represented by about 5,000 species distributed among 104-122 genera (Jung and

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Choi, 2013). Although there are no endemic genera of Cyperaceae in Brazil, it is estimated that 203 are endemic Brazilian species, of which 40 belong to *Rhynchospora*, 28 to *Cyperus*, 25 to *Scleria*, 22 to *Bulbostylis*, 19 to *Pleurostachys*, 16 to *Eleocharis* and 11 to *Hypolytrum* (Alves et al., 2009).

According to Martins et al. (2013), species of the family Cyperaceae have scientifically proven biological activities *in vivo* and *in vitro*, being related to the high content of flavonoids and phenols that occur in these plants. Adeniyi et al. (2014) report that extracts obtained from some species of this family have compounds with antimicrobial properties that can be used in the development of new drugs for the treatment of infectious diseases caused by pathogens.

Among the species of Cyperaceae cited in the literature as possible medicinal plants, the *Kyllinga odorata* rhizome has been used in Paraguay as a medicine, and that flavones and tannins would be responsible for the diuretic, antispasmodic, antidiarrheal and astringent activity attributed to this species (González et al., 2009). Pagning et al. (2016) point out that extracts and compounds isolated from a plant of the genus *Rhynchospora* (*Rhynchospora corymbosa*) have antimicrobial activity against sensitive microorganisms.

Despite the scientific evidence on the therapeutic use of some species of Cyperaceae, other representatives, such as those of the genus *Oxycaryum*, for example, have not yet had their medicinal potential scientifically tested, requiring further research. Carneiro et al. (2014) emphasize that multidisciplinary studies regarding potentially medicinal plants are essential, since there is a great need for research regarding isolation, purification, characterization of active principles, and pharmacological investigation of extracts. In this way, the present work was carried out aiming to analyze the chemical constituents and evaluate the antimicrobial and antioxidant activities of the extracts of *Kyllinga odorata* Vahl, *Oxycaryum cubensis* Poepp. & Kunth and *Rhynchospora nervosa* Boeck.

METHODOLOGY

Botanical material

Specimens of *Kyllinga odorata* Vahl; *Oxycaryum cubensis* Poepp. & Kunth and *Rhynchospora nervosa* Boeck were collected at the Agricultural Sciences Center of the Federal University of Alagoas between August and September, 2017. *Exsiccatae* of the botanical material were identified by teacher Ana Paula do Nascimento Prata and deposited in the Herbarium of the Institute of the Environment of Alagoas, under the numbers MAC-64293 (*K. odorata*), MAC-64295 (*O. cubensis*) and MAC-64294 (*R. nervosa*).

Obtaining and concentrating extracts

Both aerial and underground parts of *K. odorata, O. cubensis* and *R. nervosa* 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.

Partitioning of bioactive extracts

In order to carry out the liquid-liquid partitioning process of the crude hydroalcoholic extract of *K. odorata, O. cubensis* and *R. nervosa*, a separation funnel was used. 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.

Prospecting phytochemistry (screening)

The accomplishment of the phytochemical triage was based on the methodology proposed by Matos (1997). From the sample of crude extracts obtained from *K. odorata, O. cubensis* and *R. nervosa*, a total of 35.0 ml were set apart for phytochemical prospection, divided into seven 3.0 ml portions in test tubes numbered from "1" to "7". The qualitative and semi-quantitative tests were carried out, covering trials for phenols and tannins (by reaction with ferric chloride), anthocyanins, catechins and flavonoids (by pH variation test with sodium hydroxide and hydrochloric acid), and alkaloids (identification with Dragendorff) .

Total flavonoids

The total content of flavonoids was determined using the spectrophotometric method with 5% aluminum chloride ($AlCl_3$) reaction in methanol (Alves and Kubota, 2013). A total of 100 µl of 5% aluminum chloride was added to a 5 ml volumetric flask, then aliquots of the extracts of the aerial parts (AP) and underground parts (UP) of *K. odorata* (AP: 1212, 1010, 808, 606, 404 µg/ml and UP: 3258, 2896, 2534, 2172 and 1810 µg/ml), *O. cubensis* (AP: 3392, 2968, 2544, 2120 and 1696 µg/ml and UP: 2178, 1936, 1694, 1452 and 1210 µg/ml) and *R. nervosa* (AP: 819, 702, 585, 468 and 351 µg/ml and UP: 1674, 1488, 1302, 1116 and 930 µg/ml) at five different concentration points. The final volume of the flask was adjusted with methanol and after 30 min the absorbance was measured at 425 nm.

