

Investigating the Phytochemicals and Antimicrobial Properties of Three Sedge (Cyperaceae) Species

Tiwalade Adeyemi ADENIYI^{1*}, Peter A. ADEONIPEKUN¹, Elizabeth A. OMOTAYO²

¹University of Lagos, Department of Botany, Akoka, Nigeria; dessytwa@yahoo.com (*corresponding author); p1adeonipekun@yahoo.com

²University of Lagos, Department of Microbiology, Akoka, Nigeria; elizabethomotayo@yahoo.com

Abstract

In order to evaluate the medicinal value of notorious sedge weeds, three species: *Cyperus esculentus*, *Cyperus rotundus* and *Mariscus alternifolius* were investigated for their phytochemical constituents and antimicrobial properties. Preliminary qualitative phytochemical constituents and *in vitro* antimicrobial activities were evaluated against four fungi species: *Aspergillus niger*, *Aspergillus fumigatus*, *Penicillium chrysogenum* and *Candida albicans*, and three bacteria species: *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. Two solvents, water and ethanol, were used to produce the extracts and were screened for their antimicrobial activity. Antimicrobial activity evaluation of the extracts against pathogens was carried out at 100 mg/ml concentration by Disc Diffusion method for fungi, Disc Diffusion and Agar Well Diffusion methods for bacteria. Observed activities were related to standard antibiotics, antifungal and antibacterial, which served as controls. Phytochemically, the plant extracts showed the presence of carbohydrates, flavonoids, ketose sugars, steroids, reducing sugars and tannins. The ethanolic extract of *C. rotundus* exhibited the highest activity against *A. niger*, *E. coli* and *S. aureus*. No extract was active against *C. albicans*. From these findings, it was concluded that *C. rotundus* is a potential source of bioactive compounds for new drugs upon isolation and purification for treating infections caused by these pathogens.

Keywords: bacteria, *Cyperus esculentus*, *Cyperus rotundus*, plant extract, fungi, *Mariscus alternifolius*

Introduction

Nearly 80% of the world population depends upon traditional system of health care (Ganesan *et al.*, 2004). The indigenous traditional knowledge of medicinal plants of various ethnic communities, where it has been transmitted orally for centuries, is fast disappearing from the face of the earth due to the advent of modern technology and transformation of traditional culture (Ganesan *et al.*, 2004). However, during the last few decades there has been an increasing interest in the study of medicinal plants and their traditional uses in different parts of the world. This led to major pioneering works such as the isolation of quinine from *Cinchona* spp. (bark extracts), reserpine from *Rauwolfia serpentina* – serpent wood (root extracts) and diosgenin from *Dioscorea* spp. (tuber extracts) (Joy *et al.*, 2001). Consequently, the level of awareness of phyto-medicine in Nigeria is very large. It is estimated that more than 80% of the Nigerian population use herbal remedies in one form or the other, because less than 35% of the population has access to modern health care facilities (Awosika, 1993). Thus, medicinal plants form the basis of primary health care for majority of the people living in the rural and remote areas in Nigeria (Awosika, 1993). Most

recently, the efforts of some newspaper columnists in raising awareness over herbal medicine efficacy in curing diseases cannot be over-simplified. The availability of many of these herbal plants makes them more readily affordable to the rural and even urban dwellers. Of particular significance in herbal medicine are common weeds around homes and waste-lands, as well as farmlands where sedges form important components.

Among these common weeds is the family Cyperaceae which are ubiquitous, but may be difficult to recognize because of their closeness to the grass family, with the exception of their triangular shaped stems, that are characteristic. Lowe and Stanfield (1974) remarked that about four-fifths of the species grow in damp or wet places (including a few submerged aquatics), while one-fifth is found in drier regions, such as savannah grassland and sandy places including sand-dunes.

