

## ANTIBACTERIAL, ANTIFUNGAL AND ANTHELMINTIC PROPERTIES OF CRUDE EXTRACTS OF *CYPERUS EXALTATUS* RETZ. AND *SCIRPUS MARITIMUS* L.

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### Abstract

The present research study was conducted to assess the antimicrobial and anthelmintic potential of two sedges i.e. *Cyperus exaltatus* Retz. and *Scirpus maritimus* L., of family Cyperaceae from Lahore, Pakistan. Antibacterial, antifungal and anthelmintic properties were determined using the crude extracts of plant parts i.e., leave, stem, inflorescence and rhizome. The aqueous extract of *C. exaltatus* inflorescence exhibited the maximum zone of inhibition against bacterial strains i.e. *Bacillus subtilis* (Gram positive) and *Escherichia coli* (Gram negative), while the ethanolic extract of *C. exaltatus* inflorescence exhibited the maximum zone of inhibition against fungal strains i.e. *Candida albicans* and *Penicillium commune*. Maximum inhibition zone was found in ethanolic extract of *S. maritimus* inflorescence against bacterial strains while the maximum zone of inhibition was recorded in water extract of *S. maritimus* stem against fungal strains. Water extract of inflorescence of *S. maritimus* showed maximum anthelmintic activity while n-hexane extract of rhizomes of *C. exaltatus* showed the least activity. It is observed that these plants have ethnopharmacological potential for the investigated assays and could serve as source for synthesis of novel drugs.

**Keywords:** Antibacterial, Antifungal, Anthelminthic, *Candida albicans*, *Cyperus exaltatus*, Drugs, n-hexane, *Penicillium commune*, *Scirpus maritimus*

### INTRODUCTION

Plants contain bioactive components having a wide variety of medicinal uses, such as antioxidants, antibacterial, antipyretic, and anti-inflammatory properties (Moussouni *et al.*, 2018; Khan and Javaid, 2020). Natural compounds and their derivatives are more effective, having no side effects than manufactured products. These plant-derived substances such as, terpenoids, phenols and flavonoids have biological properties that support therapeutic actions to prevent from cancer development, heart diseases, oxidation and inflammation (Sharma *et al.*, 2020; Javaid *et al.*, 2022).

Many therapeutic herbs contain antioxidants (Khan and Javaid, 2019; Kanwal *et al.*, 2023). Antioxidants taken from plants, either as raw extracts or as chemical ingredients, are extremely powerful to halting the harmful processes induced by the oxidative process. Antioxidant compounds are also very helpful to induce the harmful radicals produced by oxidative stress, endure the plants under such conditions (Zengin *et al.*, 2011). Antimicrobial resistance is a phenomenon where microorganisms develop defense mechanisms against drugs intended to kill them, allowing the germs that are not killed to continue to grow and become more potent than ever (Shriram *et al.*, 2018).

Helminth derived from the Greek word helminths, means “worm” that lives inside the living body as parasites (Mathias *et al.*, 2021). Helminth infections are the most frequent illness in humans and animals, affecting a huge percentage of the population. Most helminth disorders are chronic and severe in nature. The identification of plants having bioactive compounds that serve as anti-helminthiasis is thus regarded as a significant step forward in the treatment of helminthiasis (Yadav and Singh, 2011).

The present research project was designed to explore the ethnopharmacological potential of locally growing two sedges, *Cyperus exaltatus* Retz. and *Scirpus maritimus* L., of family Cyperaceae. These plants have traditional medicinal uses but have limited scientific research regarding anthelmintic activity. The sedge family, Cyperaceae, is the second largest monocotyledon family, with 3000 species, 220 of which are considered weeds. Except for Antarctica, the family is found all over the planet, most frequent in tropical areas, widespread in highland areas and paddy fields.

Sedges seem like grass, but they have a triangle cross-section stem and spirally arranged leaves. Sedges range in size from 0.01 to 5 meters in length, depending on the species. Flowers are either unisexual or bisexual, and their size and complexity have decreased. It is organized around a shortened axis that emerges from the center of the stem. Although most of the sedges were pollinated by wind, a few Cyperaceae species were pollinated by insects (Abdullah *et al.*, 2021). Cyperaceae plants have an excellent source of a wide range of substances such as phenolics, stilbenes, flavonoids, phenolic acids, phenylpropanoids, terpenoids, coumarins, and quinones. Most sedges are used to treat various disorders, such as stomach and bowl

problems, bronchitis, tumors, discomfort and fever. Some edible species are taken as nutritional supplements, while others are used to weave household goods (David *et al.*, 2021). The compound  $\alpha$ -viniferin derived from *C. humilis*, showed antibacterial effect. By inhibiting the two enzymes, anthelmintic effect of resveratrol and  $\alpha$ -viniferin is mediated (David *et al.*, 2021).