Total flavonoid content was determined using a standard quercetin curve at seven points of concentrations 4, 5, 6, 7, 8, 9 and 10 µg/ml. The equation obtained from the quercetin standard curve was: $y = 0.0987x - 0.0541$, where y is the absorbance and x is the concentration; ($R^2 = 0.9993$). The total content of flavonoids was expressed as µg of quercetin equivalents (EQ/µg) per µg of the extracts of *K. odorata, O. cubensis* and *R. nervosa*, considering their dry extract content.

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 *K. odorata* (AP: 280, 420, 560, 700 and 840 µg/ml and UP: 452, 543, 633, 724 and 814 µg/ml), *O. cubensis* (AP: 255, 383, 510, 638 and 766 µg/ml and UP: 363, 423, 484, 544 and 605 µg/ml) and *R. nervosa* (AP: 76, 95, 114, 133 and 152 µg/ml e UP: 325, 372, 418, 465 and 511 µg/ml) at five different concentrations. Subsequently, 0.25 ml of the Folin-Ciocalteu reagent was added and after 2 min, 1 ml of sodium carbonate

Table 1. 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
41,97	Myricitrin		259	351
42,95	Rutin	-	256	355
48,18	Quercetin		255	369
49,13	Luteolin	-	255	349
52,13	Apigenin	239	267	339
54,32	Chrysin	-	268	313

(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 h, 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 *K. odorata*, *O. cubensis* and *R. nervosa*, considering their dry extract content.

Identification of bioactive compounds

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 *K. odorata, O. cubensis* and *R. nervosa* were injected at a flow rate of 0.6 ml/min for 72 min 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. 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 1).

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 *K. odorata* (AP: 808, 606, 404, 202, 101 and 40 µg/ml and UP: 724, 543, 362, 181, 90 and 36 µg/ml) *O. cubensis* (AP: 848, 636, 424, 212, 106 and 42 µg/ml and UP: 484, 363, 242, 121, 60 and 24 µg/ml) and *R. nervosa* (AP: 936, 702, 468, 234, 117 and 46 µg/ml and UP: 372, 279, 186, 93, 46 and 18 µg/ml) were added at six different concentration points. The final volume of the flask was filled with ethanol and after 30 min the absorbance was measured at 518 nm.

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 (Lôbo et al., 2010).

In vitro antimicrobial activity

The efficiency of the ethyl acetate and chloroformic phases obtained from *K. odorata, O. cubensis* and *R. nervosa* were tested against the following pathogenic microorganisms: *Staphylococcus aureus* (Gram-positive bacterium); *Pseudomonas aeruginosa* (Gram-negative bacterium), and *Candida albicans* (fungus).

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 *K. odorata, O. cubensis* and *R. nervosa* were diluted in DMSO at 1% in H_2O at 2500 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 MIC was determined by spectrophotometry in an Elisa reader at 560-630 nm after 24 h 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.

Statistical analyzes

Statistical analyzes were performed using GraphPad Prism 5.0 software and Microsoft Excel® 2010 software. Average tests were carried out to differentiate phenol and total flavonoid contents of the extracts of *O. cubensis, K. odorata* and *R. nervosa*. The comparison between the groups was performed through analysis of variance (ANOVA), considering all results with p below 0.05 using the Tukey

Table 2. Phytochemical prospection of the hydroalcoholic extracts of *Kyllinga odorata* Vahl; *Oxycaryum cubensis* Poepp. & Kunth, and *Rhynchospora nervosa* Boeck.