Cyperus esculentus L. (Family: Cyperaceae, common name: Tiger nut, Nigerian local names: Yoruba - Ofio, Hausa - Ayaya and Igbo - Akiausa). It is found in all tropical and warm-temperate regions except Malaysia, Australia and Oceania. In Nigeria, it is commonly found across the country. It is a weed found in cultivated areas, in moist places and waste-lands (Burkill, 1985). It is 24-55 cm tall, producing rhizomes from the base which are 2 mm thick

and bear tubers. Stem is triangular, with sides approximately 2 mm wide. Leaves are fairly numerous at the base of the stem, reaching 30 cm long and 8 mm wide, but often narrower. Inflorescence subtended by approximately four bracts, the longest approximately 20 cm long and 5 mm wide, usually tapering to a fine point. Inflorescence is a simple umbel when small, or a compound umbel when larger, with primary rays of 4-9 cm long, secondary rays if present are 2 cm long. Branches ending in spikes 1-3 cm long, composed of 6-25 golden-yellow spikelets, each 7-15 mm long, 1-1.5 mm broad, with 3-8 glumes along each side. Glumes approximately 3 mm long, with a fairly prominent midrib, and with a papery edge. They are occasionally viviparous (Lowe and Stanfield, 1974). Medicinally, they are used in healing menstrual discomforts and stomach troubles (Burkill, 1985).

Cyperus rotundus L. (Family: Cyperaceae, common name: Nut grass, Nigerian local names: Hausa - Giragiri, Fulfude - Ayaare and Yoruba - Danda). It is found in pan-tropical and sub-tropical regions in West Africa, and other damp places. Its distribution ranges from Mali to Niger, and towards Nigeria, Ghana and Sierra Leone. It is also found in warm temperate regions often as a rice-field weed (Burkill, 1985). In Nigeria it is found across the country (Lowe and Stanfield, 1974). Its rhizome is fairly narrow with fibrous brown scales. Stems are 20-40 cm high, 1-2 mm thick, with a swollen tuberous base. Leaves vary in length from half as long as the stem to equal the length of the stem in some cases; leaves are sometimes few (6) and narrow (2 mm); sometimes numerous (14) and broader (5 mm). Inflorescence is 3-8 cm long, often rather narrow with sub-erect spikelets, subtended by approximately 3 bracts similar to the leaves, and from ½ to 1 ½ times as long as the inflorescence. Inflorescence usually with a few primary rays 2-5 cm long, ending in a short spike of 3-10 spikelets, sometimes with 1 or 2 short secondary rays at the base of the spike. Spikelets are rather long and flat, generally reddish-brown, 12-20 cm long, 1.5-2 mm broad, with 5-10 glumes along each side. Glumes with a green keel and red-brown sides, 3-4 mm long (Lowe and Stanfield, 1974). In medicine, tubers have been reported to be used in treating diarrhea and dysentery; as genital stimulants or depressants, treatment of kidney troubles, stomach troubles and as diuretics (Burkill, 1985). The tubers also possess aromatic substances that are used as insecticides and arachnicides (Burkill, 1985). The presence of polyphenol, flavonol glycoside, saponin, vitamin C, sesquiterpenoids and essential oil from phytochemical investigation of *C. rotundus* rhizomes was reported by Nagulendran *et al.* (2007).

Mariscus alternifolius Vahl. (Family: Cyperaceae, common name: Mariscus, Nigerian local names: Hausa - Ayaa, Yoruba - Alubosa eranko and Igbo - Ataku mainya). It is pan-tropical and common in damp grassy places in Togo, Gambia, Nigeria, Liberia, Ivory Coast, Ghana, Gambia, Guinea and Cameroon (Burkill, 1985). It is found across Nigeria (Lowe and Stanfield, 1974). *Mariscus* is perhaps the most common species of sedge in Nigeria, except the far north. An extremely variable plant, forming shoots often thickened or almost bulb-like at the base, sometimes from a short woody rhizome, with numerous fibrous roots. May be very slender, or may reach 1 m tall with leaves 8 mm wide.

Leaf sheaths are purplish-red. Stems rounded to triangular. Inflorescence is simple umbel with a different number of rays, the longest approximately 10 cm long, and subtended by leafy bracts. Spikelets always densely crowded into heads, which may be almost globose and approximately 5 mm diameter, or elongated into spikes 3 cm long and 1 cm wide. In young inflorescences, the heads are almost sessile, but stalks (i. e. the rays of the inflorescence) soon develop to various lengths. Spikelets usually under 5 mm long with 1 or 2 ribbed glumes showing; in some specimens there may be spikelets over 5 mm long, with 2 or 3 glumes visible (Lowe and Stanfield, 1974). The chewed stem bandaged on a cut or wound for at least three days is reported to have a healing effect. The swollen stem base after washing, ground in a mortar and then mixed into some honey is a treatment for gonorrhoea (Burkill, 1985).

