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Research Article

Assessment of ethnopharmacological potential of *Cyperus difformis* L. in terms of its' phytochemistry, antibacterial, antioxidant and anticancer attributes

Shahzada Z. AHMAD*, Zaheer ud din KHAN, Safdar A. MIRZA

*Government College University, Department of Botany, Lahore 54000, Pakistan; ziaahmad85@gmail.com (*corresponding author); zaheeruddin@gcu.edu.pk; safdaralimirza@gcu.edu.pk*

Abstract

The present research was carried out on *Cyperus difformis* L., commonly found sedge weed in rice field to evaluate its' ethnopharmacological potential in terms of phytochemical constituents, antibacterial, antioxidant and anticancer activities. FTIR spectroscopy of powdered material of plant parts, rhizome (with roots), leaves and flowers showed the presence of three main chemical groups, i.e. -OH (alcohol), -C=O (carbonyl) and -CO-O-CO (anhydride). The phytochemical composition of n-Hexane, chloroform and ethanol crude extracts of same parts analysed by GC-MS and n-Hexane extract of various parts indicated 14 compounds with highest (10) in flowers extract followed by six compounds in chloroform and five in ethanol extracts of plant parts. The antibacterial activity was assessed against *Salmonella enterica*, *E. coli* and *Staphylococcus aureus* by using well diffusion method and similar resistance was shown by n-Hexane and ethanol extracts while noticeable inhibition by chloroform extract, especially by leaves showing zone of inhibition, 16.17±0.52mm, comparable with Gentamycin 18±0.11mm. The antioxidant activity in terms of DPPH Scavenging activity was found higher in n-Hexane and chloroform extracts, especially leaves i.e., 54.6±0.43 & 43.45±0.53 as compared to that of ethanol extracts. The percentage activity was increased with an increase of concentration of extracts. Antiproliferative activity checked by SRB proliferative assay and the crude extracts of plant parts of *C. difformis* showed good activity against A2780 and HCT116 cancer cells in three days. *C. difformis* having therapeutic action as well as its' ethnopharmacological history, being used as fodder, it may be recommended good herbal fodder for the dairy animals.

Keywords: antibacterial; anticancer; antioxidant; *Cyperus difformis* L.; phytochemicals

Introduction

Medicinal plants have a long history of use by human beings for therapeutic purposes against a variety of diseases. Plants are a major source of drugs. The medicinal properties of plants are associated with the variety of phytochemicals, synthesized as a result of metabolic activities in them, such as flavonoids, steroids, alkaloids, tannins, lactones, sesquiterpene, coumarins, triterpenes, carbohydrates, etc. These compounds demonstrate diverse biological activities, including antimicrobial, antioxidant, anticancer, anticonvulsants, anti-

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inflammatory, antipyretics, analgesics, antihypertensive, anticoagulant, skin and bone healing agents, cardio protective and immune modulatory (Dahanukar *et al*., 2000; Shety *et al*., 2006; Ahmed and Urooj, 2010).

Worlds Health Organization (WHO) reported that more than 80% of the world's population is mainly dependent upon herbal remedies for the treatment of the diseases (Ganesan *et al*., 2004). Phytochemicals or secondary metabolites are very important in changing the physiological processes of human body. The demand of ethnomedicinally important plants is increasing day by day (Kumar *et al*., 2015).

Herbal medicines are playing important role in modern health care system due to their cost effectiveness and low side effects. Plants are major source of new drugs discovery (Dar *et al*., 2017). Anticancerous and antioxidant potential of different plant species were determined globally, Selamoglu *et al.* (2017a) determined the *in vitro* antioxidant potential of five different plant species i.e., *Plantago lanceolata*, *P. major*, *Robinia pseudoacacia*, *Platanus arientakis* and *P. aesculus*. Selamoglu (2017b) emphasized the importance of polyphenolic compound such as flavonoids present in various plant food products and medicines in reducing the risk of serious health problems, these compounds are frequently occurring in the plants and about 6,000 flavonoids have been identified.

Erdemli *et al.* (2017) conducted an experiment on the biochemical efficacy of grape seed extract coupled with low laser therapy in intraoral wound healing effect in rat liver. The active compound, catchin was found present in grape seed. Erdemli (2018) studied the role of grape seed extract and low laser therapy on the healing of fractured mandible of rats. The biochemical agent in the extract showed very promising effects.

Nageen *et al.* (2018) revealed a research work on Eupatilin, a biologically active flavone reported from many medicinal plants that it is an excellent anticancer agent having anti-inflammatory and antioxidant potential. The derivatives of this compounds can enhance its efficacy to reduce the toxicity of drugs. Akalin and Selamoglu (2019) explained the importance of natural foods in maintenance of healthy skin, due to their pharmacological, antioxidant and antibiotic activities reducing cellular damage and stress and age-related disorders in skin health. Jiang *et al.* (2019) conducted a research to determine the pharmacological and biological potential of formononetin, a bioactive isoflavon extracted from different plants mainly from *Glycine max*, *Trifolium pretense* and *Astragalus membranaceus*. It was observed that Formononetin has anticancer, anti-inflammatory and antioxidant properties, especially cancer-causing pathways and arresting cell cycle. Salehi *et al.* (2019) determined the phytochemical, phytotherapy and pharmaceutical properties of different juices of leaves and fruits of the genus *Berberis*. It consists of several bioactive compounds which are used for the treatment of several diseases. Several oils including p-cymene, ocimene and limonene were reported in the juices by the gas chromatography. It was also observed that plants of this genus have antimicrobial and anticancer activities.

