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ORIGINAL PAPER

Antibacterial, antioxidant, and xanthine-oxidase inhibitory screening studies of selected cyperaceae species occurring in Europe

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Abstract

Plants belonging to the family Cyperaceae are used in traditional medicine worldwide to treat various diseases. The present study focuses on the antibacterial, antioxidant, and xanthine-oxidase inhibitory screening of 41 Cyperaceae species from genera *Carex, Cladium,* and *Schoenoplectus,* occurring in the Carpathian Basin. This study evaluated 123 fractions with different polarities (*n*-hexane, chloroform, and ethyl acetate) of 41 extracts for their antibacterial, antioxidant (DPPH and ORAC assays), and xanthine oxidase (XO)-inhibitory activities. Mainly the ethyl acetate fractions of *Carex elata, C. hartmaniorum, C. melanostachya, C. montana, C. morrowii, C. oshimensis, C. petriei,* and *C. siderosticta* displayed high antibacterial activity (inhibition zones > 15 mm) against one or more bacterial strains. In the case of *Schoenoplectus lacustris* and *Carex petriei*, also the ethyl acetate fractions showed remarkable antioxidant effects. In contrast, several chloroform extracts possessed XO-inhibitory activity. For *Carex siderosticta, C. sylvatica,* and *Cladium mariscus,* the results of the DPPH tests correlated well with those of the ORAC tests; these sedges demonstrated high antioxidant activities in both assays. Based on the results, especially *Carex buxbaumii, C. rostrata, C. muskingumensis, C. oshimensis, C. montana, C. siderosticta, C. sylvatica,* and *Cladium mariscus* are considered worthy of activity-guided phytochemical investigations to identify the specific compounds responsible for the observed activity.

Keywords Cyperaceae · *Carex* · *Cladium* · Antioxidant · Antibacterial · XO-inhibitory

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Introduction

Drug discovery from natural sources continues to provide new and important leads against various pharmacological targets including cancer, infectious diseases, cardiovascular diseases, and neurological disorders. Plants produce a wide variety of bioactive compounds, including flavonoids, other phenolic compounds, terpenoids, and alkaloids. Natural products (NPs) are structurally 'optimized' by evolution to serve specific biological functions, such as regulating endogenous defense mechanisms and interacting (often competitively) with other organisms. This explains their significant relevance to infectious diseases and cancer (Atanasov et al. 2015). NPs are known to regulate key pathophysiological processes, including oxidative stress, inflammation, cell proliferation, and metabolism, all of which are linked to human diseases.

Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radicals, are an entire class of highly reactive molecules derived from the incomplete

reduction of molecular oxygen. ROS can cause extensive damage to cells and tissues, during infections and various degenerative disorders, such as cardiovascular diseases, aging, neurodegenerative diseases (e.g., Alzheimer's disease), and cancer (Liu et al. 2018). Xanthine oxidase (XO) is also an important biological source of oxygen-derived free radicals that contribute to oxidative damage to living tissues involved in many pathological processes including inflammation, atherosclerosis, and gout, among others. Moreover, XO plays a role in the formation of uric acid (UA). XO is responsible for catalyzing the process from hypoxanthine to xanthine, and then to uric acid. However, oxygen reduction is faster than the oxidation reaction of xanthine to UA (Masuoka 2021). Circulating uric acid is thought to be effective against loss of bone mineral density and oxidative damage. However, excess of serum uric acid is associated with diseases such as gout, hypertension, and cardiovascular disease (Kaushal et al. 2018; Freilich et al. 2022).

Today, in the era of widespread antibiotic resistance against available antibiotics, plants are considered the most promising sources of new antimicrobial compounds (Khameneh et al. 2019; Angelini 2024). There is an increasing need for new effective antimicrobials, especially against Gram-negative bacteria. Gram-negative bacteria have significant clinical importance resulting in severe medical conditions with high risk of morbidity and mortality, often requiring the patients to be hospitalized at intensive care unit (Breijyeh et al. 2020). A large number of studies are being conducted worldwide to identify antimicrobial compounds in plants (Abdallah et al. 2023).

Screening plants from specific genera or families can lead to the identification of extracts with promising biological activities for bioactivity-guided isolation of effective constituents that can serve as starting materials for further research.

As a continuation of our screening program related to plants and bioactive natural compounds in the European Flora (Lajter et al. 2013; Orbán-Gyapai et al. 2015, 2017; Tóth et al. 2016, 2017), the present work describes for the first time a systematic in vitro antibacterial, antioxidant, and XO inhibitory assay of extracts prepared from 41 species belonging to three genera of the Cyperaceae family. Cyperaceae species are distributed worldwide, comprising approximately 5000 species in 100 genera. Members of Cyperaceae are grass-like, annual, or perennial, herbaceous plants commonly known as sedges, with the greatest diversity in the humid and semi-humid tropics. However, they are also often dominant in temperate or cold temperate regions of the world, at almost all latitudes, except Antarctica (Goetghebeur 1998; Fiorentino et al. 2008a; Reznicek 2024). The largest genera are *Carex, Cyperus,* and *Rhynchospora* with approximately $n = 2000$, 650, and 250 species, respectively (Reznicek 2024). There are 180 *Carex* species native or naturalized to Europe (Chater 2005), 67 of them occurring in the Carpathian Basin as well. Several species are traditionally used in folk medicine, e.g., *Cyperus rotundus* and *C. scariosus* for abdominal tumors (Hartwell 1969). In addition, many sedges are used in traditional medicines for the treatment of different diseases, e.g., stomach and bowel disorders, hematopoietic disorders (Ito et al. 2012), tumors, infectious diseases, bacteria-related dermatologic conditions, dysentery, and enteritis, caused by different microorganisms, pain, and fever, diabetes, and problems concerning the circulation, respiratory and reproductive organs (Simpson et al. 2001, Al-Snafi 2016; Kamala et al. 2018).

Although Cyperaceae is one of the largest plant families in the world, phytochemical or pharmacological investigations have been carried out with only a limited number of species. The most investigated Cyperaceae species is *Cyperus rotundus.* To date, nearly 200 compounds have been identified from this plant, including terpenoids, flavonoids, stilbenes, aromatic compounds, and fatty acids (Babiaka et al. 2021). Pharmacological studies have reported antioxidant, antimicrobial, anti-inflammatory, cardioprotective, and antiproliferative activities of different plant parts (tuber, rhizome, leaf, essential oil) (Kilani et al. 2008a; b; Sundaram et al. 2008; Dang et al. 2011). Cyperaceae species are rich sources of bioactive constituents (e.g., flavonoids, phenolic acids, phenylpropane derivatives, stilbenes, coumarins, quinones, and terpenoids) that contribute to a wide range of their medicinal properties (Nakajima et al. 1978; Kurihara et al. 1990; Fiorentino et al. 2006, 2008a; Arraki et al. 2013; Gamal et al. 2015; Dhar et al. 2017; Cho et al. 2018). From the aqueous MeOH extract of *Schoenoplectus lacustris* 49 compounds were isolated, among them polyphenols, cinnamic acid, and dihydrocinnamic acid derivatives, flavonoids, and nor-isoprenoids (D'Abrosca et al. 2006). According to Harborne's research, luteolin, tricin and glycoflavones are characteristic flavonoids in the leaves of the sedges (e.g., *Carex riparia, Carex acutiformis, Carex trifida, Carex nigra, Carex sylvatica,* and *Cyperus longus)* (Harborne 1971). A survey of the flavonoids of 92 Australian *Cyperus* species has confirmed the occurrence of luteolin 5-methyl ether, tricin, luteolin, and glycosides of the last two, in the leaves of the plants. Quercetin occurred in the analyzed species either in glycosidic form or as a methyl ether. The 3-monomethyl and 3,7-dimethyl ethers of kaempferol and quercetin and the 3,7,3'-trimethyl ether of quercetin have also been reported from *Cyperus* species (Harborne et al. 1982). Besides flavonoids, stilbenes of Cyperaceae species possess noteworthy biological activities (Dávid et al. 2021).

This paper reports the investigation of the effect of fractions prepared from MeOH extracts of *Carex acutiformis,*

C. appropinquata, C. bohemica, C. buxbaumii, C. caryophyllea, C. conica, C. davalliana, C. diandra, C. distans, C. divulsa, C. elata, C. flacca, C. foliosissima, C. grayi, C. halleriana, C. hartmaniorum, C. hirta, C. lasiocarpa, C. melanostachya, C. michelii, C. montana, C. morrowii, C. muskingumensis, C. ornithopoda, C. oshimensis, C. paniculata, C. pendula, C. petriei, C. praecox, C. pseudocyperus, C. remota, C. riparia, C. rostrata, C. siderosticta, C. stenophylla, C. supina, C. sylvatica, C. umbrosa, C. vesicaria, Cladium mariscus, and *Schoenoplectus lacustris* in the ORAC (Oxygen Radical Absorbance Capacity) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay, xanthine oxidase inhibitory assay, and antimicrobial tests on eight bacterial strains.