To verify the claimed and reported medicinal uses of some sedges, the antimicrobial properties and phytochemicals present in the extracts of *Cyperus esculentus*, *Cyperus rotundus* and *Mariscus alternifolius* were investigated.

Materials and methods

Plant collection

Plant samples were collected from the University of Lagos Campus, Akoka, Lagos State and neighbouring surroundings. The plants were collected wholly with roots, shoots and inflorescences. The plants were confirmed in the Herbarium of the Department of Botany, University of Lagos. The collected plants are *Cyperus esculentus*, *C. rotundus* and *Mariscus alternifolius*.

Test microorganisms

The test bacteria: *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* were obtained from Lagos University Teaching Hospital, Idi-Araba, Lagos and the test fungi: *Aspergillus niger* and *Aspergillus fumigatus* were obtained from Microbiology Department, University of Lagos. However, *Candida albicans* and *Penicillium chrysogenum* were obtained from the Nigerian Institute for Medical Research, Yaba, Lagos. The bacteria strains were maintained on Nutrient Agar while the fungi were maintained on Potato Dextrose Agar at 4 °C in a refrigerator.

Preparation of plant extracts

The plant materials were cut into pieces and air-dried for a week in an oven. Dried samples were ground into powder with a grinding machine. For the aqueous extraction, 50 g of each powdered plant sample was soaked in 500 ml of cold distilled water in a conical flask and left undisturbed for 48 hours. Thereafter, the extracts were filtered off using Whatman No 1 filter paper. The filtrate was concentrated under vacuum below 40 °C using a rotary evaporator (Bag *et al.*, 2009). For organic solvent extraction, 50 g of air-dried powder was soaked in 500 ml of ethanol in a conical flask and left undisturbed for 48 hours. Thereafter, the extract was filtered off using Whatman No 1 filter paper. The filtered extract was concentrated under vacuum below 40 °C using a rotary evaporator (Bag *et al.*, 2009). The extracts thus obtained were stored in well-labeled sterile bottles and kept in the freezer at 4 °C until further

use for the screening of antimicrobial activity.

Antimicrobial Assay

Antifungal Activity Test of Plant Extracts

This was done using the Agar Diffusion Test Method of Irobi and Daramola (1993).

About 10 ml of Potato Dextrose Agar (PDA) was poured into petri dishes and allowed to solidify. Liquid inoculum was prepared by pouring cooled sterile distilled water on already grown fungal plates. The suspension was transferred into a test tube. This allowed for fungal spores to be easily removed from the pure culture. A drop pipette was used to transfer two drops of fungal inoculum into each of the agar plates. A sterile glass rod was used for even spreading. To prepare discs, Whatman No 1 filter paper was perforated using a paper punch to form paper discs 6 mm in diameter. The discs were wrapped in aluminum foil and sterilized in an autoclave at 121 °C for 15 minutes. After sterilization, the discs were dropped in each extract (100 mg/ml).

Two controls were used: sterile distilled water and *Fulcin*. *Fulcin* control was prepared by dissolving 12.5 g of *Fulcin* powder in 10 ml of sterile distilled water. Four of the discs from each of the three extracts, antibiotic and water controls were aseptically placed with the aid of a sterile forceps on PDA and inoculums in petri dishes. For each fungus, two replicates were produced by repeating the above processes. This is to reduce experimental errors by calculating the average of the values obtained. All the plates containing the extracts and fungi were incubated at 25 °C. The zones of inhibition were measured after 48 hours of incubation and the results analyzed statistically.

Antibacterial Activity Test of Plant Extracts

Disc Diffusion Assay

This was done using the Agar Diffusion Test Method of Bauer *et al.* (1966). About 10 ml of Nutrient Agar (NA) was poured into petri dishes and allowed to solidify. Petri dishes were inoculated by streaking bacteria over their surfaces with the aid of an inoculating loop. To prepare discs, Whatman No 1 filter paper was perforated using a paper punch to form paper discs of 6 mm in diameter. The discs were wrapped in aluminum foil and sterilized in an autoclave at 121 °C for 15 minutes. After sterilization, they were dropped in each extract (100 mg/ml).