Phytochemical compounds	OCEAP	KOEAP	RNEAP	OCEUP	KOEUP	RNEUP
Phenols	+	+++	-	+	+	++
Tannins	-	-	-	-	-	-
Flobafenic tannins	-	-	+	-	-	-
Anthocyanin	-	-	-	-	-	-
Antocyanidin	-	-	-	-	-	-
Leucoantocianidines	-	-	-	-	-	-
Catechins	+	+++	+++	+	-	+++
Flavanones	++	+++	+	+	-	++
Flavones	-	-	+	+	-	++
Flavonols and xanthones	+++	-	++	+	+	-
Chalcones and auronas	-	-	-	-	-	-
Flavononols	++	++	++	+	-	+
Alkaloids	++	++	+	+	+++	-

OCEAP: *O. cubensis* extract - Aerial Part; **OCEUP**: *O. cubensis* extract – Underground part; **KOEAP**: *K. odorata* extract - Aerial part; **KOEUP**: *K. odorata* extract – Underground part; **RNEAP**: *R. nervosa* extract - Aerial part; **RNEUP**: *R. nervosa* extract – Underground part.
(+) weakly positive reaction, (++) positive reaction, (+++) reaction strongly positive, (-) absent.

test at 5% probability.

RESULTS AND DISCUSSION

Phytochemical prospecting

From the preliminary phytochemical analyses of the hydroalcoholic extracts of *K. odorata, O. cubensis,* and *R. nervosa*, it was possible to evidence the occurrence of some groups of bioactive compounds of therapeutic and alimentary importance (Table 2). The chemical reactions observed in the extracts of the aerial and underground parts of the three species studied suggested the occurrence of phenols, flobafenic tannins, catechins, flavanones, flavones, flavonols and xanthones, flavononols, and alkaloids.

In these analyses, the substances known as anthocyanin, anthocyanidin and leucoanthocyanidins were not identified. The absence of these compounds in extracts of green coloration (for the aerial parts) and brown (for the underground parts) is justifiable, for anthocyanins are pigments belonging to a subgroup within the flavonoids that occur mainly in many fruits, vegetables and flowers, showing a great variety of colors that oscillate between intense red to violet and blue (Patras et al., 2010; Petroni and Tonelli, 2011; Ribeiro et al., 2011).

It was evidenced that extracts of the underground parts of *K. odorata* presented a greater amount of precipitate by the Dragendorff test in relation to the other species, suggesting that the rhizomes of this plant are rich in alkaloids. In phytochemical analyses performed by Majumder (2013), it was possible to identify the occurrence of alkaloids from the ethanolic extracts of the *Kyllinga nemoralis* roots. Verma et al. (2016) also observed the presence of alkaloids from a phytochemical prospection using the methanolic extract of *Kyllinga triceps*, indicating that plants of the genus *Kyllinga* are sources of this bioactive compound.

Total flavonoids

From the results obtained from the quantification test of the total flavonoids present in the crude extracts of the species studied, it was possible to evidence that the aerial parts of *R. nervosa* (1.521 µg EQ/µg) and *K. odorata* (1.024 µg EQ) presented a higher content of flavonoids when compared to *O. cubensis* (0.284 µg EQ/µg). In relation to the underground parts of these species, the amount of total flavonoids is lower when compared to the aerial parts, except for *O. cubensis* (0.330 µg EQ/µg), which did not differ statistically between the two extracts analyzed (Figure 1).

In general, flavonoids are found in all vascular plants and represent one of the most important and diversified phenolic groups among products of natural origin. In addition, these substances are of great interest for human health and nutrition (Hichri et al., 2011; Carrera et al., 2014). Although the data of this research point to *R. nervosa* as the species with the highest flavonoid content in its aerial parts, no records were found in the literature that prove this same tendency. Another species that showed good results was *K. odorata*. Studies indicate that the methanolic extract of *K. erecta* (a representative