Nawaz *et al.* (2020) studied the role of Cardamonin, chalconoid role in human wellbeing. It was found that chalcone has great role in anticancer activity in boosting immune system, killing various pathogens, through the modulation of cell pathways. Khan *et al*. (2021) studied that lycopene, an antioxidant acquired importance in preventing autoxidation of fats and related products. Tomatoes are an important agricultural product for the source of lycopene. It was observed that the lycopene taken in the diet showed positive effects in many stages of atherosclerosis. The serum lipid levels, endothelial dysfunction, inflammation, blood pressure, and antioxidative potential are mainly affected by lycopene. Ozdemir *et al.* (2021) conducts an experiment to investigate the antioxidant potential of caffeic acid phenethy ester, propolis and pollens on heart of rats, as they hypothesized that heart and vessels are affected by the high blood pressure. It was observed that BP of rats treated with pollens, CAPE and propolis were significantly reduced at 0.05 significance level, thus, found having vasorelaxant properties. Adnan *et al.* (2021) studied pharmacologically active compounds physcion and physcion 8-O-B-D-glycopyranoside (PG) and anthraquinones which showed excellent potential against cancerous activities. It was concluded on the basis of reviewed literature that PG and physcion regulated multitudinous cell mechanism of actions and regulating multitudinous cell signaling pathways, cell cycle, kinase

(protein), apoptosis limited proteins, transcriptional factor, micro RNAs and effective killing of cancerous cells found *in vitro* as well as *in vivo* studies.

The weeds, growing nearby our houses, on agricultural and waste or farm lands are very good source of herbal medicine. Some important medicinal weed sedges belonging to family Cyperaceae, are found growing mostly in wet, damp places and rest in dry places. Family Cyperaceae having more than 4000 species of 70 genera are mostly traditionally used as medicine, due to a large number of bioactive compounds in them such as, caryophyllene oxide, cyperotundone, germacrene D, α-cyperone, α-corymbolol, α-pinene, mustakone and zierone, which enhance the impact of these species as therapeutic plants. The crude extracts of *Cyperus* sp. exhibit antidepressive, antiarthritic, antimicrobial, anti-inflammatory, antioxidant, anticancer, neuroprotective, antiobesity, vasodilator, spasmolytic, bronchodilator, and estrogenic properties (Yasman *et al*., 2021).

In the present research work the phytochemical screening of *Cyperus difformis* L. was carried out through the FTIR analysis to elaborate the functional groups of the chemical compounds and GCMS for the identification of chemical compounds, and also its ethnopharmacological potential, in terms of the antibacterial, antioxidant and anticancer activities was evaluated using standard techniques.

Cyperus difformis L. commonly known as small-flowered nut sedge or rice sedge, is an annual weed with fibrous roots of predominately red colour and stem is erect, triangular 2-3mm in thickness, basal leaves 3-4 in number, united at base with sheaths about 15-45 cm long. It is considered as annual weed with fibrous roots of predominately red colour, having length of 100 cm (Lanidon *et al*., 2011).

Materials and Methods

Plant collection

Cyperus difformis was collected from tehsil and district Narowal, Punjab, identified using Flora of Pakistan (Kukkenon, 2001) and its' voucher specimen was deposited in Botanical herbarium, GC university, Lahore. The plant parts, i.e., rhizome (with roots), leaves and flowers were separated, dried in shade at room temperature and ground into fine powder.

Preparation of plants extracts

The n-hexane, chloroform and ethanol were used to get the crude extracts of powder of different plant parts using maceration technique (Paliwal *et al*., 2017).

Phytochemical screening

Identification of functional groups by FTIR Spectra

The powder of different plant parts roots and rhizome, leaves and flowers were analysed by FTIR spectroscopy for the identification of functional groups (Pharmawati and Wrasiati, 2020).

GCMS analysis

The chemical compounds in the targeted sedge weed were identified through GC-MS (Model GC-MS-QP2010, Shimadzu Co., Japan), with capillary column equipped with DB-1 column containing length (0.25 μm film x 0.25mm ID x 30m length) having ionization volt 70 eV, at 230 °C injector temperature and 280 °C detector temperature, the carrier gas, helium (99.9% purity) flowing at the rate of 1 mL/min, initially oven temperature programmed at 80 °C (isothermal for 5min.), then increasing to 200 °C at 5 °C/min and finally to 280 °C at 5 °C/min (isothermal for 16min). The compounds found present in 1 μL of the sample injected were identified using the available mass spectral records of NIST and WILEY libraries (Abo-Altemem *et al*., 2019).

Exploration of antibacterial potential

The well diffusion susceptibility method was employed to test the antibacterial activity of the weed extracts against 18-24 hours old cultures of pathogenic bacteria, such as *Salmonella enterica, E. coli* and *Staphylococcus aureus*. Four wells (8 mm in diameter) were made in each petri plate containing agar medium, using sterilized cork-borer. Each plant sample (24 µL) containing 4 mg of dried extract was poured into each well. A standard antibiotic, Gentamycin (4 mg per 24 µL) and blank solvents (each 24 µL per well) were used as negative and positive control, respectively. Inoculated plates were then incubated at 37 °C for 24 hrs. Three replicates of each sample extracts were used to record the average value of zone of inhibition (Adeniyi *et al*., 2014; Aneja and Joshi, 2009).

Evaluation of antioxidant potential using DPPH radical scavenging assay

Antioxidant potential was assessed through DPPH Radical Scavenging activity. The plant extracts were serially diluted ranging from 0.5 µg/mL to 5 µg/mL. 0.1 mM solution of DPPH was prepared by adding 95% ethanol. The test solution was prepared by adding 0.4 mL of serial dilutions and 2.6 mL of DPPH solution in the test tubes. After 30 minutes, spectrophotometric absorbance was recorded at 517nm. The control was having the same quantity of DPPH solution but not the extract while using ascorbic acid (5 mg/mL) as reference (Kakarla *et al*., 2016).

The following equation was used to find out the % Scavenging activity:

$$\text{Scavenging Activity (\%)} = [(\text{Absorbance of the control} - \text{Absorbance of the test sample})/ \text{Absorbance of the control}] \times 100$$

Anticancer potential

Cell lines (A2780 and HCT 116 Cells), maintained in culture medium (DMEM or RPMI) supplemented with 10% FBS and 1 Å~ Antibiotic-Antimycotic at 37 °C in humified incubators passaged with 5% CO_2, for less than 6 months before replacement from early passage frozen stocks, were tested for their antiproliferative activity after treating with two dilutions, i.e. 0.5mg/mL and 1mg/mL of n-Hexane, chloroform and ethanol crude extracts of rhizome (with roots), leaves and flowers of *C. differmis* for 72 hours in 96-well plates, using Sulforhodamine B (SRB) assay. Cells were then fixed with ice-cold TCA (3% final concentration) for 2 hours at 4 °C and stained with 0.06% SRB. The O.D. of SRB bound to the stained cells, being solubilized in 10 mM Tris with pH 10.5 was measured at 490 nm using microplate reader (BioTek) after Manzoor *et al*. (2018).