Two controls were used: sterilized distilled water and *Tetracycline* – an antibiotic. *Tetracycline* control was prepared by dissolving 12.5 g of tetracycline powder in 10 ml of sterile distilled water. Four of the discs from each extract, antibiotic and water control were then aseptically placed with the aid of sterile forceps on petri dishes with NA and inocula. For each bacterium, the experiment was performed in duplicates. All the plates containing the extracts and bacteria were then incubated at 37 °C. The zones of inhibition around the disc were measured after 24 hours of incubation and the results analyzed statistically.

Agar Well Diffusion Assay

The Agar Diffusion Test Method of Aneja and Joshi (2009) was applied. About 10 ml of Nutrient Agar (NA) was poured into petri dishes and allowed to solidify. The medium was inoculated with bacteria by streak plate method. Wells were prepared in the plates with a cup-borer (0.85 cm) and 100 µl of the test compound was pipetted directly into the well. This was repeated for all the extracts and controls. Two controls were used: sterilized distilled water and *Ciprofloxacin*. *Ciprofloxacin* control was prepared by dissolving 12.5 g of ciprofloxacin powder in 10 ml of sterile distilled water. Prior to incubation at 37 °C for 24 hours, the petri dishes were kept at room temperature for 15 minutes in order to promote diffusion of the extracts into the agar. All the tests were made in duplicates and the mean diameter of the inhibition zones were analyzed statistically.

Phytochemical Screening

Phytochemical screenings of the plant samples were carried out qualitatively using the method described by Harbone (1998). These screenings are: Fehling's Solution test for reducing sugars, Molisch test for carbohydrate sugars, Barfoed's test for monosaccharides, Resorcinol test (or Seliwanoff's test) for keto-sugars, Phloroglucinol test for pentose sugars, test for Alkaloids, general test for Glycosides, tests for saponin, tannins, anthraquinones, flavonoids, steroids and terpenoids.

Statistical Analysis

Tests were carried out in duplicate for the three separate experiments. Analysis of the standard error was used for comparison between the mean values of the studies parameters.

Tab. 1. Effects of water and ethanolic extracts of sedges on fungi

Extracts	Inhibition zones of fungi (cm)			
	<i>A. niger</i>	<i>A. fumigatus</i>	<i>P. chrysogenum</i>	<i>C. albicans</i>
<i>C. esculentus</i> (water extract)	na	1.00±0.38	4.00±0.85	na
<i>C. esculentus</i> (ethanol extract)	na	4.63±1.18	6.13±1.22	na
<i>C. rotundus</i> (water extract)	na	7.88±1.73	0.88±0.74	na
<i>C. rotundus</i> (ethanol extract)	9.38±0.98	na	2.38±1.08	na
<i>M. alternifolius</i> (water extract)	na	4.88±1.39	2.38±0.71	na
<i>M. alternifolius</i> (ethanol extract)	na	na	6.75±0.94	na
Fulcin	10.00±0.33	13.88±0.72	11.63±0.38	20.00±0.42
Water	na	na	na	na

*na = not active. Data are presented as the mean ± SE of each duplicate test.

Tab. 2. Effects of water and ethanolic extracts of sedges on bacteria (disc method)

Extracts	Inhibition zones of bacteria (cm)		
	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Salmonella typhi</i>
<i>C. esculentus</i> (water extract)	2.13±0.79	0.75±0.41	0.75±0.49
<i>C. esculentus</i> (ethanol extract)	1.38±0.80	1.25±0.49	0.25±0.16
<i>C. rotundus</i> (water extract)	2.00±0.68	0.50±0.19	0.50±0.50
<i>C. rotundus</i> (ethanol extract)	6.75±0.77	2.25±0.56	na
<i>M. alternifolius</i> (water extract)	0.63±0.38	0.50±0.38	1.00±0.57
<i>M. alternifolius</i> (ethanol extract)	0.50±0.33	1.00±0.50	0.13±0.13
Tetracycline	19.75±0.98	31.00±1.13	20.00±0.87
Water	na	na	na

*na = not active. Data are presented as the mean ± SE of each triplicate test.

Results

Antimicrobial results

Phytochemical results

Cyperus esculentus contained the highest number of phytochemicals; *C. rotundus* contained tannins and reducing sugars, while *M. alternifolius* contained relatively the least number of phytochemicals (only reducing sugars). Alkaloids, saponins, anthraquinones and terpenoids were not found in the three sedges (Tab. 3).