*Cyperus exaltatus* Retz. is a pantropical species occurring on all the major continents. It is native to South America, Africa, Australia and Asia (India and Nepal). It is a hardy perennial with tightly packed culms, about 1cm of thickness and 1.5 m tall that arise from short, thick, woody rhizomes. The leaves are entirely basal and can reach lengths up to 80 cm with width of 0.1 cm. The inflorescence grows up to 40 cm of length subtended by leafy bracts that may reach 60 cm in length and 1 cm in width (Haines and Lye, 1983).

Its rhizomes are grated and consumed, used to treat malaria and as dressings on scarifications over the spleen (Subramanian *et al.*, 2021). *Scirpus maritimus* L. is a glabrous, rhizomatous perennial with fleshy roots and stems up to 1-150 cm long. It is widely distributed in temperate zone. It grows up to altitudes of 600-950m in freshwater wetlands such as streams, ditches, swamp edges, shallow pools from irrigation canal overflow, and occasionally in saline areas, as well as a weed in rice fields (Erkal, 2016; Charpentier *et al.*, 2000). As all available species of Cyperaceae are considerably used for different treatments by local people, they are worthy for scientific investigations.

## MATERIALS AND METHODS

### Collection of Plant Material

The locally growing sedges *Cyperus exaltatus* Retz. and *Scirpus maritimus* L. collected

from the outskirts of Lahore city, i.e. Kala Shah Kaku (31. 724° N, 74.268° E). Collections were made in months of February-March. The plant specimens were identified and authenticated by Prof. Dr. Zaheer-ud-din Khan, Botany department, Government College University, Lahore and stored them in departmental herbarium. The plants were properly cleaned with tap water to get rid of dust and then separated into their parts, i.e. leaf, stem, inflorescence and rhizomes. All plant parts were under shade, dried at room temperature for two weeks. The dried plant parts were then ground in powdered form separately using mortar and pestle. The powdered plant parts were then stored in airtight containers at room temperature for further analysis.

The powder of plant material was weighted and then soaked in the non-polar and polar solvents based on increasing polarity order as: n-Hexane < Chloroform < Ethanol < Distilled water. The powder was immersed in each of the solvents for 7-8 days, shaken daily and filtered using Whatman filter paper. The filtrate was kept in pre-weighed jars, while the residue after air drying was macerated using the next solvent (Tiwari *et al.*, 2009).

### Appraisal of Antimicrobial Activity

#### Collection of sample microbes

The bacterial strains *Bacillus subtilis* and *Escherichia coli* while the fungal strains, *Candida albicans* and *Penicillium commune* collected from laboratories of GC University Lahore, were used to assess the anti-bacterial and antifungal activity by using Nutrient Agar medium and Malt Extract medium, respectively.

#### Agar Well Diffusion Method

Agar well diffusion method was used to measure the zone of inhibition following Jorgensen

and Ferraro, (1998). Standard antibiotic disc was used as a positive control while solvents (n-Hexane, Chloroform, Ethanol and Distilled water) as negative control.

Under aseptic conditions, the inoculum was spread on sterilized and solidified medium in the petri plate using a sterile spreader. The four wells were made in each petri plate using a sterilized cork borer and 0.1-0.3 ml of plant extract was added into the respective wells using sterilized dropper. The petri plates were incubated at 37°C for antibacterial activity and at 27°C for 48 hours for antifungal activity.

The zone of inhibition where there was no growth of microbes thus developed around the well by the plant extract was measured in millimeter.

### Determination of Anthelmintic Activity

Anthelmintic activity of crude plant extracts was determined by following the method of Ajaiyeoba *et al.*, (2001). The alive worms (*Haemonchus contortus*) were collected from the abomasum of freshly slaughtered sheep and then placed in freshly prepared 0.9% NaCl solution. After this, eleven worms were placed on each petri plate containing 10 mL of plant extract. Albendazole was used as a standard anthelmintic drug and each solvent as a negative control. For accurate results, the experiment was performed in triplicates. Time of paralysis was recorded in minutes when no movement could be seen in worms and time of death was recorded when worms lost their motility and fading of their body color.

## RESULTS

### Appraisal of Antimicrobial Activity

The antibiotic and antimycotic discs were used as positive control while extraction solvents as a negative control. In comparison to extracts, the

results revealed that standard discs showed inhibitory effect against the selected strains.