Materials and methods

Plant materials

The plants were collected during the flowering period between June and August 2020 and 2021 in the Botanical Garden of Eötvös Loránd University (Illés u. 25, 1083 Budapest, Hungary). Botanical identification was performed by László Papp (Botanical Garden, Eötvös Loránd University). Voucher specimens for each plant (Nos 902–940, *Carex acutiformis, Carex appropinquata, Carex bohemica, Carex buxbaumii, Carex caryophyllea, Carex conica, Carex davalliana, Carex diandra, Carex distans, Carex divulsa, Carex elata, Carex flacca, Carex foliosissima, Carex grayi, Carex halleriana, Carex hartmaniorum, Carex hirta, Carex lasiocarpa, Carex melanostachya, Carex michelii, Carex montana, Carex morrowii, Carex muskingumensis, Carex ornithopoda, Carex oshimensis, Carex paniculata, Carex pendula, Carex petriei, Carex praecox, Carex pseudocyperus, Carex remota, Carex riparia, Carex rostrata, Carex siderosticta, Carex stenophylla, Carex supina, Carex sylvatica, Carex umbrosa, Carex vesicaria, Cladium mariscus, Schoenoplectus lacustris)* were deposited at the Herbarium of the Institute of Pharmacognosy, University of Szeged, Szeged, Hungary.

Preparation of the extracts and fractions

Extracts were prepared from 20 g of air-dried, powdered plant material with 200 mL of methanol by ultrasonication (5 × 15 min). After filtration, the solutions were evaporated to dryness under reduced pressure (yield of the methanolic extracts prepared from the different sedges was between 3.2 and 9.4%). The residues were dissolved in 50 mL of 50% aqueous methanol and subjected to solvent–solvent partition consecutively with *n*-hexane (4 × 50 mL) (fraction A), chloroform (4 × 50 mL) (fraction B), and ethyl acetate (4 × 50 mL) (fraction C). The extracts and fractions were stored in the fridge, at 6 °C until investigations.

Antioxidant assays

DPPH assay

Free radical scavenging activity of the plant fractions was assessed using DPPH assay (Fukumoto and Mazza 2000). The measurement was carried out on a 96-well microplate. In brief, a microdilution series of samples (1 mg/mL, dissolved in MeOH) were prepared starting with 150 µL. To obtain 200 µL of sample, 50 µL of DPPH reagent (100 µM) was added to each sample. The microplate was stored at room temperature in darkness. The absorbance was measured after 30 min at 550 nm using a FLUOStar Optima microplate reader (BMG Labtech). MeOH and ascorbic acid (0.01 mg/mL) were used as blank control and standard, respectively.

The antiradical activity was calculated using the following equation:

$$I\% = \frac{A_0 - A_1}{A_0} \bullet 100,$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. The antiradical activity of the samples was expressed as IC_{50} (concentration of the compounds that caused 50% inhibition). IC_{50} values (µg/mL) were determined using GraphPad Prism 8.0 software.

ORAC assay

The oxygen radical absorbance capacity (ORAC) assay was carried out on a 96-well microplate according to the method described by Mielnik et al. (2011). Samples (20 µL) at a 0.01 mg/mL concentration were mixed with 60 µL of 2,2′-azobis(2-methyl-propionamidine)-dihydrochloride (AAPH) (12 mM final concentration) and 120 µL of fluorescein solution (70 nM final concentrations), then the fluorescence was measured for 3 h with 1.5-min cycle intervals with a FLUOStar Optima plate-reader. All experiments were carried out in triplicate; (±)-6-hydroxy-2,5,7,8-tetramethyl-chromane-2-carboxylic acid (Trolox) was used as a standard. AAPH free radical and Trolox standard were purchased from Sigma-Aldrich (Budapest, Hungary). Fluorescein was purchased from Fluka Analytical (Tokyo, Japan). The antioxidant capacity was expressed as mmol Trolox equivalent per g of dry extract (mmol TE/g), using GraphPad Prism 8.0 software.

XO-inhibitory activity

The method, based on a modified protocol created by Sigma, is a continuous spectrophotometric rate determination. The absorbance of XO-induced uric acid production from xanthine was measured at 290 nm for 3 min in a 96-well plate, using the FLUOStar OPTIMA plate reader. The XO inhibitory effect was determined via the decreased production of uric acid. The reagents were 50 mM potassium phosphate buffer, pH 7.5 with 1 M KOH, 0.15 mM xanthine solution (pH 7.5) and XO solution (0.2 units/mL). XO, isolated from bovine milk (lyophilized powder) and xanthine powder were purchased from Sigma-Aldrich Co. The different plant fractions (12 mg/mL) were prepared in dimethyl sulfoxide (DMSO). For enzyme activity control, the final reaction mixture consisted of 100 µL of xanthine, 150 µL of buffer, and 50 µL of XO in a 300 µL well. The reaction mixture for inhibition was made with 100 µL of xanthine, 140 µL of buffer, 10 µL of sample, and 50 µL of XO. Allopurinol served as a positive control. Extracts were added in appropriate volumes so that the final concentration of DMSO in the assay did not exceed 3.3% of the total volume. All experiments were conducted in triplicate. The reaction was initiated by the automatic addition of 0.050 mL of XO solution to a final concentration of 0.006 units/mL. The IC_{50} values were calculated by analyzing the inhibition (%) of each concentration, by using GraphPad Prism 8.0 software (GraphPad Software Inc.) with non-linear regression.

Antibacterial assay

Antibacterial activity of the plant fractions was tested against four gram-positive bacterial strains *(Staphylococcus aureus* ATCC 29213, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633) and four gram-negative strains *Moraxella catarrhalis* ATCC 25238, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218, and *Klebsiella pneumoniae* ATCC 700603). The antibacterial activities of the extracts were tested for their inhibitory zones using the disc-diffusion method as described previously (Bauer et al. 1966). The test organisms (*S. aureus, MRSA, S. epidermidis, B. subtilis, M. catarrhalis, P. aeruginosa, E. coli,* and *K. pneumoniae)* were cultured on Mueller–Hinton agar plates (bio-Mérieux) at 37 °C. Columbia agar + 5% sheep blood (COS) plates (bio-Mérieux) were used to grow *M. catarrhalis.* A few bacterial colonies were picked from overnight agar plate cultures and suspensions were prepared in a sterile saline solution. The turbidity was adjusted to match the 0.5 McFarland standard, resulting in a concentration of $1–2 \times 10^8$ CFU/mL. The sterile filter paper discs (6 mm in diameter) impregnated with the fractions (10 µL of dried extracts dissolved in DMSO at 50 mg/mL) were placed on the agar plates seeded with the respective bacteria. The solvent (DMSO) served as a negative control, while ciprofloxacin susceptibility disks (5 µg/disc) were used as a positive control. The plates were then incubated at 37 °C for 24 h under aerobic conditions. The entire diameters of the inhibition zones (including the disc) produced by the plant fractions were measured and recorded. It was observed that DMSO did not inhibit the growth of microorganisms in this used concentration. All experiments were carried out in triplicate.

Data analysis

The GraphPad Prism version 8.0.1 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com) was utilized to process and analyze the entire dataset. The normal distribution of the data was checked using the Shapiro–Wilk test. One-way ANOVA followed by Bonferroni's multiple comparison test was performed to evaluate the differences in activities of fractions. The differences were considered significant when $p < 0.05$ (*), $p < 0.01$ (**), and $p < 0.001$ (***). The correlation between the two groups was analyzed using Pearson's correlation test.

Results and discussion

This study aimed to investigate extracts of 41 species of Cyperaceae (39 *Carex,* 1 *Cladium,* and 1 *Schoenoplectus)* for their antioxidant, XO inhibitory, and antibacterial activities. The results of the assays are presented in Tables 1 and 2, together with graphical presentations in Fig. 1–3. The available scientific literature on these species is scarce. The chemical profiles and pharmacological activities of analyzed species are poorly characterized, which underscores that studies investigating the pharmacological effects and characteristic constituents of these species are of great importance. To the best of our knowledge, this is the first comprehensive study of Cyperaceae, and especially *Carex* species.

The extracts were prepared with methanol from whole plant materials, and then solvent–solvent partition was performed using *n*-hexane (A), chloroform (B), and ethyl acetate (C), respectively. Altogether, the analysis of 41 species resulted in 123 fractions which were screened in the in vitro tests. Although there was no generally accepted threshold for efficacy in our experiments, in the DPPH assay IC_{50} values less than 25 µg/mL, in the ORAC assay values greater than 5 mmol TE/g, and in the XO inhibitory assay percentages above 80% were considered as substantial activity, and IC_{50} values were determined for these extracts. On the other

Table 1 Antioxidant and xanthine oxidase inhibitory activities of Cyperaceae species