Results

Phytochemical analysis

FTIR spectra

The results of FTIR analysis indicated three main groups, i.e. -OH (alcohol), -C=O (carbonyl) and -CO-O-CO (anhydride) in all the powdered plant parts samples. The position of -OH stretching was found slightly varied in the samples, may be due to the variation of hydrogen bonding strength and steric hindrance within the molecules of samples. The values were ranging between 3280-3260 cm^{-1} (Figure 1). The stretching of alkane -C-H in samples showed better intensity of peaks indicating less steric hindrance among molecules. Broad peaks of -C=O (carbonyl, 1610-1590 cm^{-1}) and -CO-O-CO (anhydride, 1028-1013 cm^{-1}) were observed in all the samples indicating the complexity in molecular structure.

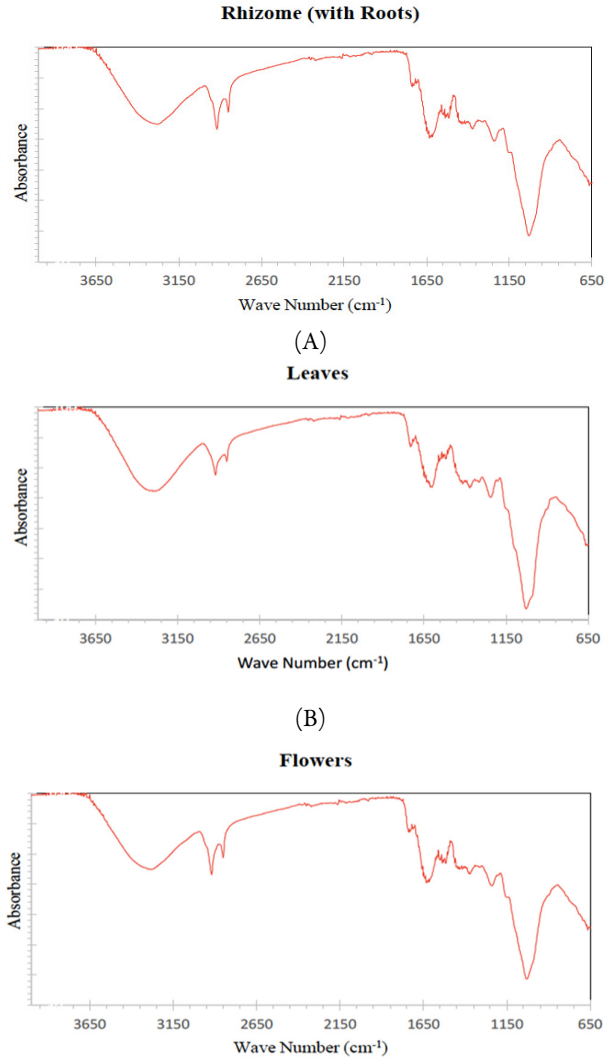

Figure 1. FTIR spectral absorbance in: (A) rhizome (with roots) of *Cyperus difformis* L. between wave number of 4000–650 cm^{-1}; (B) leaves of *Cyperus difformis* L. between wave number of 4000–650 cm^{-1}; (C) flowers of *Cyperus difformis* L. between wave number of 4000–650 cm^{-1}

GC-MS analysis

GC-MS analysis identified the following chemical constituents in the extracts of *C. difformis* L.:

n-Hexane extracts of plant parts

The n-hexane extract of rhizome (with roots) showed two peaks of two phytochemicals, i.e. n-Octane and 1,2-Benzenedicarboxylic acid while the leaves extract exhibited three compounds, i.e. n-Hexan-1-ol, Bicyclo [4.2.0] octa-1,3,5-triene and 1,2-Benzenedicarboxylic acid and the flowers extract indicated 9 compound, i.e. Heptane-2,4-dimethyl, Trans-1,2-Dimethylcyclohexane, Cis-1,4-Dimethylcyclohexane, 1,4-Dimethylbenzene, 1,3,5,7-Cyclooctatetraene, Benzenamine-3-methyl-, Methyl hexadecanoate, Methyl (11E, 14E)-11,14-icosadienoate and (6Z)-6-Octadecenoic acid (Figure 2; Table 1).

Table 1. GCMS of n-Hexane extracts of rhizome (with roots), leaves and flowers

S. No.	R.T.	Peak ID	Compounds	Mol. Wt.	Formula	Nature of compound
Extract of rhizome (with roots)						
I)	3.092	a	n-Octane	114	C_8H_{18}	Alkane
II)	11.667	b	1,2-Benzenedicarboxylic acid	222	$C_{12}H_{14}O_4$	Aromatic carboxylic acid
Extract of leaves						
I)	3.058	c	n-Hexan-1-ol	102	$C_6H_{14}O$	Alcohol
II)	4.667	d	Bicyclo[4.2.0]octa-1,3,5-triene	104	C_8H_8	Cyclic alkene
III)	11.667	e	1,2-Benzenedicarboxylic acid	222	$C_{12}H_{14}O_4$	Aromatic carboxylic acid
Extract of flowers						
I)	3.092	f	Heptane-2,4-dimethyl	128	C_9H_{20}	Alkane
II))	3.142	g	Trans-1,2-Dimethylcyclohexane	112	C_8H_{16}	Cyclic alkene
III)	3.242	h	Cis-1,4-Dimethylcyclohexane	112	C_8H_{16}	Cyclic alkene
IV)	4.308	i	1,4-Dimethylbenzene	106	C_8H_{10}	Aromatic
V)	4.667	j	1,3,5,7-Cyclooctatetraene	104	C_8H_8	Cyclic alkene
VI)	7.05	k	Benzenamine-3-methyl-	107	C_7H_9N	Aromatic amine
VII)	13.817	l	Methyl hexadecanoate	270	$C_{17}H_{34}O_2$	Ester
VIII)	14.825	m	Methyl (11E, 14E)-11,14-icosadienoate	322	$C_{21}H_{38}O_2$	Ester
IX)	16.225	n	(6Z)-6-Octadecenoic acid	282	$C_{18}H_{34}O_2$	Carboxylic acid