**Table 1: Zone of inhibition (mm) showed by antibiotic disc against bacterial strains and standard antimycotic discs against fungal strains**

Antibiotic standard discs	Conc. (mg)	Zone of inhibition(mm)	
		Gram positive <i>Bacillus subtilis</i>	Gram negative <i>Escherichia coli</i>
Augmentin	30	17	22
	15	9	12
Erythrocin	30	15	15
	15	10	11
Antimycotic standard discs	Conc. (mg)	<i>Candida albicans</i>	<i>Penicillium commune</i>
Fluconazole	30	12	17
	15	9	13
Griseofulvin	30	20	16
	15	11	12

The effectiveness of crude plant extracts against the respective strains was evaluated using the agar well diffusion method and measured their zone of inhibition. The aqueous extract of *C. exaltatus* inflorescence exhibited the maximum zone of inhibition against bacterial strains, while the ethanolic extract of *C. exaltatus* inflorescence exhibited the maximum zone of inhibition against fungal strains. Maximum inhibition zone was found in ethanolic extract of *S. maritimus* inflorescence against bacterial strains while the maximum zone of inhibition was recorded in water extract of *S. maritimus* stem against fungal strains.

**Determination of Anthelmintic Activity**

The anthelmintic activity of crude plant extracts was investigated against the gastrointestinal nematode *Haemonchus contortus* and results were recorded by observing paralysis and death time of worms. Water extract of inflorescence of

*S.maritimus* showed maximum anthelmintic activity while n-hexane extract of rhizomes of *C.exaltatus* showed the least activity.

**DISCUSSION**

Secondary metabolites are produced in plants in result of their different biochemical processes. Different kinds of secondary metabolites have medicinal importance and have potential to be used for treatment of various diseases (Dhandapani *et al.*, 2008). Different parts of same plant may use for the preparation of drugs, therapeutic agents, anti-inflammatory agents, etc. Numbers of plants are reported to possess medicinally important phytochemicals. Secondary metabolites and phytochemicals include: hydrogen cyanide, elemental sulphur, indoles, glucosinolatesterpenoids, flavonoids, saponins, terpenoids, alkaloids, cardiac glycosides, phenolics, steroids, phenyl propanoids, phlobatannin, etc.

**Table 2: Zone of inhibition of different solvents of *Cyperus exaltatus* and *Scirpus maritimus***

Plant Parts	Extracts	Bacterial strains		Fungal strains	
		Gram positive <i>Bacillus subtilis</i>	Gram negative <i>Escherichia coli</i>	<i>Candida albicans</i>	<i>Penicillium commune</i>
		Diameter of Zone of inhibition(mm)			
Leaves	n-Hexane	4	---	3	---
	Chloroform	---	3	---	2
	Ethanol	5	---	12	11
	Water	14	---	6	5
Stem	n-Hexane	6	---	---	4
	Chloroform	---	---	4	---
	Ethanol	---	6	7	14
	Water	12	16	10	10
Inflorescence	n-Hexane	---	2	---	1
	Chloroform	3	---	---	---
	Ethanol	4	7	19	12
	Water	10	18	8	10
Rhizomes	n-Hexane	6	---	2	---
	Chloroform	---	5	---	---
	Ethanol	5	9	6	6
	Water	13	11	10	10
<i>Scirpus maritimus</i>					
Leaves	n-Hexane	---	---	2	---
	Chloroform	8	6	---	3
	Ethanol	4	6	5	---
	Water	2	4	---	5
Stem	n-Hexane	---	---	---	6
	Chloroform	3	5	3	7
	Ethanol	10	14	10	4
	Water	9	11	14	18
Inflorescence	n-Hexane	7	2	---	---
	Chloroform	10	12	---	---
	Ethanol	16	13	15	12
	Water	6	9	6	13