Species	Sample	DPPH assay IC_{50} values (µg/ml)	ORAC assay mmol TE/g	XO-inhibitory assay IC_{50} values (µg/ml)
Carex acutiformis (AC)	A	43.70 ± 1.48	–	–
	B	**5.80 ± 0.07**	7.90 ± 0.19	**2.55 ± 0.04**
	C	**4.60 ± 0.21**	6.91 ± 0.45	8.24 ± 0.00
Carex appropinquata (AP)	A	–	–	–
	B	22.08 ± 2.43	3.08 ± 0.08	**1.72 ± 0.09**
	C	42.42 ± 2.23	1.86 ± 0.01	–
Carex bohemica (CB)	A	–	–	–
	B	13.37 ± 0.18	3.36 ± 0.16	**1.63 ± 0.19**
	C	13.26 ± 0.19	3.93 ± 0.04	–
Carex buxbaumii (BU)	A	82.32 ± 0.26	–	–
	B	20.47 ± 0.58	3.30 ± 0.04	0.72 ± 0.01
	C	**6.26 ± 0.01**	6.19 ± 0.15	–
Carex caryophyllea (CA)	A	65.05 ± 5.52	–	–
	B	37.89 ± 0.07	2.39 ± 0.22	–
	C	11.72 ± 0.11	9.37 ± 0.69	–
Carex conica (CCO)	A	–	–	–
	B	–	1.12 ± 0.04	22.24 ± 0.42
	C	82.96 ± 2.38	2.75 ± 0.10	93.31 ± 0.00
Carex davalliana (DA)	A	–	–	–
	B	–	1.57 ± 0.00	–
	C	–	–	–
Carex diandra (DI)	A	–	–	–
	B	13.36 ± 0.00	2.85 ± 0.03	–
	C	26.01 ± 0.57	3.17 ± 0.09	4.03 ± 0.15
Carex distans (DIS)	A	–	–	–
	B	26.66 ± 0.77	3.71 ± 0.14	**1.87 ± 0.10**
	C	16.47 ± 0.40	**13.37 ± 0.88**	**2.70 ± 0.28**
Carex divulsa (CD)	A	52.09 ± 0.90	–	28.83 ± 1.59
	B	11.63 ± 0.46	2.95 ± 0.12	**2.33 ± 0.08**
	C	14.28 ± 0.05	3.62 ± 0.15	8.94 ± 0.15
Carex elata (CC)	A	78.17 ± 2.64	–	–
	B	32.34 ± 0.75	3.09 ± 0.05	6.00 ± 0.01
	C	20.00 ± 0.34	**11.59 ± 0.05**	34.25 ± 0.28
Carex flacca (CFL)	A	40.69 ± 0.61	1.15 ± 0.11	7.03 ± 0.33
	B	13.94 ± 0.39	5.05 ± 0.11	**1.47 ± 0.03**
	C	**4.48 ± 0.08**	6.91 ± 0.23	3.60 ± 0.24
Carex foliosissima (CFO)	A	–	–	–
	B	67.09 ± 1.91	1.55 ± 0.03	5.61 ± 0.33
	C	–	2.26 ± 0.03	–
Carex grayi (CG)	A	–	–	10.47 ± 0.02
	B	32.22 ± 1.21	4.00 ± 0.33	**1.24 ± 0.07**
	C	37.38 ± 0.56	**12.93 ± 0.63**	–
Carex halleriana (CHA)	A	44.47 ± 1.03	–	–
	B	18.06 ± 0.79	4.02 ± 0.16	5.04 ± 0.02
	C	11.07 ± 0.14	5.82 ± 0.00	15.51 ± 1.46
Carex hartmaniorum (CH)	A	–	–	–
	B	27.82 ± 1.15	1.06 ± 0.06	7.25 ± 0.47
	C	25.95 ± 1.50	9.34 ± 0.01	–
Carex hirta (CHI)	A	–	–	17.08 ± 2.52
	B	30.47 ± 1.89	3.87 ± 0.24	**1.53 ± 0.00**
	C	12.76 ± 0.10	7.20 ± 0.23	–
Carex lasiocarpa (LA)	A	49.20 ± 2.96	1.16 ± 0.08	11.25 ± 1.29
	B	24.30 ± 1.17	3.64 ± 0.12	**2.28 ± 0.15**
	C	21.77 ± 0.20	5.62 ± 0.33	49.8 ± 4.00

Table 1 (continued)

Species	Sample	DPPH assay IC_{50} values (µg/ml)	ORAC assay mmol TE/g	XO-inhibitory assay IC_{50} values (µg/ml)
Carex melanostachya (CM)	A	–	–	18.22 ± 0.09
	B	24.59 ± 2.02	5.15 ± 0.00	**0.94 ± 0.03**
	C	22.15 ± 0.64	6.49 ± 0.23	22.39 ± 1.41
Carex michelii (MI)	A	–	–	–
	B	39.08 ± 0.29	1.90 ± 0.07	4.45 ± 0.20
	C	16.62 ± 0.31	5.02 ± 0.52	–
Carex montana (CMN)	A	–	–	–
	B	31.11 ± 0.64	1.66 ± 0.12	**2.21 ± 0.04**
	C	13.69 ± 0.18	**14.38 ± 0.27**	17.37 ± 0.83
Carex morrowii (CMR)	A	–	–	–
	B	25.96 ± 0.63	4.42 ± 0.64	–
	C	46.84 ± 1.70	5.52 ± 1.31	–
Carex muskingumensis (CMU)	A	79.18 ± 2.71	–	–
	B	15.11 ± 0.14	3.94 ± 0.27	**2.93 ± 0.00**
	C	**8.21 ± 0.31**	5.64 ± 0.01	12.26 ± 1.35
Carex ornithopoda (COR)	A	–	–	–
	B	–	–	21.45 ± 0.70
	C	34.28 ± 0.05	9.33 ± 0.32	72.91 ± 14.89
Carex oshimensis (CO)	A	84.49 ± 1.23	–	–
	B	40.13 ± 3.26	3.52 ± 0.04	7.19 ± 0.03
	C	33.23 ± 0.79	8.37 ± 0.27	11.2 ± 0.78
Carex paniculata (PA)	A	–	–	–
	B	22.48 ± 0.29	2.39 ± 0.04	**2.23 ± 0.00**
	C	24.21 ± 1.15	5.06 ± 0.2	–
Carex pendula (RP)	A	59.06 ± 5.48	–	–
	B	27.06 ± 1.23	2.85 ± 0.02	3.68 ± 0.24
	C	13.35 ± 0.09	3.68 ± 0.00	–
Carex petriei (CPE)	A	–	1.40 ± 0.22	–
	B	11.05 ± 0.00	1.77 ± 0.08	3.31 ± 0.20
	C	64.51 ± 1.23	**14.99 ± 0.30**	57.65 ± 0.41
Carex praecox (CP)	A	–	–	–
	B	12.65 ± 0.03	2.59 ± 0.72	**1.85 ± 0.06**
	C	15.53 ± 0.67	1.71 ± 0.48	–
Carex pseudocyperus (CPS)	A	–	–	–
	B	26.14 ± 0.11	3.82 ± 0.25	**2.18 ± 0.18**
	C	14.62 ± 0.44	4.22 ± 0.26	32.99 ± 4.25
Carex remota (CRE)	A	–	–	–
	B	24.05 ± 0.19	3.23 ± 0.08	6.71 ± 0.32
	C	21.30 ± 0.10	5.29 ± 0.09	24.91 ± 0.08
Carex riparia (CR)	A	–	–	–
	B	36.50 ± 1.92	3.02 ± 0.04	6.29 ± 0.47
	C	38.46 ± 0.45	9.42 ± 0.26	–
Carex rostrata (RO)	A	65.17 ± 10.26	–	–
	B	45.00 ± 0.01	–	**1.51 ± 0.17**
	C	34.57 ± 0.59	**14.34 ± 0.35**	24.75 ± 1.33
Carex siderosticta (CSI)	A	55.04 ± 0.50	–	–
	B	14.22 ± 0.69	2.50 ± 0.01	22.72 ± 1.77
	C	**4.29 ± 0.14**	**11.69 ± 0.65**	–
Carex stenophylla (ST)	A	62.86 ± 0.40	1.06 ± 0.12	–
	B	21.13 ± 0.27	3.22 ± 0.01	–
	C	32.71 ± 0.11	–	–
Carex supina (SU)	A	–	–	–
	B	24.99 ± 0.00	3.01 ± 0.14	4.34 ± 0.04
	C	**9.44 ± 0.41**	4.72 ± 0.06	60.23 ± 12.32

Table 1 (continued)

Species	Sample	DPPH assay IC_{50} values (µg/ml)	ORAC assay mmol TE/g	XO-inhibitory assay IC_{50} values (µg/ml)
Carex sylvatica (CS)	A	–	–	–
	B	37.51 ± 0.36	1.81 ± 0.27	6.69 ± 0.27
	C	**8.30 ± 0.07**	**13.19 ± 0.31**	41.15 ± 4.12
Carex umbrosa (CU)	A	–	–	–
	B	50.00 ± 2.12	1.52 ± 0.08	10.81 ± 0.57
	C	67.80 ± 2.22	1.12 ± 0.04	–
Carex vesicaria (VE)	A	–	–	–
	B	59.70 ± 3.29	3.02 ± 0.07	–
	C	55.24 ± 3.01	3.03 ± 0.13	–
Cladium mariscus (MA)	A	82.84 ± 4.75	–	10.52 ± 0.38
	B	15.12 ± 0.10	3.89 ± 0.33	**0.44 ± 0.04**
	C	**4.31 ± 0.04**	**11.39 ± 1.20**	19.00 ± 0.73
Shoenoplectus lacustris (SL)	A	25.43 ± 1.05	–	–
	B	7.71 ± 0.10	4.30 ± 0.03	5.92 ± 0.40
	C	**2.59 ± 0.04**	6.95 ± 0.23	5.74 ± 0.08
Rutin		2.26 ± 0.13	10.32 ± 0.49	–
Allopurinol		–	–	0.26 ± 0.01

Bold values highlight fractions that possess remarkable antioxidant and XO inhibitory properties. Data are presented as mean ± SD of values. (IC_{50}: half-maximal inhibitory concentration; DPPH, (2,2-diphenyl-1-picrylhydrazyl); ORAC: oxygen radical absorbance capacity; TE: Trolox Equivalent; XO: xanthine oxidase)

hand, in the DPPH assay, values above 100 µg/mL, in the ORAC assay values less than 1 mmol TE/g, and in the XO inhibitory assay values less than 10% were considered inactive, and therefore, these values are not presented in Table 1 and marked as inactive.