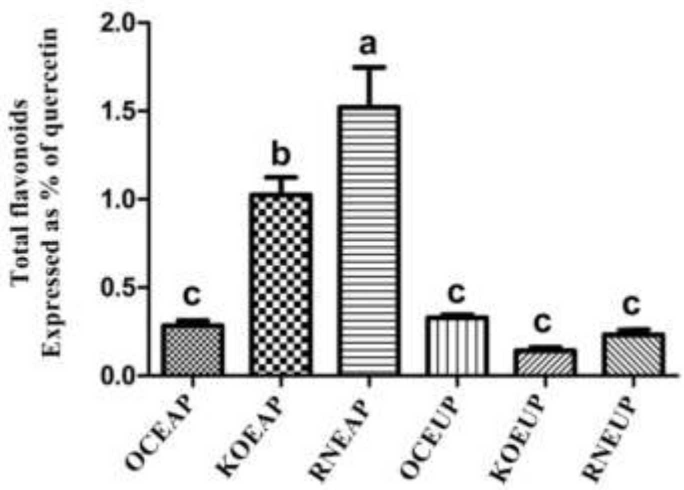

Figure 1. Total flavonoid content of the hydroalcoholic extracts of *Kyllinga odorata* Vahl; *Oxycaryum cubensis* Poepp. & Kunth and *Rhynchospora nervosa* Boeck.
OCEAP: *O. cubensis* extract - Aerial Part; **OCEUP**: *O. cubensis* extract – Underground part; **KOEAP**: *K. odorata* extract - Aerial part; **KOEUP**: *K. odorata* extract – Underground part; **RNEAP**: *R. nervosa* extract - Aerial part; **RNEUP**: *R. nervosa* 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.

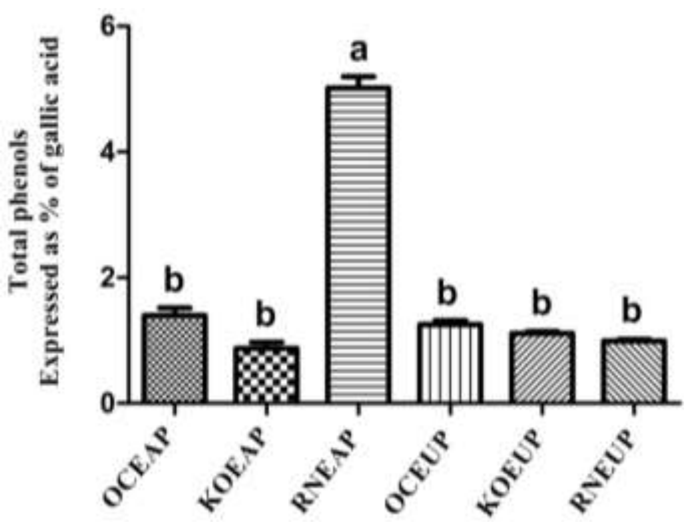

Figure 2. Total phenol content of the hydroalcoholic extracts of *Kyllinga odorata* Vahl; *Oxycaryum cubensis* Poepp. & Kunth and *Rhynchospora nervosa* Boeck.
OCEAP: *O. cubensis* extract - Aerial Part; **OCEUP**: *O. cubensis* extract – Underground part; **KOEAP**: *K. odorata* extract - Aerial part; **KOEUP**: *K. odorata* extract – Underground part; **RNEAP**: *R. nervosa* extract - Aerial part; **RNEUP**: *R. nervosa* 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.

of the genus Kyllinga) has a high content of flavonoids and phenolic content. Consequently, it has high antioxidant activity (Augustus et al., 2015).

Total phenols

The analyses concerning the quantification of the total phenol content of the hydroalcoholic extracts of the three species studied are in agreement with the results found for total flavonoids, since the aerial parts of *R. nervosa* differ statistically from the other samples, indicating a much higher amount of phenolic compounds (5.020 µg EGA/µg) (Figure 2). It is important to note that reports about the chemical constitution of *R. nervosa* are scarce in the literature, which makes the present study important for the phytochemical knowledge of this species and the genus *Rhynchospora* itself. Natural products have stood out as therapeutic and alimentary alternatives. Thus, the aerial parts of *R. nervosa* can serve as the basis for the development of new pharmacological research, since phenolic compounds are known for their many benefits (Crozier et al., 2010; Cartea et al., 2011).

Identification of chemical compounds

According to the chromatograms obtained by the high performance liquid chromatography (HPLC) of the aerial parts of *K. odorata*, *O. cubensis* and *R. nervosa*, substances of therapeutic and nutritional importance were identified. In the ethyl acetate phase of *K. odorata* (Figure 3), it was possible to identify only myricitrin. According to research conducted by Tucker et al. (2006), twenty-three different chemical constituents were identified as dominant components of dihydroquinone and aristolochene in *K. odorata*. These authors also emphasize that dihydroquinone and aristolochene were not previously reported in the essential oils of Cyperaceae.