Tab. 3. Phytochemical screening of sedges extracts

Test	Sedges		
	<i>C. esculentus</i>	<i>C. rotundus</i>	<i>M. alternifolius</i>
Fehling's	-	+	+
Molisch	+	-	-
Barfoed's	-	-	-
Resorcinol	+	-	-
Phloroglucinol	-	-	-
Alkaloids (Dragendoff's)	-	-	-
Saponin	-	-	-
Tannins	+	+	-
Anthraquinones	-	-	-
Flavonoids	+	-	-
Steroids	+	-	-
Terpenoids	-	-	-

Key: Inference of the test is indicated in parenthesis: (+) indicates present; (-) indicates absent.

Discussion

Burkill (1985) observed that nuts of *Cyperus esculentus* and *C. rotundus* have medicinal properties which have been harnessed by traditional medicine practitioners, but only a few of these properties have been proven scientifically. However, in this present study, only the shoots, roots and inflorescence of *Cyperus esculentus* and *C. rotundus* were utilized. This was done to further assess other floral parts for their medicinal importance, due to their fast growth rate which make them more readily available for use, since the tubers take a longer time to develop. Traditional medicine practitioners make use of water primarily as a solvent, but studies have shown that alcohol extracts of plants are much better and powerful. This may be due to the better solubility of the active components in organic solvent (De Boer et al., 2005). The two extracts (ethanolic and aqueous) used in this study showed either inhibitory or no activities against

the different species of fungi and bacteria tested. *A. niger* is one of the most common species of *Aspergillus* that causes aspergillosis in humans. Aspergillosis develops in the lungs of humans who are immuno-compromised and results in symptoms such as cough, fever, chest pains and breathing difficulties. In plants, *Aspergillus niger* causes black mould of onion bulbs. Due to the high potency of *Cyperus rotundus* (ethanolic extract) against *A. niger*, it can be suggested that isolation of the bioactive compounds responsible for the inhibitory activity and the purification of this compound may provide another reliable antimicrobial just like Fulcin, which will be used specifically for controlling *A. niger*-related infections in humans and plants.

The inactivity of *C. rotundus* (ethanolic extract) against *A. fumigatus* and its potency against *A. niger* may be attributed to the fact that both species produce different mycotoxins. *Aspergillus fumigatus* produces a mycotoxin known as gliotoxin, which is different from ochratoxin produced by *A. niger*. *Aspergillus fumigatus* is the most common cause of aspergillosis in the lungs of immuno-compromised humans. Its effectiveness is as a result of the mycotoxin – gliotoxin, which is genotoxic and cytotoxic (Susana et al., 2002). Aqueous extract of *C. rotundus* was the most effective plant extract against the fungus (7.88±1.73 cm). This suggests that further isolation and purification of the bioactive compounds responsible for the inhibitory activity may lead to the development of effective drugs which can be used as a combination therapy with Fulcin against the fungus.

Penicillium chrysogenum is a wonderful source of antibiotic – Penicillin; it causes no diseases in humans, except few cases of allergies, but may cause mould on plant stored products. All plant extracts showed relatively mild inhibitory activities against *Penicillium chrysogenum* compared to the antifungal control – Fulcin (Tab. 1). This is probably due to the fact that the fungus itself produces an antibiotic contributing to its weak potency and susceptibility. The resistance of *Candida albicans* (Tab. 1) to most antimicrobial agents was clearly demonstrated in this study as no extract showed inhibitory activity against the yeast-like fungus. *Candida albicans* is the causative agent of candidiasis: an opportunistic infection very difficult to control in immuno-compromised patients, but easily cured in patients not immuno-compromised. According to Awodele et al. (2007), the use of some common disinfectants such as "savlon" and "jik" against *C. albicans*

revealed that unless 100% concentration of these substances are used, *C. albicans* cannot be controlled.