Antioxidant activity

Various assays are used to test antioxidant activity. However, the most widely used methods involve generating free radical species, which are then neutralized by antioxidant compounds (Arraki et al. 2013). In the present study, two different experiments, the DPPH and ORAC assays, were carried out to characterize the antioxidant potential of Cyperaceae species. DPPH radical is commonly used as a substrate to evaluate antioxidant activity. Using the ORAC antioxidant assay, the process of free radical scavenging can be followed from the time the sample is added, at regular intervals, until the end of the reaction, providing information about the inhibition time and the degree of inhibition.

The IC_{50} values for DPPH activity together with the antioxidant activity of 1 g fraction expressed as mmol of tocopherol equivalent (mmol TE/g) measured using ORAC assay are summarized in Table 1 and Figs. 1 and 2. Notably, EtOAc fractions were overrepresented among the fractions with higher activity (IC_{50} < 5.0 µg/mL, n = 5; IC_{50} = 5.0–15.0 µg/mL, n = 12) in the DPPH assay compared to chloroform fractions (IC_{50} < 5.0 µg/mL, n = 0; IC_{50} = 5.0–15.0 µg/mL, n = 9), while all of the active hexane fractions had an IC_{50} value higher than 25.0 µg/mL. The IC_{50} values ranged from 2.59 µg/mL *(S. lacustris)* to 84.49 µg/mL *(C. oshimensis)*. Among the 41 sedges, the EtOAc (C) extract of five species *(C. acutiformis, C. flacca, C. mariscus, C. sid-erosticta,* and *S. lacustris)* was highly effective (IC_{50} values < 5 µg/mL), while the hexane fractions had a weak free radical scavenging effect (IC_{50} > 25 µg/mL). In the case of *C. acutiformis* (IC_{50} 5.80 ± 0.07 (B) and 4.60 ± 0.21 (C) µg/mL) and *S. lacustris* (IC_{50} 7.71 ± 0.10 (B), and 2.59 ± 0.04 (C) µg/mL], both the chloroform (B) and EtOAc (C) extracts showed promising activity. The antioxidant activity of chloroform fractions was lower, in general; however, comparing chloroform (B) and ethyl acetate fractions (C) with at least moderate activity (IC_{50} < 25 µg/mL) the Pearson's correlation results in significant positive correlation ($r(30) = 0.649$, $p = 0.007$). Although the ethyl acetate fractions (C) were the most promising one, the statistical comparison using one-way ANOVA demonstrated significant difference ($F(5,12) = 218.9$, $p < 0.0001$) between groups (Fig. 2A). Comparison of discussed ethyl acetate (C) fractions using Bonferroni's multiple comparison test showed that the activity of rutin, used as positive control, had significantly lower IC_{50} value (2.26 ± 0.13 µg/mL) compared even to the most effective *Shoenoplectus lacustris* fraction ($p = 0.0312$, 95% C.I. [–0,6347, –0,02532]).

In the ORAC test, similar to the DPPH results, the EtOAc (C) fractions demonstrated the highest activity (Fig. 1). The one-way ANOVA ($F(9,20) = 19.16$, $p < 0.0001$) showed that activity of nine fractions were comparable to that of rutin (10.32 mmol TE/g) (Fig. 2B). Bonferroni's multiple comparison test showed that the activity of *C. elata*

Table 2 Antibacterial activity of the most active Cyperaceae extracts (inhibition zones in mm)

Species	Samples	Diameter of inhibition zones (mm ± SD)							
		S. aureus ATCC 29213	*S. epidermidis* ATCC 12228	*MRSA* ATCC 43300	*B. subtilis* ATCC 6633	*M. catarrhalis* ATCC 25238	*E. coli* ATCC 35218	*K. pneumoniae* ATCC 700603	*P. aeruginosa* ATCC 27853
Carex acutiformis AC	A	–	7.3 ± 0.6	–	9.7 ± 2.08	–	8.7 ± 0.6	7.0 ± 1.0	7.8 ± 0.68
	B	–	–	–	7.7 ± 0.6	–	–	–	–
	C	9.3 ± 0.6	10.0 ± 0.0	9.3 ± 0.6	8.0 ± 0.0	7.3 ± 0.6	–	–	–
Carex appropinquata (AP)	A	–	–	–	7.0 ± 0.0	–	–	8.5 ± 1.5	–
	B	–	–	–	–	7.0 ± 0.0	–	–	–
	C	–	–	–	–	–	–	–	–
Carex bohemica (CB)	A	7.0 ± 1.0	7.0 ± 0.0	7.0 ± 0.0	8.3 ± 1.5		7.7 ± 0.6	8.7 ± 0.6	–
	B	–	–	–	–	–	–	–	–
	C	8.0 ± 0.0	10 ± 0	7.7 ± 0.6	8.7 ± 0.6	7.3 ± 0.6	–	–	–
Carex buxbaumii (BU)	A	–	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–
	C	–	–	–	–	–	–	–	–
Carex caryophyllea (CA)	A	–	–	–	–	–	–	8.5 ± 0.5	–
	B	–	–	–	7.0 ± 0.0	7.0 ± 0.0	–	–	–
	C	10.3 ± 0.6	12.0 ± 1.0	10.3 ± 0.6	8.3 ± 0.6	7.3 ± 0.6	–	–	–
Carex conica (CCO)	A	7.33 ± 0.6	–	7.33 ± 0.6	8.67 ± 2.08	–	8.0 ± 1.0	10.0 ± 1.0	7.7 ± 0.6
	B	–	–	–	–	6.0 ± 0.0	–	–	–
	C	–	–	–	–	–	–	–	–
Carex davalliana (DA)	A	–	–	–	7.5 ± 0.5	–	7.5 ± 0.5	7.3 ± 0.6	8.5 ± 1.5
	B	–	–	–	–	10.0 ± 0.0	–	–	–
	C	–	–	–	–	–	–	–	–
Carex diandra (DI)	A	7.3 ± 0.6	–	8.3 ± 0.6	–	7.3 ± 0.6	9.0 ± 1.0	9.3 ± 1.2	7.0 ± 1.0
	B	–	–	–	–	–	–	–	–
	C	8.3 ± 0.6	8.0 ± 0.0	7.3 ± 0.6	7.0 ± 0.0	7.3 ± 0.6	–	–	–
Carex distans (DIS)	A	7.0 ± 0.0	–	–	11.3 ± 1.5	7.0 ± 1.0	7.7 ± 0.6	9.3 ± 0.6	8.3 ± 1.5
	B	–	–	–	7.3 ± 0.6	–	–	–	–
	C	7.0 ± 0.0	11.3 ± 0.6	8.0 ± 0.0	8.0 ± 0.0	7.0 ± 0.0	–	–	–
Carex divulsa (CD)	A	–	7.0 ± 0.0	7.0 ± 0.0	8.0 ± 1.0	–	7.0 ± 0.0	–	7.7 ± 0.6
	B	–	–	–	8.0 ± 0.0	–	–	–	–
	C	–	7.0 ± 0.0	–	7.0 ± 0.0	–	–	–	–
Carex elata (CC)	A	8.0 ± 1.0	–	7.0 ± 0.0	7.7 ± 1.2	–	7.7 ± 0.6	8.7 ± 1.5	9.0 ± 1.0
	B	–	–	–	–	–	–	–	–
	C	13.3 ± 0.6	18.3 ± 0.6	13.0 ± 0.0	10.7 ± 0.6	8.0 ± 0.0	–	–	–
Carex flacca (CFL)	A	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	–	7.0 ± 0.0	7.0 ± 0.0	–
	B	8.0 ± 0.0	10.0 ± 0.0	8.0 ± 0.0	8.0 ± 0.0	–	–	–	7.0 ± 0.0
	C	11.0 ± 0.0	14.0 ± 0.0	12.0 ± 0.0	10.0 ± 0.0	7.0 ± 0.0	–	–	–
Carex foliosissima (CFO)	A	8.0 ± 1.0	7.0 ± 0.0	7.0 ± 0.0	7.3 ± 1.5	7.0 ± 1.0	7.7 ± 0.6	7.7 ± 1.2	7.7 ± 1.2
	B	–	–	–	8.3 ± 0.6	–	–	–	–
	C	–	–	–	–	–	–	–	–
Carex grayi (CG)	A	7.0 ± 0.0	7.0 ± 0.0	–	–	–	–	–	7.0 ± 0.0
	B	–	–	–	–	–	–	–	–
	C	–	–	–	–	–	–	–	–
Carex halleriana (CHA)	A	7.3 ± 0.6	–	–	7.3 ± 1.5	7.0 ± 0.0	–	7.7 ± 1.2	7.7 ± 1.2
	B	–	–	–	7.0 ± 0.0	–	–	–	–
	C	–	–	–	7.0 ± 0.0	–	–	–	–
Carex hartmaniorum (CH)	A	7.7 ± 0.6	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	8.0 ± 1.0	7.3 ± 0.6	8.7 ± 2.08	7.3 ± 0.6
	B	–	–	–	–	–	–	–	–
	C	11.0 ± 0.0	17.0 ± 0.0	13.0 ± 0.0	9.7 ± 0.6	8.0 ± 0.0	–	–	–
Carex hirta (CHI)	A	7.0 ± 0.0	7.0 ± 0.0	–	–	7.0 ± 1.0	7.3 ± 0.6	8.7 ± 2.08	7.0 ± 0.0
	B	–	–	–	–	–	–	–	–
	C	7.0 ± 0.0	10.0 ± 0.0	7.3 ± 0.6	–	–	–	–	–