Catechin and chlorogenic acid were the only substances identified in the chromatogram of the aerial parts of *O. cubensis* (Figure 4). It was not possible to identify the major compounds that occur in this species by means of the techniques used. It was possible to identify catechin and apigenin (Figure 5) from the chromatographic profile of the ethyl acetate phase of the aerial parts of *R. nervosa*. Apigenin is one of the most bioactive flavones and is widely distributed in the plant kingdom. Its consumption is highly recommended for a healthy diet (Shukla and Gupta, 2010). According to Begum and Prasad (2012), it is plausible to assume that apigenin has a protective effect against oxidative stress induced by radiation, and this may be related to its antioxidant action. These data can serve as a basis to explain the potent antioxidant effect evaluated in the present study, from hydroalcoholic extracts of *R. nervosa*.

The major compound identified in the chloroformic

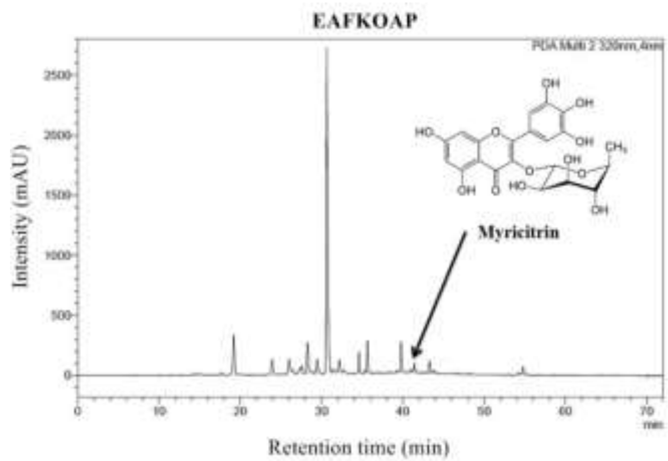

Figure 3. Chromatographic profile of the ethyl acetate phase of the aerial parts of *Kyllinga odorata* Vahl (EAFKOAP) at 320 nm wavelength.

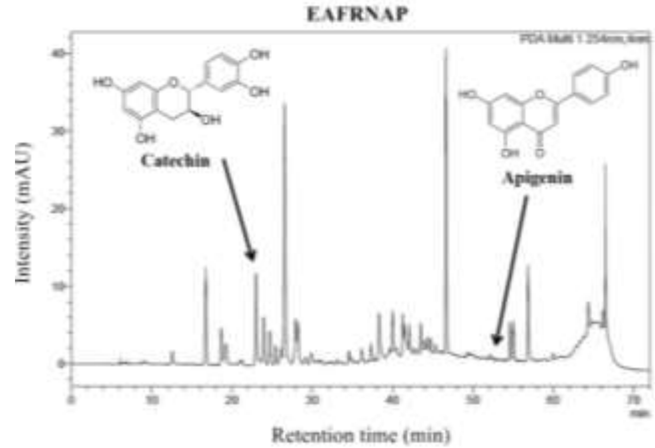

Figure 5. Chromatographic profile of the ethyl acetate phase of the aerial parts of *Rhynchospora nervosa* Boeck (EAFRNAP) at 254 nm wavelength.

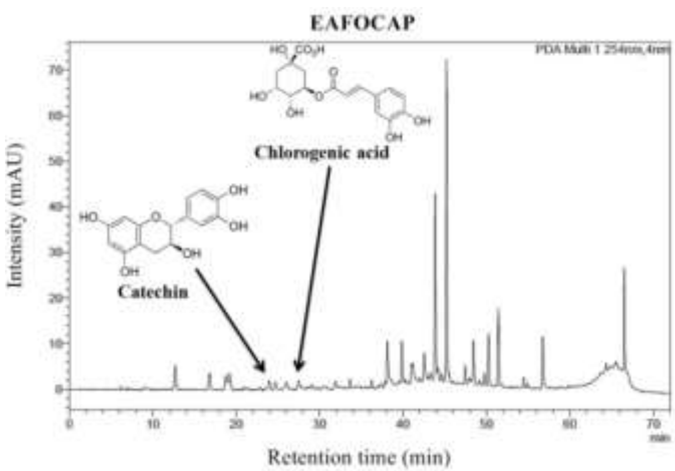

Figure 4. Chromatographic profile of the ethyl acetate phase of the aerial parts of *Oxycaryum cubensis* Poepp. & Kunth (EAFOCAP) at 254 nm wavelength.