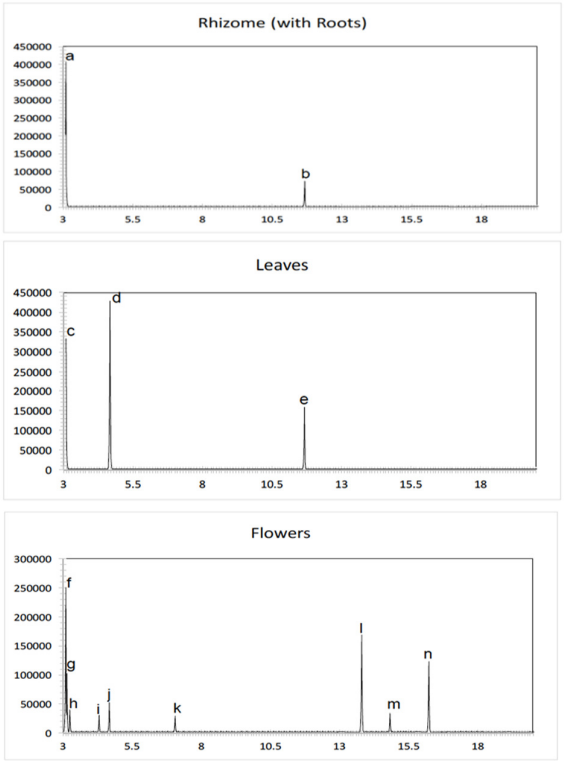

Figure 2. GC-MS of n-Hexane extracts

Chloroform extracts of plant parts

The chloroform extract of rhizome (with roots) showed the presence of two compounds, i.e., 1,2,4,5-tetramethylbenzene and 4-methylheptanamide while the leaves extract indicated the presence of four compounds, i.e., 1,2,4-Trimethylbenzene, 4-Ethyl-1,2-dimethylbenzene, Benzene, 1,3-dimethyl-5-(1-methylethyl)- and 9-Octadecenamide, N, N-dimethyl- (Table 2 and Figure 3).

Table 2. Compounds identified in chloroform extracts of Rhizome (with roots) and Leaves by GC-MS

S. No.	R.T.	Peak Id	Compounds	Mol. Wt.	Formula	Nature of compound
Extract of rhizome (with roots)						
I)	9.458	p	1,2,4,5-tetramethylbenzene	134	$C_{10}H_{14}$	Aromatic
II)	22.7	q	4-methylheptanamide	171	$C_{10}H_{21}NO$	Amides
Extract of leaves						
I)	7.417	r	1,2,4-Trimethylbenzene	120	C_9H_{12}	Aromatic
II)	8.458	s	4-Ethyl-1,2-dimethylbenzene	134	$C_{10}H_{14}$	Aromatic
III)	10.558	t	Benzene, 1,3-dimethyl-5-(1-methylethyl)-	148	$C_{11}H_{16}$	Aromatic
IV)	22.692	u	9-Octadecenamide, N,N-dimethyl-	309	$C_{20}H_{39}NO$	Amide

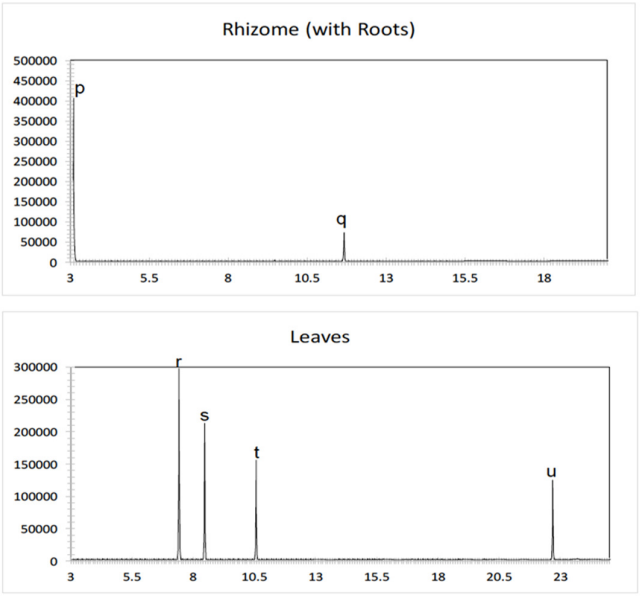

Figure 3. GC-MS of chloroform extracts

Ethanol extract

The rhizome (with roots) ethanol extract was found containing Di-n-octyl adipate while leaves extract contained four compounds, i.e. Pentadecanoic acid, n-Eicosanoic acid, Ethyl tridecanoate and Tributyl butane-1,2,4-tricarboxylate (Table 3 and Figure 4).