Table 2 (continued)

Species	Samples	Diameter of inhibition zones (mm ± SD)							
		S. aureus ATCC 29213	*S. epidermidis* ATCC 12228	*MRSA* ATCC 43300	*B. subtilis* ATCC 6633	*M. catarrhalis* ATCC 25238	*E. coli* ATCC 35218	*K. pneumoniae* ATCC 700603	*P. aeruginosa* ATCC 27853
Carex lasiocarpa (LA)	A	–	–	–	–	–	7.0 ± 0.0	7.3 ± 0.6	7.0 ± 0.0
	B	–	–	–	–	7.0 ± 0.0	–	–	–
	C	–	–	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	–	–	–
Carex melanostachya (CM)	A	–	–	–	–	7.3 ± 0.6	–	7.0 ± 0.0	–
	B	–	–	–	–	–	–	–	–
	C	11.0 ± 0.0	16.0 ± 1.0	13.7 ± 0.6	9.0 ± 0.0	8.7 ± 0.6	–	–	–
Carex michelli (MI)	A	–	–	–	–	7.0 ± 0.0	7.0 ± 0.0	–	7.0 ± 0.0
	B	–	–	–	–	7.0 ± 0.0	–	–	–
	C	–	–	–	–	–	–	–	–
Carex montana (CMN)	A	–	8.0 ± 0.0	8.0 ± 0.0	9.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0
	B	7.0 ± 0.0	7.0 ± 0.0	–	7.0 ± 0.0	7.0 ± 0.0	–	–	–
	C	16.0 ± 0.0	18.0 ± 0.0	15.0 ± 0.0	14.0 ± 0.0	9.0 ± 0.0	–	–	–
Carex morrowii (CMR)	A	–	–	8.0 ± 0.0	–	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0
	B	12.0 ± 0.0	15.0 ± 0.0	12.0 ± 0.0	10.0 ± 0.0	9.0 ± 0.0	–	–	–
	C	10.0 ± 0.0	17.0 ± 0.0	15.0 ± 0.0	15.0 ± 0.0	7.0 ± 0.0	–	–	–
Carex muskingumensis (CMU)	A	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0
	B	–	–	–	–	7.0 ± 0.0	–	–	–
	C	12.0 ± 0.0	15.0 ± 0.0	12.0 ± 0.0	10.0 ± 0.0	8.0 ± 0.0	–	–	–
Carex ornithopoda (COR)	A	8.3 ± 1.5	7.3 ± 0.6	7.3 ± 0.6	8.7 ± 1.2	–	8.3 ± 0.6	9.3 ± 0.6	8.0 ± 1.0
	B	–	–	–	–	–	–	–	–
	C	11.3 ± 0.6	13.3 ± 0.6	10.3 ± 0.6	9.3 ± 0.6	7.7 ± 0.6	–	–	–
Carex oshimensis (CO)	A	7.0 ± 0.0	–	–	8.0 ± 0.0	8.0 ± 0.0	7.0 ± 0.0	8.0 ± 0.0	7.0 ± 0.0
	B	7.0 ± 0.0	9.0 ± 0.0	–	7.0 ± 0.0	7.0 ± 0.0	–	–	–
	C	15.0 ± 0.0	20.0 ± 0.0	15.0 ± 0.0	15.0 ± 0.0	9.0 ± 0.0	–	–	8.0 ± 0.0
Carex paniculata (PA)	A	8.5 ± 0.5	–	–	–	7.3 ± 0.6	–	7.5 ± 0.5	7.5 ± 0.5
	B	–	–	–	–	7.0 ± 0.0	–	–	–
	C	–	–	–	–	–	–	–	–
Carex pendula (RP)	A	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	8.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	–	–
	B	–	7.0 ± 0.0	–	–	7.0 ± 0.0	–	–	–
	C	10.0 ± 0.0	15.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	7.0 ± 0.0	–	–	–
Carex petriei (CPE)	A	7.0 ± 0.0	8.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	–
	B	–	–	–	–	–	–	–	–
	C	14.0 ± 0.0	18.0 ± 0.0	14.0 ± 0.0	12.0 ± 0.0	9.0 ± 0.0	–	–	–
Carex praecox (CP)	A	7.3 ± 0.6	–	7.0 ± 0.0	10.0 ± 2.0	8.0 ± 1.0	8.3 ± 0.6	10.0 ± 1.7	7.3 ± 0.6
	B	–	–	–	–	–	–	–	–
	C	–	–	–	–	–	–	–	–
Carex pseudocyperus (CPS)	A	7.7 ± 1.2	7.7 ± 0.6	7.3 ± 0.6	9.0 ± 2.0	8.0 ± 0.0	7.7 ± 0.6	8.3 ± 0.6	–
	B	–	–	–	–	–	–	–	–
	C	7.0 ± 0.0	8.7 ± 0.6	7.0 ± 0.0	10.0 ± 0.0	7.0 ± 0.0	–	–	–
Carex remota (CRE)	A	–	–	7.3 ± 0.6	10.0 ± 2.0	7.0 ± 1.0	7.0 ± 0.0	7.7 ± 1.2	7.0 ± 0.0
	B	–	–	–	7.0 ± 0.0	–	–	–	–
	C	7.3 ± 0.6	–	8.0 ± 0.0	7.3 ± 0.6	7.0 ± 0.0	–	–	–
Carex riparia (CR)	A	–	–	7.0 ± 0.0	–	7.3 ± 0.6	8.0 ± 1.0	–	–
	B	–	–	–	–	–	–	–	–
	C	9.3 ± 0.6	–	–	8.7 ± 0.6	8.0 ± 0.0	8.3 ± 0.6	–	–
Carex rostrata (RO)	A	7.5 ± 0.0	–	7.0 ± 0.0	–	–	–	7.7 ± 0.6	8.0 ± 1.0
	B	–	–	–	–	–	–	–	–
	C	11.0 ± 0.0	14.0 ± 1.0	10.0 ± 0.0	11.3 ± 0.6	8.0 ± 0.0	7.0 ± 0.0	–	–
Carex siderosticta (CSI)	A	7.0 ± 0.0	–	–	8.0 ± 0.0	7.0 ± 0.0	–	–	7.0 ± 0.0
	B	–	–	–	7.0 ± 0.0	8.0 ± 0.0	–	–	–
	C	14.0 ± 0.0	16.0 ± 0.0	14.0 ± 0.0	11.0 ± 0.0	12.0 ± 0.0	–	–	7.0 ± 0.0

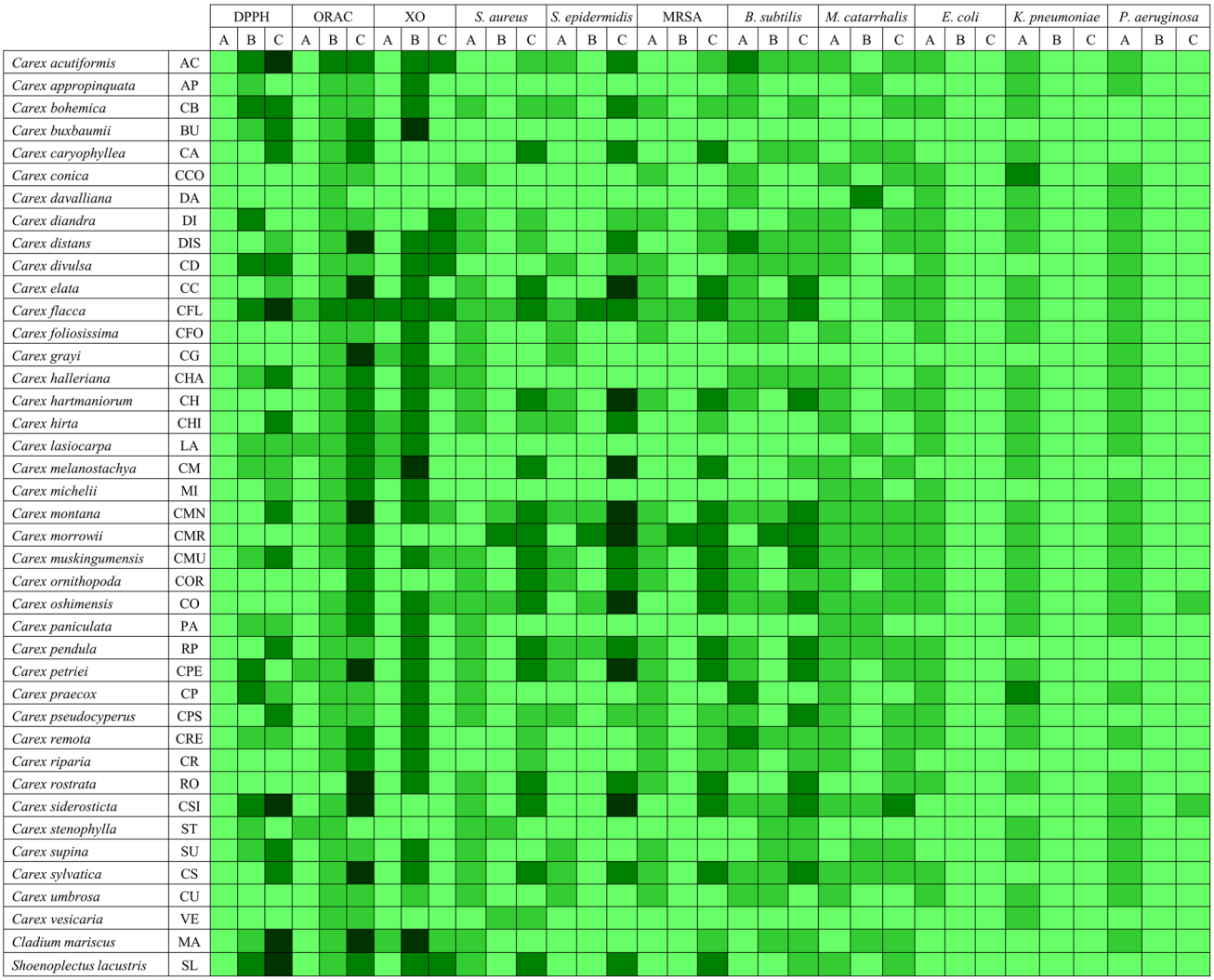
Table 2 (continued)