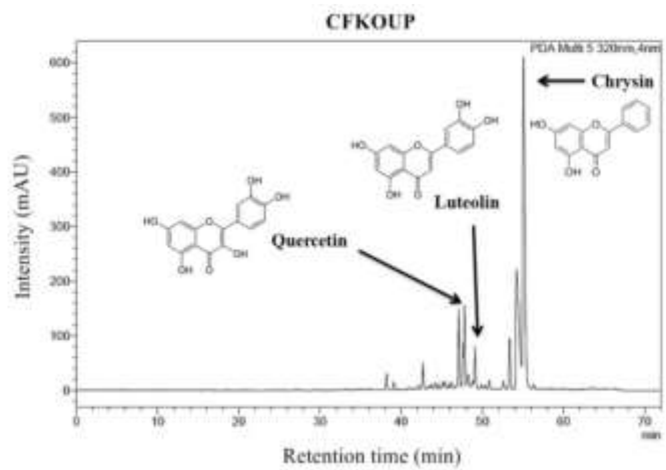

Figure 6. Chromatographic profile of the chloroformic phase of the underground parts of Kyllinga *odorata* Vahl (CFKOUP) at 320 nm wavelength.

phase chromatogram of the underground part of *K. odorata* is a flavonoid known as Chrysin (Figure 6). From the phytochemical studies performed by Noori et al. (2015), it was evidenced that Chrysin was also identified in other species of the family Cyperaceae. Khoo et al. (2010) highlighted the importance of this substance in the process of inhibiting the proliferation and induction of apoptosis in cancer cells, being more potent than other flavonoids in the treatment of leukemia.

Some of the major substances in the chloroformic phase of the underground parts of *O. cubensis* have not been fully elucidated by the standards used (Figure 7). Luteolin was an important compound identified in this chromatographic run. According to Seelinger et al. (2008), luteolin is a flavone that can be found in many medicinal plants. These authors also report that this substance, like other flavonoids, is often found in plants in the form of glycosides. According to López-Lázaro (2009), numerous preclinical studies have demonstrated that luteolin has a wide range of biological activities and several mechanisms of action have been elucidated and even used in the treatment of cancer.

The major compound present in the chloroformic phase of the underground part of *R. nervosa* presented wavelengths different from the standard used for the recognition of the substances analyzed by liquid chromatography (Figure 8). Thus, it is necessary to carry out analyses using infrared and nuclear magnetic resonance (NMR) techniques in order to elucidate the chemical structure of this substance.

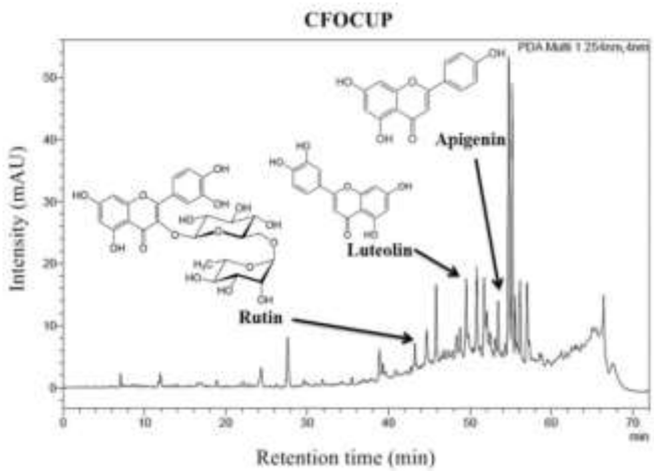

Figure 7. Chromatographic profile of the chloroformic phase of the underground parts of *Oxycaryum cubensis* Poepp. & Kunth (CFOCUP) at 254 nm wavelength.