Table 3. Compounds identified in the ethanol extracts of rhizome (with roots) and leaves by GC-MS

S. No.	R.T.	Peak Id	Compound name	Mol. Wt.	Formula	Nature of compound
Extract of rhizome (with roots)						
I)	16.375	v	Di-n-octyl adipate	370	$C_{22}H_{42}O_4$	Ester
Extract of leaves						
I)	13.833	w	Pentadecanoic acid	270	$C_{17}H_{34}O_2$	Carboxylic
II)	14.033	x	n-Eicosanoic acid	312	$C_{20}H_{40}O_2$	Carboxylic
III)	14.233	y	Ethyl tridecanoate	242	$C_{15}H_{30}O_2$	Ester
IV)	16.617	z	Tributyl butane-1,2,4-tricarboxylate	358	$C_{19}H_{34}O_6$	Ester

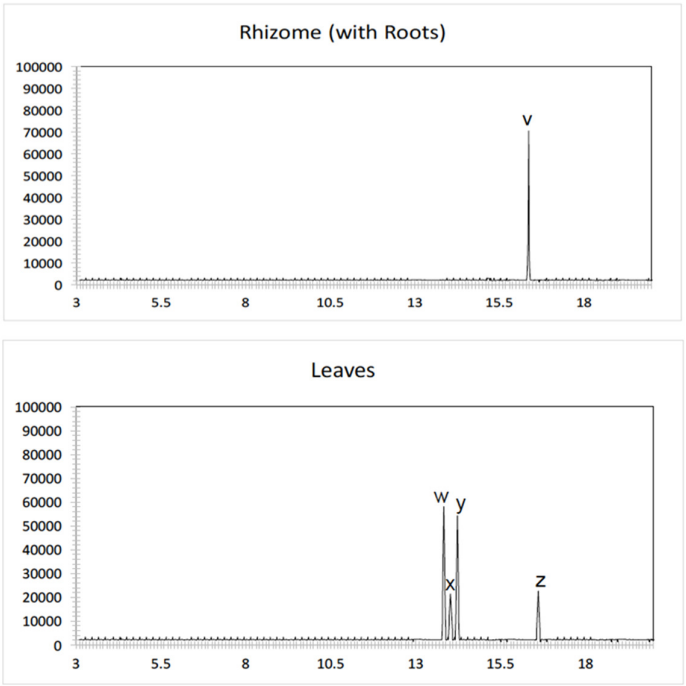

Figure 4. GC-MS of ethanol extracts

Antibacterial potential

The antibacterial activity of n-hexane, chloroform, and ethanol extracts of rhizome (with roots), leaves and flowers of *Cyperus difformis* as well as of standard antibiotic, Gentamycin was assessed against *Salmonella enterica, E. coli* and *Staphylococcus aureus* by using well diffusion susceptibility method. The extracts were found effective in suppressing bacterial growth (Table 4 and Figure 5). The n-hexane and ethanol extracts of different plant parts, i.e., rhizome (with roots), leaves and flowers showed almost similar zone of inhibition against *Salmonella enterica*, *E. coli* and *Staphylococcus aureus,* with the results of ethanol crude extracts slightly higher. A noticeable difference in zone of inhibition was exhibited in the chloroform extract of various plant parts, the leaves showing highest activity comparable to that of the standard antibiotic, the Gentamycin showing 18 mm of zone of inhibition.

Table 4. Zone of inhibition of crude extracts of rhizome (with roots), leaves and flowers

Name of extract		Zone of inhibition (mm)		
		Salmonella enterica	*Escherichia coli*	*Staphylococcus aureus*
n-Hexane	Rhizome (with roots)	12.47±0.20	11.83±0.43	12.6±0.43
	Leaves	12.73±0.36	12.63±0.22	11.57±0.22
	Flowers	12.71±0.24	9.59±0.37	12.77±0.37
Chloroform	Rhizome (with roots)	12.4±0.17	12.33±0.18	13.4±0.18
	Leaves	19.82±0.38	14.36±0.29	16.43±0.29
	Flowers	9.45±0.22	10.4±0.21	9.46±0.21
Ethanol	Rhizome (with roots)	14.76±0.34	14.03±0.27	12.72±0.27
	Leaves	16.17±0.52	15.27±0.26	13.77±0.26
	Flowers	12.11±0.31	10.4±0.05	9.81±0.05
Gentamycin		18±0.11	18±0.11	18±0.11

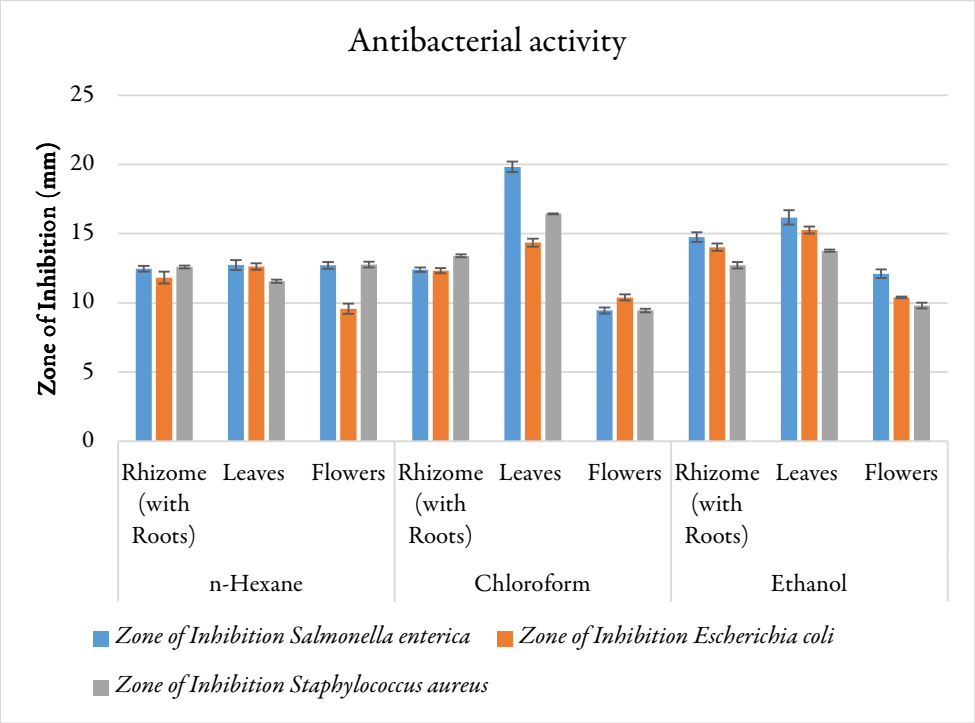

Figure 5. Zone of inhibition of crude extracts of rhizome (with roots), leaves and flowers against *Salmonella enterica, Escherichia coli* and *Staphylococcus aureus*

Antioxidant Potential: DPPH Radical Scavenging Activity

The n-hexane extract of leaves showed comparatively higher DPPH scavenging activity as compared to that of rhizome (with roots) and flowers (Table 5 and Figure 6). Percentage activity increased with an increase of concentration of extracts. The highest percentage (90.06±0.49%) was exhibited by the n-hexane extract of leaves.