Species	Samples	Diameter of inhibition zones (mm ± SD)							
		S. aureus ATCC 29213	*S. epidermidis* ATCC 12228	*MRSA* ATCC 43300	*B. subtilis* ATCC 6633	*M. catarrhalis* ATCC 25238	*E. coli* ATCC 35218	*K. pneumoniae* ATCC 700603	*P. aeruginosa* ATCC 27853
Carex stenophylla (ST)	A	7.3 ± 0.6	–	–	–	–	–	7.0 ± 0.0	7.7 ± 0.6
	B	–	–	–	7.0 ± 0.0	–	–	–	–
	C	7.7 ± 0.6	–	–	7.0 ± 0.0	–	–	–	–
Carex supina (SU)	A	7.3 ± 0.6	7.0 ± 0.0	7.0 ± 0.0	–	–	–	9.0 ± 1.0	7.5 ± 0.5
	B	–	–	–	7.0 ± 0.0	7.5 ± 0.5	–	–	–
	C	–	–	–	7.5 ± 0.5	7.0 ± 0.0	–	–	–
Carex sylvatica (CS)	A	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	8.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	–	7.0 ± 0.0
	B	–	–	–	7.0 ± 0.0	7.0 ± 0.0	–	–	–
	C	12.0 ± 0.0	15.0 ± 0.0	12.0 ± 0.0	10.0 ± 0.0	8.0 ± 0.0	–	–	–
Carex umbrosa (CU)	A	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	9.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0	7.0 ± 0.0
	B	–	–	–	7.0 ± 0.0	–	–	–	–
	C	–	–	–	–	–	–	–	–
Carex vesicaria (VE)	A	–	–	–	–	–	–	9.0 ± 1.0	–
	B	7.0 ± 0.0	–	–	–	–	–	–	–
	C	7.0 ± 0.0	–	–	–	–	–	–	–
Cladium mariscus (MA)	A	7.0 ± 0.0	–	–	–	–	–	–	7.7 ± 1.2
	B	–	–	–	8.0 ± 0.0	7.0 ± 0.0	–	–	–
	C	–	–	–	–	–	–	–	–
Shoenoplectus lacustris (SL)	A	8.0 ± 0.0	–	–	–	–	–	–	–
	B	–	–	–	–	–	–	–	–
	C	10.0 ± 0.0	10.0 ± 0.0	10.0 ± 0.0	7.3 ± 0.6	8.0 ± 0.0	–	–	–
Ciprofloxacin		30.0 ± 0.0	34.0 ± 0.0	27.3 ± 0.6	28.3 ± 0.3	30.0 ± 0.0	30.0 ± 1.04	24.4 ± 1.3	28.3 ± 0.3

The values are expressed as mean ± SD. (A: *n*-hexane fraction, B: chloroform fraction, C: ethyl acetate fraction)

(11.59 ± 0.05 mmol TE/g), *Cl. mariscus* (11.39 ± 1.20 mmol TE/g), and *C. siderosticta* (11.69 ± 0.65 mmol TE/g) exerted similar activity to rutin, while the activity of *C. grayi* (12.93 ± 0.63 mmol TE/g), *C. sylvatica* (13.19 ± 0.31 mmol TE/g), *C. distans* (13.37 ± 0.88 mmol TE/g), *C. rostrata* (14.34 ± 0.35 mmol TE/g), *C. montana* (14.38 ± 0.27 mmol TE/g), and *C. petriei* (14.99 ± 0.30 mmol TE/g) were significantly higher compared to rutin ($p < 0.001$). The activity observed in *C. petriei, C. montana,* and *C. rostrata* is particularly noteworthy. The EtOAc (C) extract of *Cl. mariscus* and *C. siderosticta* possessed high activity in both antioxidant assays.

Previously, the antioxidant activity of some Cyperaceae species and their constituents has been investigated. The MeOH extract of *Cyperus longus* showed DPPH radical scavenging activity at $SC_{50} = 22$ µg/mL (the concentration required for a 50% reduction of 40 µM DPPH radical) (Morikawa et al. 2010). Total oligomer flavonoid-enriched and EtOAc extracts of Cyperus rotundus proved to be potent radical scavengers. These extracts decreased nitroblue tetrazolium (NBT) photoreduction by 89.8% and 86.0% at 10 mg/mL concentration, and showed IC_{50} values of 68 and 90 µg/mL, respectively, being more active than the positive control, quercetin. The activity can be related to the presence of phenolic compounds such as flavonoids, tannins, and coumarins in the extracts (Kilani et al. 2008b). The polyphenol content of the EtOH and aqueous extracts of *C. rotundus* was determined as 26.89 and 51.84 mgGAE/g, respectively by Ngoc et al. The flavonoid content of the same extracts was 78.03 and 20.28 mgQE/g, respectively (Ngoc and Minh 2021). Among the stilbenes isolated from the rhizome of *C. rotundus,* cyperusphenol B was the most active (65% at 5 µg/mL) in scavenging free radicals in DPPH assay. The other components showed 58% [(*E*)-mesocyperusphenol A)], 49% [(+)-(*E*)-cyperusphenol A, and (–)-(*E*)-cyperusphenol A], 47% (cyperusphenol D) and 37% (scirpusin A) free radical scavenging activity. All compounds, except scirpusin A possessed higher activity than the positive control ascorbic acid (46%) (Ito et al. 2012). The compound *trans*-scirpusin B, isolated from *Scirpus californicus,* exhibited the most potent DPPH radical scavenging activity with an SC_{50} of 2.8 µM (Schmeda-Hirschmann et al. 1996). The scavenging activities of various stilbene dimers from *Cyperus longus* were as follows: scirpusin A (SC_{50} 8.2 µM), scirpusin B (SC_{50} 2.8 µM), longusone A (SC_{50} 4.6 µM), longusol A (SC_{50} 9.3 µM), longusol B (SC_{50} 4.3 µM), longusol C (SC_{50} 5.0 µM), cassigarol E (SC_{50} 3.2 µM), and cassigarol G (SC_{50} 4.5 µM) (Morikawa et al. 2010). Notably, these



DDPH	IC_{50} (μg/mL)	>25	15–25	5–15	<5
ORAC	IC_{50} (mmol TE/g)	<1	1–5	5–10	>10
XO	IC_{50} (μg/mL)	>20	10–20	1–10	<1
Inhibition zone	mm	<7	7–10	10–15	>15

Fig. 1 In vitro biological activities of hexane (**A**), chloroform (**B**), and ethyl acetate (**C**) fractions of methanolic extracts obtained from Cyperaceae species

values were lower than those of the monomers, i.e. *trans*-resveratrol (SC_{50} 24 μM) and piceatannol (SC_{50} 11 μM), except for pallidol, which had an SC_{50} of 29 μM. The antioxidant activity of MeOH extract of *Carex distachya* roots was measured by DPPH assay (IC_{50} value 4.2 μg/mL) and it was comparable to those of ascorbic acid, α-tocopherol and butylated hydroxytoluene (BHT) (IC_{50} values 4.3, 5.1, and 3.9 μg/mL, respectively). This high antioxidant power of the extract is due, at least partly to its stilbenoids, resveratrol diglucoside, and its methyl ether, and hydroxy pallidol. Resveratrol derivatives showed a strong nitric oxide radical scavenging capacity (> 80% and 58.5% for ascorbic acid and α-tocopherol, and 62.2% for BHT, respectively) (Fiorentino et al. 2008b). Distachyasin isolated from *C. distachya* possessed radical scavenging activity against superoxide radical by 60% at 0.5 mg/mL, hydrogen peroxide at 0.1 and 0.2 mg/mL by 32% and 39%, and NO radical at 0.5 mg/mL by 10.7%. Moreover, it inhibited the formation of reactive oxygen species to thiobarbituric acid over 59.0% at 0.5 mg/mL (Fiorentino et al. 2006). Piceatannol was found to interact with several molecular targets when its antioxidant activity was investigated. It promoted the nuclear translocation and target gene transcription of the antioxidant transcription factor, nuclear factor erythroid 2-related factor 2 (Nrf2) while reducing intracellular reactive oxygen species (ROS) levels (Zhu et al. 2020).