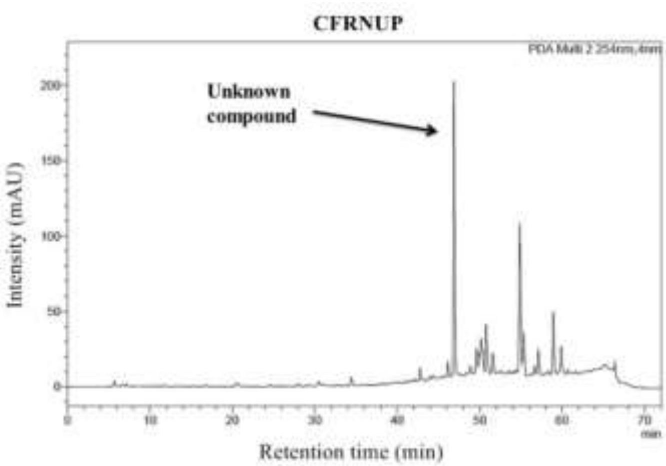

Figure 8. Chromatographic profile of the chloroformic phase of the underground parts of *Rhynchospora nervosa* Boeck (CFRNUP) at 254 nm wavelength.

In vitro antioxidant activity

Among the analyzed samples, it was observed that the extract of the aerial parts of *R. nervosa* presented the best result, requiring a concentration of only 122.11 µg/ml for the reduction of 50% of the DPPH radical. As for the hydroalcoholic extracts of *K. odorata* and *O. cubensis*, concentrations above 280 µg/mL were required for the occurrence of the same reduction of the DPPH radical and, therefore, present a lower antioxidant activity than that observed in the aerial parts of *R. nervosa* (Table 3).

According to Palacios et al. (2011), phenolic compounds are responsible for the antioxidant activity. Thus, considering that the aerial parts of *R. nervosa* presented high levels of phenols and total flavonoids, it was expected that their antioxidant activity would also be higher when compared to the other extracts analyzed by the DPPH method. The scavenging potential of free radicals has been evaluated from extracts of the Cyperaceae family. Forero-Doria et al. (2014) elucidated the antioxidant properties of *Cyperus digitatus*, indicating that extracts from this plant may be useful in preventing the progress of various disorders related to oxidative stress. According to Aeganathan et al. (2015), the chloroformic fractions of the rhizomes of *Cyperus rotundus* also presented an excellent antioxidant activity.

In vitro antimicrobial activity

The ethyl acetate (aerial parts) and chloroformic phases (underground) of *K. odorata*, *O. cubensis* and *R. nervosa* were evaluated against *Candida albicans* (fungus), *Staphylococcus aureus* (Gram-positive bacteria) and *Pseudomonas aeruginosa* Gram-negative). From the results obtained (Table 4), it was observed that the phases of the aerial parts of the three species studied did not present any significant effect when tested against the pathogenic microorganisms by means of serial microdilutions. The best results were observed in the chloroformic phases of the underground parts of *O. cubensis* against *C. albicans* (15.6 µg/ml), *S. aureus* (31.2 µg/ml) and *P. aeruginosa* (62.5 µg/ml).

The *C. albicans* pathogen was susceptible to all chloroformic phases of the analyzed plant underground parts. It is noteworthy that CFOCUP inhibited fungal growth in MIC of 15.6 µg/ml. From these data, a perspective is created for later analyses of the main chemical constituents present in the underground parts of *K. odorata*, *O. cubensis* and *R. nervosa* that are responsible for the control of *C. albicans*, considering that this microorganism is the causative agent of candidiasis: an opportunistic infection of difficult control in immunocompromised patients (Adeniyi et al., 2014). In general, the underground parts of some Cyperaceae species may be important sources of anti-candida bioactive products (Duarte et al., 2005). Adeonipekun et al. (2014) also reported in their work that the ethanolic extract of the roots of *Pycreus smithianus* (family Cyperaceae) showed strong activity in relation to the control against *C. albicans*.