Table 5. Antioxidant potential of n-Hexane extracts of rhizome (with roots), leaves and flowers

Conc. µg/mL	DPPH Scavenging (%)		
	Rhizome (with Root)	Leaves	Flowers
0.5	25.83±0.68c	54.6±0.34b	−3.22±0.37b
1	28.1±0.47b	60.25±0.49b	2.39±0.24a
1.5	29.62±0.26a	67.21±0.39b	4.27±0.26a
2	31.09±0.08a	70.87±0.42c	5.71±0.32a
2.5	33.35±0.22a	72.54±0.29a	8.37±0.3a
3	36.41±0.21a	74.82±0.24a	12.1±0.5c
3.5	42.37±0.39ab	78.25±0.2a	16.37±0.33ab
4	45.07±0.31ab	85.15±0.25a	20.79±0.56c
4.5	47.8±0.61c	88.19±0.31ab	27.28±0.34ab
5	50.57±0.72c	90.06±0.49c	35.49±0.58c

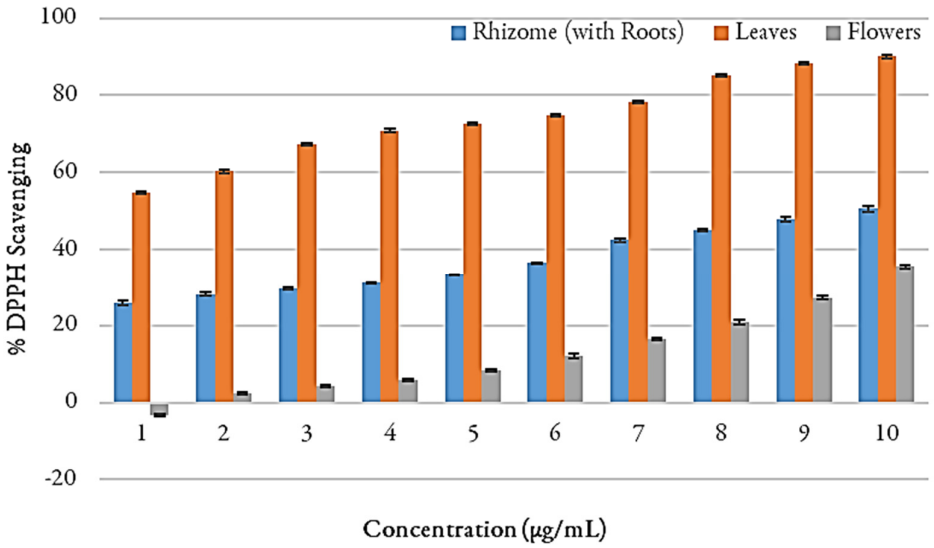

Figure 6. Comparative antioxidant potential of n-Hexane extract of rhizomes with roots, leaves and flowers

The chloroform extracts of leaves exhibited highest DPPH scavenging activity as compared to other plant parts while the lowest by the flower extract (Table 6 and Figure 7). The percentage activity was increased with an increase of concentration of extracts. The highest activity, i.e., 81.3±0.59 % was that of 5 µg/mL leaves extract while the lowest, i.e. 41.9±0.13% of 5 µg/mL of flowers extract.

Table 6. Antioxidant potential of chloroform extract of rhizome (with roots), leaves and flowers

Conc. µg/mL	DPPH Scavenging (%)		
	Rhizome (with Roots)	Leaves	Flowers
0.5	24.3±0.29a	43.45±0.53c	−13.67±0.31a
1	26.79±0.66c	50.34±0.45c	3.05±0.37ab
1.5	29.66±0.3a	57.53±0.32b	12.99±0.47c
2	34.68±0.52b	62.66±0.25a	14.98±0.52d
2.5	37.96±0.47b	67.43±0.28a	16.83±0.48c
3	41.38±0.79d	70.19±0.18a	20.73±0.37ab
3.5	45.08±0.33a	72.24±0.37b	26.13±0.42c
4	50.11±0.51b	74.19±0.54c	31.89±0.25a
4.5	54.37±0.29a	77.33±0.27a	35.36±0.21a
5	60.66±0.39a	81.3±0.59c	41.9±0.13a

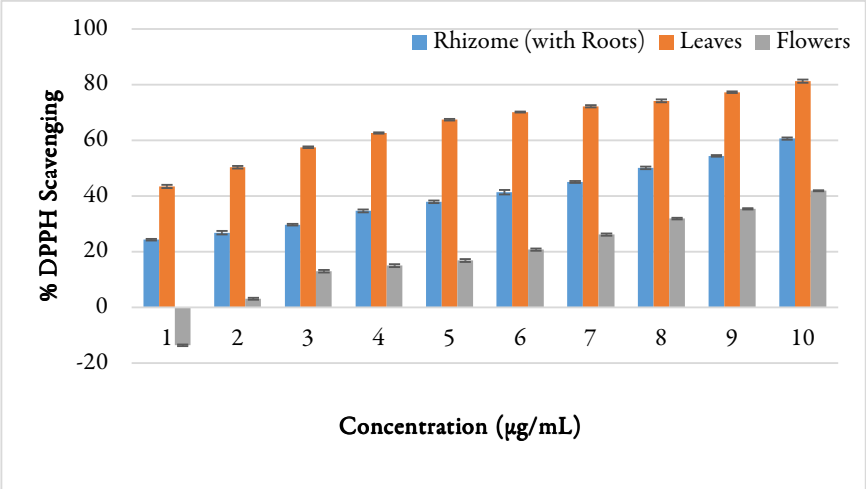

Figure 7. Antioxidant potential of chloroform extract of rhizome (with roots), leaves and flowers

The ethanol extracts of rhizome (with roots), leaves and flowers exhibited lowest % Scavenging activity as compared to n-hexane and chloroform extracts, i.e., 22.57±0.35%, 5.84±0.33% and −21.30±0.23% at 0.5 µg/mL concentration, respectively (Table 7 and Figure 8). Maximum activity was revealed by ethanol extracts of rhizome (with roots), leaves and flowers at 5 µg/ml, i.e., 54.9±0.38%, 65.35±0.52% and 35.44±0.32%, respectively, whereas the highest DPPH scavenging activity was exhibited by n-Hexane extracts of rhizome (with roots), leaves and flowers. The leaves extracts revealed highest while the flowers extracts showed lowest activity (Tables 5-7 and Figures 6-8).