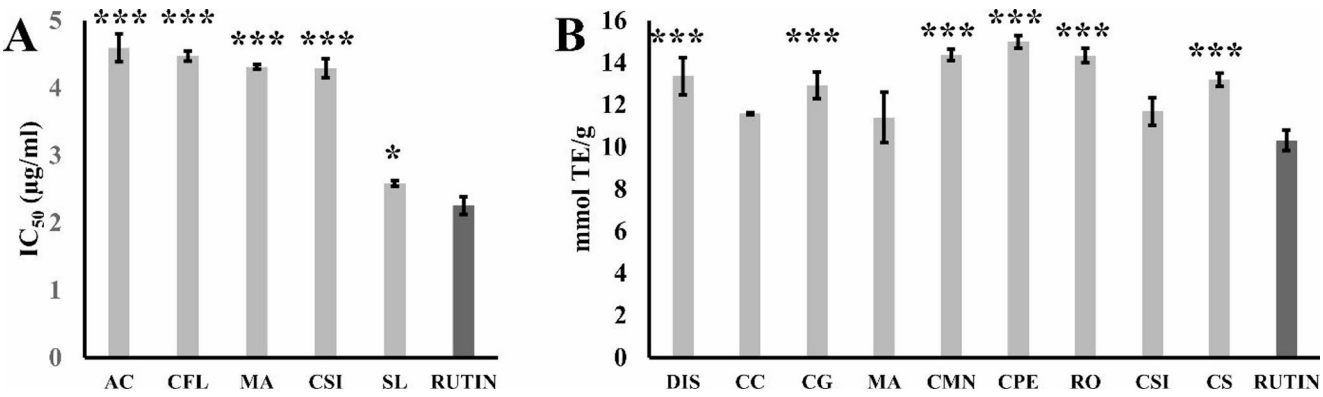


Fig. 2 Antioxidant activity of ethyl acetate fractions using DPPH (**A**) and ORAC (**B**) assays. The values are expressed as mean ± SD. (IC_{50}: half-maximal inhibitory concentration; TE: Trolox Equivalent; AC: *C. acutiformis;* CC: *C. elata;* CFL: *C. flacca;* CG: *C. grayi;* CS: *C. syl-vatica;* CSI: *C. sidérosticta;* CMN: *C. montana;* CPE: *C. petriei;* DIS: *C. distans;* MA: *Cl. mariscus;* SL: *Shoenoplectus lacustris;* RO: *C. rostrata;* *$p < 0.05$, ***$p < 0.001$)

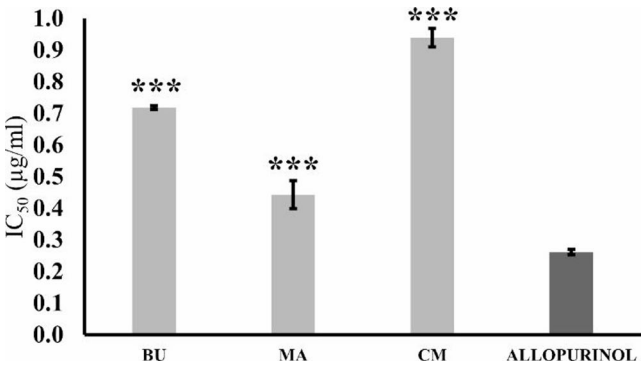


Fig. 3 Xanthine oxidase inhibitory activity of ethyl acetate fractions. The values are expressed as mean ± SD. (IC_{50}: half-maximal inhibitory concentration; BU: *C. buxbaumii,* MA: *Cl. mariscus,* CM: *C. melanostachya;* ***$p < 0.001$)

XO-inhibitory activity

At 400 µg/mL, 83 extracts demonstrated moderate XO-inhibitory activity (≥50% inhibition), while 62 exhibited a substantial (>80%) inhibitory effect (Table S1). For these extracts, IC_{50} values were also determined (Table 1). Among the fractions with different polarities, fractions B (containing $CHCl_3$-soluble lipophilic constituents) and C (EtOAc fractions) proved to be active (Fig. 1). The *n*-hexane extracts (fraction A) demonstrated moderate XO inhibitory effects (>50% inhibition) in only a few cases *(C. bohemica, C. buxbaumii, C. divulsa, C. flacca, C. foliosissima, C. grayi, C. hirta, C. lasiocarpa, C. muskingumensis, C. ornithopoda,* and *C. petriei)*. Extracts showing the best IC_{50} values (<3.0 µg/mL) are marked in bold in Table 1. In the XO-inhibitory assay, the $CHCl_3$ (B) extract of *C. acutiformis, C. appropinquata, C. bohemica, C. buxbaumii, C. distans, C. divulsa, C. flacca, C. grayi, C. hirta, C. lasiocarpa, C. melanostachya, C. montana, C. muskingumensis, C. paniculata, C. praecox, C. pseudocyperus, C. rostrata,* and *Cl. mariscus* with IC_{50} in the range of 0.44–2.93 µg/mL were the most active. Under the assay conditions, the IC_{50} of allopurinol, a clinically used XO inhibitory drug, was 0.26 µg/mL. Although the aforementioned fractions were the most potent XO inhibitors, the observed IC_{50} values were significantly higher compared to allopurinol ($F = 3,8 = 366.7$, $p < 0.0001$) (Fig. 3). Among the EtOAc (C) fractions the activity of *C. distans* was the best with an IC_{50} of 2.70 µg/mL. Besides this extract IC_{50} values <10.0 µg/mL were detected for five extracts. Although a strong and significant positive correlation was observed in activities of fractions B and C ($r(40) = 0.712$, $p < 0.0001$); however, EtOAc fractions (C) were those with weaker inhibitory activity. Interestingly, the XO-inhibitory activity of B and C extracts of *S. lacustris* was almost the same with IC_{50} values of 5.92 and 5.74 µg/mL, respectively. In the case of *C. flacca,* all three extracts showed high activity (IC_{50} values 7.03 µg/mL for A, 1.47 µg/mL for B, and 3.60 µg/mL for C). It can be concluded, that the XO-inhibitory compounds are enriched mainly in the $CHCl_3$ (B) extracts; however, the xanthine oxidase inhibition of $CHCl_3$ fractions did not correlate with activity measured with DPPH test ($r(32) = 0.140$, $p = 0.438$) and weak negative correlation could be observed with ORAC (($r(34) = –0.430$, $p = 0.01$) activity.

Based on literature data, the major compounds that inhibit XO are flavonoids. The structural similarity of flavonoid rings A and C with xanthine as a substrate causes hydrophobic interactions, hydrogen bonds, and van der Waals forces between flavonoids and XO. Therefore, the active compounds bind to the XO active site, thereby preventing the formation of UA. The planar structure of flavonoids, the presence of double bonds between C-2 and C-3, and the hydroxy substitution at C-5 and C-7 play a crucial role in the XO inhibition of flavonoids (Lin et al. 2015). Stilbenes, similar to flavonoids, contain a conjugated aromatic system and can also bind to the enzyme's active site.

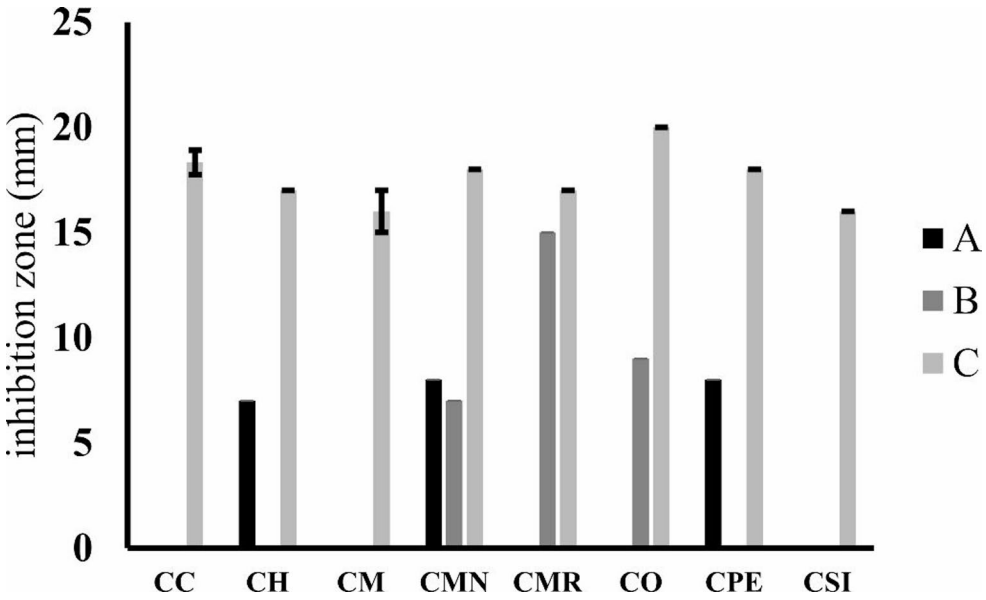


Fig. 4 Antibacterial activity on *S. epidermis* strain of *n*-hexane (**A**), chloroform (**B**), and ethyl acetate (**C**) fractions. The values are expressed as mean ± SD. (CC: *C. elata;* CH: *C. hartmaniorum;* CM: *C. melanostachya;* CMN: *C. montana;* CMR: *C. morrowii;* CO: *C. oshimensis;* CPE: *C. petriei;* CSI: *C. siderosticta)*

It was observed that the free phenolic hydroxy group of the stilbenoids was important for the discerned enzyme inhibitory actions, and glycosylation could substantially lower the activity against XO (Zhou et al. 2001). The stilbenes piceatannol, scirpusins A and B, isolated from the rhizome of *Scirpus californicus* (Cyperaceae), showed XO-inhibitory activity (IC_{50} values 3.9, 3.6, and 6.0 µg/mL, respectively) (Schmeda-Hirschmann et al. 1996). This plant is one of the most abundant and well-represented species in Peru and Ecuador, with edible rhizomes. The inhibitory effect of stilbenes on XO reactions have been investigated by Masuoka (Masuoka 2021). The results have indicated that stilbenes specifically bind to the flavin adenine dinucleotide (FAD) site in XO, consequently they inhibit the O_2^- generation. In case of piceatannol, the suppression of O_2^- generation is induced by this specific binding to the FAD site and the subsequent reduction of XO.