In addition to being effective against fungal growth, CFOCUP also had the best inhibitory activity at the concentration of 31.2 µg/ml when tested against *S. aureus*. The ground phases of *K. odorata* and *R. nervosa* also showed good results against Gram-positive bacteria, but at higher concentrations (250 µg/ml). Data that prove the effectiveness of the extracts or phases of the analyzed species are scarce in the literature, suggesting that the present study has an innovative character. From the studies carried out with *Cyperus rotundus*, one of the most extensively studied species of Cyperaceae, it was observed that extracts of this whole plant showed high

Table 3. *In vitro* antioxidant activity of the hydroalcoholic extracts of *Kyllinga odorata* Vahl; *Oxycaryum cubensis* Poepp. & Kunth and *Rhynchospora nervosa* Boeck.

Hydroalcoholic extracts – AP	IC_{50} – µg/mL	AA (% ± SD)[1]
K. odorrata	546.70	46.62 ± 0.09
O. cubensis	293.30	54.94 ± 0.26
R. nervosa	122.11	61.00 ± 0.05
Hydroalcoholic extracts – UP	**IC_{50} – µg/mL**	**AA (% ± SD)[1]**
K. odorata	608.85	42.76 ± 0.41
O. cubensis	268.27	43.49 ± 0.09
R. nervosa	287.67	45.12 ± 0.12

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

Table 4. Determination of the Minimal Inhibitory Concentration (MIC) of the ethyl acetate and chloroformic fractions of the aerial and underground parts of *Kyllinga odorata* Vahl; *Oxycaryum cubensis* Poepp. & Kunth and *Rhynchospora nervosa* Boeck.

Strains	EAFKOAP µg/ml	CFKOUP µg/ml	EAFOCAP µg/ml	CFOCUP µg/ml	EAFRNAP µg/ml	CFRNUP µg/ml
C. albicans	-	31.2	-	15.6	-	125
S. aureus	-	125	-	31.2	-	125
P. aeruginosa	-	500	-	62.5	-	-

EAFKOAP: Ethyl Acetate Phase of *K. odorata* - Aerial part; **CFKOUP**: Chloroformic Phase of *K. odorata* - Underground part; **EAFOCAP**: Ethyl Acetate Phase of *O. cubensis* - Aerial part; **CFOCUP**: Chloroformic Phase of *O. cubensis* – Underground part; **EAFRNAP**: Ethyl Acetate Phase of *R. nervosa* - Aerial part; **CFRNUP**: Chloroformic Phase of *R. nervosa* – Underground part; (-): No Inhibition.

activity against Gram-positive bacteria, including *S. aureus* (Kabbashi et al., 2015).

Concerning *P. aeruginosa*, only the chloroformic phases of the underground parts of *K. odorata* (500 µg/ml) and *O. cubensis* (62.5 µg/ml) showed inhibitory activity, suggesting that this gram-negative bacterium has greater resistance when compared to the other evaluated microorganisms. Several studies have demonstrated the resistance of *P. aeruginosa* to the currently available antibiotics, so it is extremely important to make advances in research aimed at discovering more effective drugs to fight the infections caused by this super bacterium (Breidenstein et al., 2011; Gellatly and Hancock, 2013).

Conclusion

The ethyl acetate and chloroformic phases of *K. odorata*, *O. cubensis* and *R. nervosa* were identified as substances of great pharmacological importance by means of the CLAE-DAD technique, such as: myricitrin, catechin, apigenin, quercetin, luteolin, chrysin and rutin. However, it is worth mentioning that new experiments using nuclear magnetic resonance (NMR) techniques are necessary to elucidate the chemical structures of unknown compounds that were not identified by the standard used in liquid chromatography.

The hydroalcoholic extracts of the aerial parts of these species presented higher levels of flavonoids and total phenols when compared with the extracts of the underground parts. In regard to the antioxidant activity, it was clear that the hydroalcoholic extract of the aerial parts of *R. nervosa* presented the best result in relation to the other samples evaluated. This may be directly related to the high content of phenols and flavonoids that occur in the aerial parts of this species. Only the chloroformic phases of the underground parts of the three species studied showed inhibitory activity against *S. aureus*, *C. albicans* and *P. aeruginosa*. It is important to mention that CFOCUP stood out among the other analyzed fractions, exhibiting control of the pathogens in much lower concentrations. Thus, it can be suggested that the underground parts of *K. odorata, O. cubensis* and *R. nervosa* can be sources of important bioactive substances against fungi and bacteria that affect human health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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