Table 7. Antioxidant activity of ethanol extract of rhizome (with roots), leaves and flowers

Conc. µg/mL	DPPH Scavenging (%)		
	Rhizome (with roots)	Leaves	Flowers
0.5	22.57±0.35a	5.84±0.33a	−21.30±0.23a
1	24.42±0.33a	16.51±0.52c	−18.53±0.66d
1.5	28.44±0.4b	28.68±0.33a	−17.64±0.64d
2	32.02±0.53b	32.21±0.56c	−13.53±0.66d
2.5	34.01±0.49b	37.83±0.42b	−8.55±0.46b
3	38.38±0.39a	45.34±0.31a	−2.63±0.47b
3.5	41.47±0.28a	50.11±0.43b	11.6±0.67d
4	44.48±0.4b	55.97±0.25a	20.8±0.56c
4.5	49.47±0.73c	61.19±0.35a	30.73±0.7d
5	54.9±0.38a	65.35±0.52c	35.44±0.32a

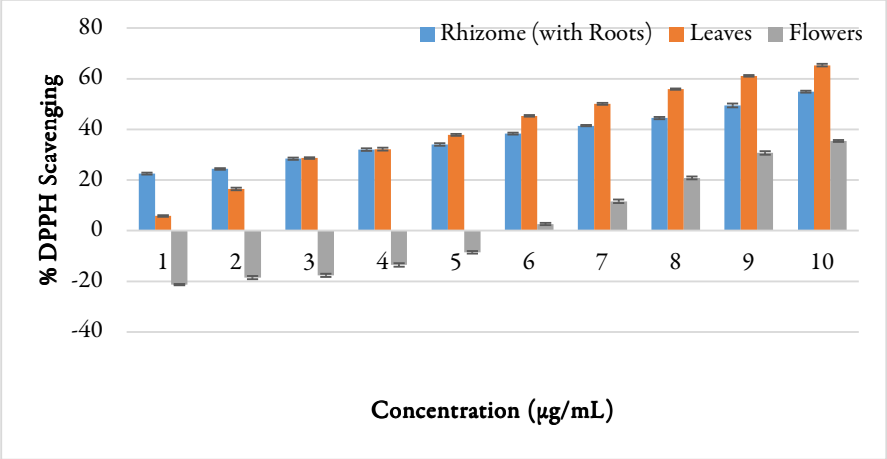

Figure 8. Comparative antioxidant potential of ethanol extract of rhizome (with roots), leaves and flowers

Anticancer activity

The n-hexane, chloroform and ethanol extracts of rhizome (with roots), leaves and flowers of *C. differmis* were evaluated for anti-proliferative activity in three days against A2780 cancer cells and HCT116 using SRB proliferation assay. Most of the crude extracts inhibited cell proliferation by more than 70% at 1mg/mL and 0.5mg/mL concentrations. n-Hexane extract of leaves and flowers failed significantly in inhibiting proliferation of A2780 and HCT116 cancer cells at 0.5mg/mL concentration (Figures 9 and 10).

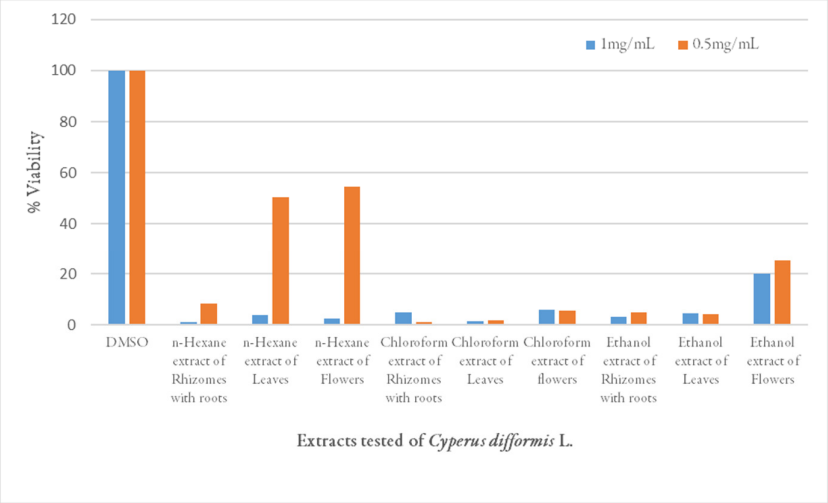

Figure 9. Antiproliferative activity of A2780 cancer cells in two concentrations (0.5mg/mL and 1mg/mL) of n-Hexane, chloroform and ethanol crude extracts of rhizome (with roots), leaves and flowers of *Cyperus difformis* L., followed by staining with SRB. Percentage inhibition was calculated with reference to the DMSO treated control cells. The graph represents results from two independent experiments, done in duplicates