The ethanolic extract of *C. rotundus* was the most active plant extract against *E. coli* (6.75 ± 0.77 cm, Tab. 2) and this probably supports the reported use of the plant by traditional medicine practitioners in treating diarrhoea and other stomach troubles. Isolation and purification of the bioactive compounds responsible for this inhibitory activity are necessary to produce more antibiotics which can be used effectively against *E. coli*. *Cyperus rotundus* ethanolic extract also showed the highest inhibitory action against *S. aureus* (2.25 ± 0.56 cm). This further enunciates the anti-microbial potency of this plant extract. The relatively low inhibitory activities of the plant extracts against *Salmonella typhi* compared to the antibiotic control – Tetracycline demonstrates the difficulty involved in controlling *Salmonella typhi*.

Mariscus alternifolius ethanolic extract was the most active against *E. coli*, and it showed similar results to *C. rotundus*. The authors are not aware of any reported use of *Mariscus alternifolius* in treating stomach troubles or diarrhoea. Further purification and isolation of the active compounds in this extract are required for pharmacological evaluation. This plant may be a potential source of a new type of antibiotic. The ethanolic and aqueous extracts of *C. rotundus* showed the highest inhibitory activities against *S. aureus* compared to the other four plant extracts. This result again further illustrates the high potency of *C. rotundus* against micro-organisms. The ethanol extract of *C. esculentus* was the most active against *Salmonella typhi* and the result was similar to that obtained from the antibiotic control – Ciprofloxacin. This indicates the high potency of the plant extract. Isolating and purifying the bioactive compounds may lead to the development of another suitable antibiotic against *S. typhi*.

The observed mild inhibitory activities of most of the tested extracts on the growth of the microbes possibly suggest that the use of these extracts at higher concentrations may be very effective. This is indicated by their use by the traditional medicine practitioners. Amongst the gram-positive and gram-negative bacteria, the gram-positive bacterium (*Staphylococcus aureus*) was more susceptible to the extracts with 100% susceptibility as compared to the gram-negative bacteria (*Salmonella typhi* and *Escherichia coli*) 66.7% and 91.8% susceptibility respectively. This observation agrees with previous works that plant extracts are more active against gram-positive bacteria than gram-negative bacteria (Vlietinck et al., 1995; Rabe and Van Staden, 1997).

The result of phytochemical screening showed that *C. esculentus* contained a higher number and variety of phytochemicals than *C. rotundus* and *Mariscus alternifolius*. *C. esculentus* had a record of five phytochemicals (carbohydrates, ketose sugars, tannins, flavonoids and steroids), *C. rotundus* and *Mariscus alternifolius* had two (tannins and reducing sugars) and one (reducing sugars) respectively (Tab. 3). According to the antimicrobial assays, it can be suggested that most of the phytochemicals in *C. esculentus* do not possess bioactive forms except against *S. typhi*. The steroids, carbohydrates and ketose sugars may be

responsible for this activity because these compounds are used in the production of phytoalexins in plants. Phytoalexins are antimicrobial substances synthesized by plants that accumulate rapidly at areas of incompatible pathogen infection (Yukihiro et al., 2002). The presence of tannins and flavonoids suggests that the class of tannins present in this plant is made up of non-hydrolysable or condensed tannins, which are effective anti-infective compounds (Okigbo et al., 2009). Further research needs to be conducted in this area to determine the phytoalexins and tannins present in this plant through their isolation and purification. *C. rotundus* contained reducing sugars and tannins. The class of tannins may be responsible for the anti-diarrhoeal potential of the plant (Akiyama et al., 2001).

Also, the combination of the reducing sugars constitute a building block for the production of phytoalexins and this may be responsible for the high antimicrobial potency of the plant (Yukihiro et al., 2002). *Mariscus alternifolius* contained only reducing sugars. This phytochemical is probably responsible for the plants' high inhibitory activity against *E. coli*. Also, further research needs to be done on this plant to exploit its antimicrobial potential. None of the plant extracts contained alkaloids, anthraquinones, saponins and terpenoids, which have been reported in other plants with anti-microbial properties (Opara et al., 2012) suggesting the diversity of possible anti-microbial drugs.

Conclusions

Cyperus esculentus, *Cyperus rotundus* and *Mariscus alternifolius* could be important sources of natural antimicrobials. The results of the present study support the traditional usage of the studied plants in the treatment of diarrhoea and stomach troubles. This suggests that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and further pharmacological evaluation carried out on them. Among these sedge species, particularly *C. rotundus* is potential sources of bioactive compounds needed in the synthesis of antibiotics against the tested pathogens.

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