Antibacterial activity

Antibacterial experiments were performed on eight standard strains using the disc diffusion method. According to our results, the antimicrobial activity of most of the plant samples of Cyperaceae species has different intensities. In our experiment, the extracts with inhibition zones smaller than 7 mm were considered inactive. Inhibition zones between 7 and 10 mm indicated low activity, those greater than 10 mm indicated moderate activity, and fractions with inhibition zones larger than 15 mm were considered to have high antibacterial activity (Table 2, Fig. 1). Among the investigated sedges, eight species – namely, *C. muskingumensis, C. praecox, C. montana, C. morrowii, C. oshimensis, C. siderosticta, C. pendula, C. sylvatica* – showed the highest antibacterial activity against one or more bacterial strains. Based on our experiment, the EtOAc (C) extract of *C. oshimensis* demonstrated the highest antibacterial capacity against all Gram-positive strains tested with inhibitory zones of 15.0 mm for *S. aureus, MRSA,* and *B. subtilis,* and 20.0 mm for *S. epidermidis.* In this study, *M. catarrhalis, B. subtilis,* and *S. aureus* proved to be the strains most susceptible to the examined extracts. However, the highest activities were observed against *S. epidermidis,* with inhibition zones of > 15 mm from the EtOAc extracts of *C. elata, C. hartmaniorum, C. melanostachya, C. montana, C. morrowii, C. muskingumensis, C. oshimensis, C. petriei,* and *C. siderosticta* (Fig. 4). Among the extracts with different polarities mainly the hexane (A) and EtOAc (C) extracts demonstrated antibacterial potency against one or more bacterial strains. The most active extracts were among the EtOAc (C) fractions. The chloroform fractions of the investigated sedges were inactive or showed low activity except for the $CHCl_3$ (B) extract of *C. flacca* and *C. morrowii.*

The Gram-negative bacteria were less sensitive to the tested plant extracts, which might be due to their unique and complex cell wall structure and defense mechanisms (Breijyeh et al. 2020; Koohsari et al. 2015). The tested bacteria were less susceptible to the extracts of *C. appropinquata, C. buxbaumii, C. grayii, C. halleriana, C. lasiocarpa,* and *C. michelii.* Interestingly, the EtOAc (C) extract of *C. davalliana* was inactive against all tested bacteria. The $CHCl_3$ extract of the plant possessed moderate activity against *M. catarrhalis* (inhibition zone = 10 mm), while its hexane extract was active against *B. subtilis, E. coli, K. pneumoniae* and *P. aeruginosa* with inhibition zones between 7.3 and 8.5 mm, respectively.

Our findings suggest that Gram-positive bacteria were more susceptible to the investigated plant fractions compared to Gram-negative bacteria. These results are in accordance with data reported in literature (Vlietinck et al. 1995; Rabe and Van Staden 1997). However, in our work, the Gram-negative bacterium *M. catarrhalis* was also sensitive to the tested sedges extracts, 90 extracts (73%) possessed some activity, the EtOAc (C) fraction of *C. siderosticta* being the most active one with an inhibition zone of 12 mm.

Previously, the antimicrobial activity of different extracts and isolated compounds of Cyperaceae species has been verified by studies reported in literature. Total oligomer flavonoids (TOF) enriched extract of *C. rotundus* tubers has proved to be active against *S. aureus* and *S. enteritidis,* with an MIC value of 0.5 mg/mL for each, and has shown significant inhibitory activity against *Salmonella typhimurium* with an MIC value of 1 mg/mL. The highest MICs have been reported for *E. coli* and *Enterococcus faecalis* with MIC values of 5 and 2.5 mg/mL, respectively. The EtOAc extract of the tubers has shown remarkable activity against *S. aureus* and *E. faecalis* (MIC value 0.5 mg/mL) (Kilani et al. 2008b). The extract of *Carex dimorpholepis,* and its constituent resveratrol, possessed antibiofilm activity against enterohemorrhagic *E. coli* (EHEC) at 10 µg/mL. Moreover, the extract has decreased the adhesion of EHEC cells to human colonic epithelial (HT-29) cells without affecting the viability of these cells (Lee et al. 2013). The ethanol extract of the rhizomes has displayed antibacterial activity against *S. aureus,* with an inhibitory zone of 8 mm (Sinjali et al. 2022). Miyabenol A, a stilbene of *Carex fedia* var. *miyabei,* has shown antimicrobial activities against *S. aureus* and *B. subtilis* at a level of less than 10 µg/8 mm diameter paper disc (Suzuki et al. 1987). The antitubercular activity of α-viniferin, isolated from *Carex humilis,* was tested against drug-susceptible and –resistant *Mycobacterium tuberculosis*. The compound has shown an antibacterial effect against both strains at MIC_{50}s of 4.6 µM in culture broth medium and MIC_{50}s of 2.3–4.6 µM inside macrophages and pneumocytes. An additive effect and partial synergism were observed against *M. tuberculosis* H37Rv, when it was applied in combination with streptomycin and ethambutol. Moreover, α-viniferin inhibited the proliferation of methicillin-susceptible (MSSA), methicillin-resistant *S. aureus* (MRSA), and methicillin-resistant *S. epidermidis* (MRSE) with MIC values of 9.2–18.4 µM (Seo et al. 2017). The resveratrol dimer ε-viniferin inhibited the biofilm formation of enterohemorrhagic *E. coli* O157:H7 (EHEC) at 10 µg/mL by 98% without affecting planktonic growth (Cho et al. 2013).

Conclusions for future biology

In the present study, the antioxidant, XO-inhibitory, and antimicrobial activity of 123 fractions of 41 Cyperaceae species extracts were screened. *S. lacustris* exhibited the highest antioxidant activity in the DPPH free radical scavenging assay, while *C. petriei* showed the strongest activity in the ORAC assay. The highest XO-inhibitory activity was observed in the EtOAc fractions of *Cl. mariscus* and *C. buxbaumii.* Eight bacterial strains were used to test the antimicrobial activity. The Gram-positive bacteria were more susceptible to sedge extracts than Gram-negative bacteria. Among the plant fractions with different polarities tested, the EtOAc (C) extracts exhibited the strongest antimicrobial activity, with the extract of *C. oshimensis* being the most active. The antibacterial activity of *C. montana* is also promising and it shows high activity in both the ORAC (EtOAc, C) and XO-inhibitory ($CHCl_3$, B) assays. Our findings suggest that Cyperaceae species might have great potential as antimicrobial compounds and might serve as a source of natural antioxidants. The compounds responsible for the detected activities have neither identified or not fully identified yet, therefore our screening investigations provided a series of results that can be the starting point for the future phytochemical, pharmacological studies. Main limitation of present study is that no chemical profiling was performed on the investigated fractions. Previous studies about investigated genera could be a good starting point for shedding light biologically active compounds; however, further phytochemical analysis, including various chromatographical methods together with wide range of spectroscopical methods (i.e. PDA, MS, and NMR) could provide sufficient data for identification of specific phytochemicals. Metabolomics might also contribute to narrowing the phytochemicals responsible for biological activity.

Authors' contributions CZD contributed to present study with conceptualization, preparation of extracts and fractions, performing antioxidant activities (DPPH, ORAC) and xanthin oxidase activity of fractions. AK contributed by conceptualization of experiments dealing with antibacterial activity of fractions, she performed the testing of antibacterial activity of the fractions and evaluated the data. TK and GG contributed to conceptualization of statistical analysis of data obtained during the analysis and performed calculations for statistical evaluation. LP collected and identified the plant species. JH contributed to present study with conceptualization, preparation of manuscript, supervision, and funding acquisition. AV contributed to present study with conceptualization, supervision, manuscript preparation, funding acquisition and project administration. All authors reviewed the manuscript and approved to the published version of the manuscript.

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Data availability The data on which the study is based were generated during the course of this study. The xanthine oxidase inhibitory activity of fractions is summarized in Supplementary Information (Table S1). Raw data are available from the corresponding author upon request.

Declarations

Conflict of interest The authors declare no conflict of interest.

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