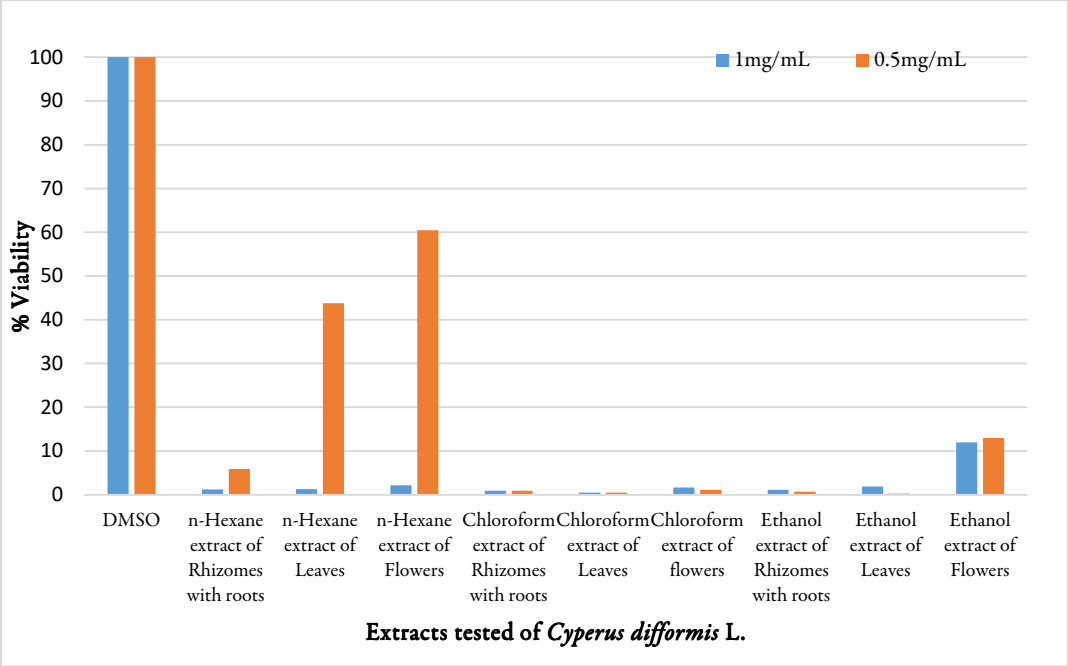

Figure 10. Antiproliferative activity of HCT 116 human colon cancer cell line in two concentrations (0.5mg/mL and 1mg/mL) of n-Hexane, chloroform and ethanol crude extracts of rhizome (with roots), leaves and flowers of *Cyperus difformis* for 72 hours, followed by staining with SRB. Percentage inhibition was calculated with reference to the DMSO treated control cells. The graph represents results from two independent experiments done in duplicates

Discussion

Despite the prevalence of *Cyperus difformis* traditional use in medicine, the chemical components responsible for its efficacy are poorly understood. The FTIR spectroscopy of powder of its' three different parts i.e. rhizome (with roots), leaves and flowers showed the presence of alcoholic, carbonyl and anhydride groups. FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition.

GC-MS analysis of n-Hexane crude extracts of rhizome (with roots), leaves and flowers exhibited the presence 14 compounds, while 6 in chloroform extracts and 5 in ethanol extracts. The chloroform extract of flowers was found containing no compound. These phytochemicals are responsible for various pharmacological actions like antimicrobial, antioxidant, anti-inflammation, anti-cancer, hepato-protective, diuretic, anti-asthma etc.

Babu and Savithramma (2014) reported the presence of glycosides, lignins, quinones, tannins and terpenoid in the crude extract of leaves of *C. difformis*. Glycoside are important in medicine because of their action on heart and are used in cardiac insufficiency (Balch and Balch, 2000). Lignins are significant components in the global carbon cycle; the resistance of lignin to microbial degradation enhances its persistence in soils (Cambell and Sederoff, 1996). Quinones are known to act as mobile electron carriers within the lipid phase of the membrane in photosynthetic and respiratory electron transport chains (Fato *et al*., 1996).

Selamoglu (2018) described oxidative stress as a process of all the time faced by our bodies in damaging of the natural oxidation process resulting in the toxic effects, such as arteriosclerosis, Parkinson's disease, myocardial and Alzheimer's disease, etc. In this particular research, importance of the plant extracts was highlighted to combat the issue related to the oxidative stress. The main function of quinones is to prevent damage of the cell by free-radical formation (Barr *et al*., 1992). Tannins as the main component of astringent used for treating intestinal disorders such as diarrhea and dysentery (Dharmananda, 2003). Tannins and tannic acid form a pellicle of coagulated protein over the lining of the alimentary tract. Terpenoids are famous for analgesic and anti-inflammatory activities.

Results of antibacterial activity of n-Hexane, chloroform, and ethanol extracts of rhizome (with roots), leaves and flowers suggested that *Salmonella enterica* was the most susceptible strain followed by *Staphylococcus aureus* and *E. coli*. Moreover, the leaves extracts were the most effective against bacteria and showed strong antibacterial activity. Chloroform extract of leaves were highly antibacterial.

The most effective DPPH scavenging potential was shown by the n-Hexane, chloroform and ethanol extract of leaves compared to rhizome (with roots) and flowers at 5 µg/ml concentration while the highest activity was shown by n-hexane extract of leaves.

Results of antiproliferative activity suggested that n-Hexane extracts of rhizome (with roots), leaves and flowers inhibited the proliferation of A2780 cell lines better than chloroform and ethanol extracts of these parts. The highest inhibition was observed by the n-Hexane extract of rhizomes with roots. Moreover, the chloroform extracts of all the targeted parts of plant were most effective in inhibiting the HCT116 cell line proliferation compared to their n-Hexane and ethanol extracts. Selamoglu (2017c) elaborated various biotechnological approaches used in anticancer activity by flavonoids, large group of phenolic compounds derived from plants. Many plant extracts show antioxidant activity and can thus be used in food products and helpful for good health.

The highest anticancer potential was observed in chloroform extract of leaves with cell viability 0.46% showing 99.5% inhibition.

Conclusions

The above mentioned ethnopharmacological studies showing the targeted sedge weed possessing medicinal value may represent new sources of pharmaceutical industries for stable, pharmacologically active secondary metabolites which can be used as healers in the field of medicine, that can be used for the treatment of many diseases including pathogenic disorders. However, further research is required to establish the *in vivo* actions and therapeutic index of this sedge with respect to the management of various pathogenic diseases to improve the health of livestock.

Authors' Contributions

Conceptualization: ZK, SZA; Execution: SZA; Review; ZK; SAM; Advice & Supervision: ZK and SAM.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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