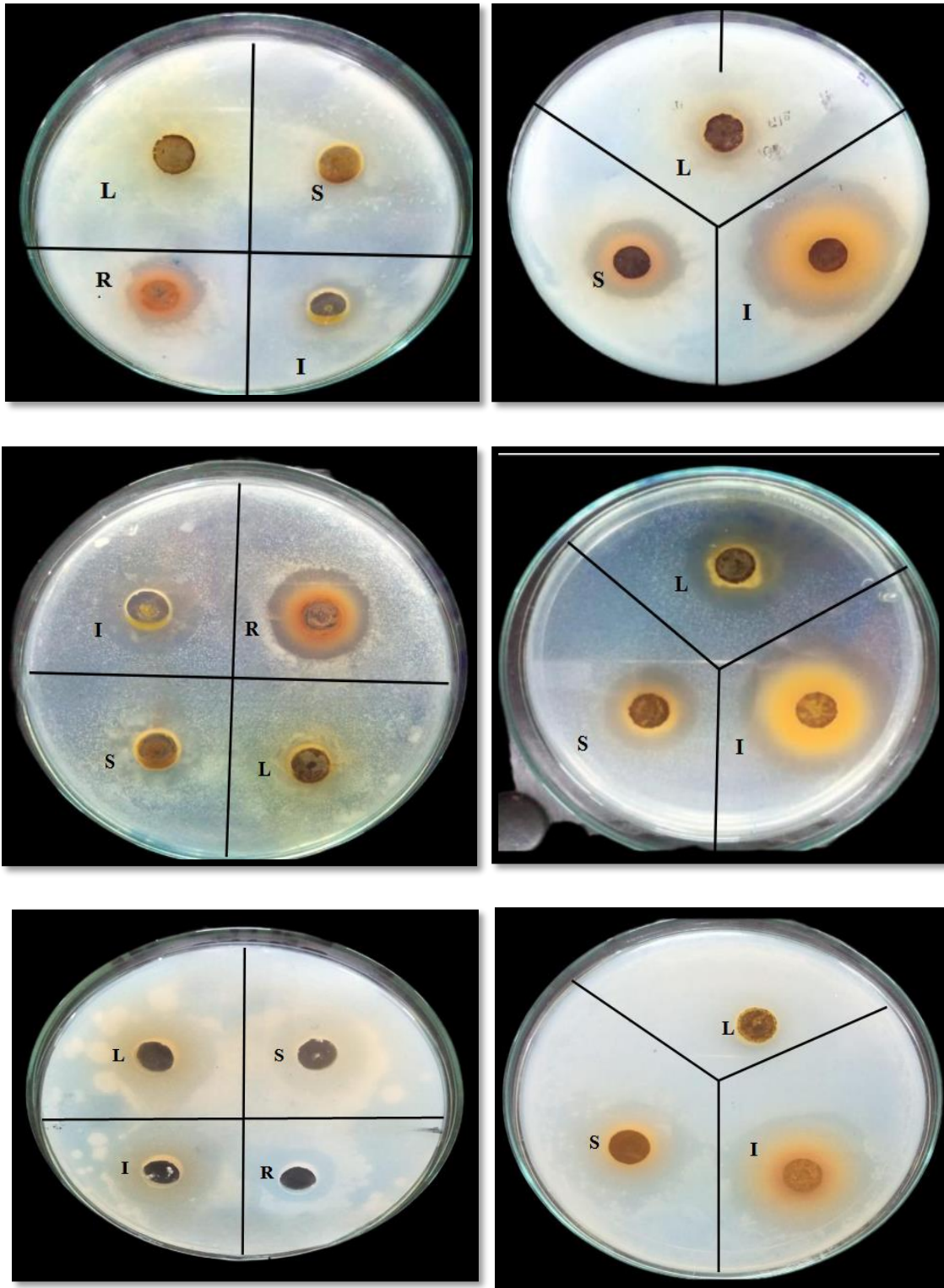


Fig: (a+b) Ethanolic extracts of *Cyperus exaltatus* and *Scirpus maritimus* against *Bacillus subtilis* (c) Ethanolic extracts of *Cyperus exaltatus* against *Escherichia coli* (d) Chloroform extracts of *Scirpus maritimus* against *Escherichia coli* (e) Aqueous extracts of *Cyperus exaltatus* against *Candida albicans* (f) Ethanolic extracts of *Scirpus maritimus* against *Candida albicans*.

**Table 3: Time taken by standard drug and control for paralysis and death of worms**

Treatment	Time of Paralysis (min)	Time of Death (min)
<b>Standard</b>		
Albendazole	-	-
<b>Control</b>		
n-Hexane	24.33±0.333	48.66±1.333
Chloroform	19.33±0.333	40.66±0.666
Ethanol	14.66±0.333	30.66±0.666
Distilled water	-	-
Saline	-	-

**Table 4: Time taken by the extracts of *Cyperus exaltatus* and *Scirpus maritimus* for paralysis and death of worms.**

Plant Parts	n-Hexane		Chloroform		Ethanol		Diswater	
	P(min)	D(min)	P(min)	D(min)	P(min)	D(min)	P(min)	D(min)
<i>Cyperus exaltatus</i>								
Leaf	18.33±1.763	38.00±1.154	16.33±0.333	36.00±0.577	10.66±0.333	22.00±0.577	09.00±0.577	20.00±0.577
Stem	15.33±0.881	33.66±0.881	17.66±0.333	37.00±0.577	12.00±0.57	24.66±0.881	09.66±0.333	21.00±0.577
Inflorescence	17.33±0.881	35.00±1.527	14.33±0.333	30.33±0.333	11.66±0.333	24.00±0.577	11.66±0.333	24.33±0.666
Rhizomes	21.00±0.577	42.66±1.201	10.66±0.333	25.00±0.577	7.66±0.333	15.00±0.577	12.00±0.577	26.00±0.577
<i>Scirpus maritimus</i>								
Leaf	16.00±1.00	36.66±0.666	16.33±0.881	38.66±0.333	07.33±0.666	17.00±0.577	08.00±0.577	14.33±0.333
Stem	15.00±0.577	31.66±0.333	15.66±0.333	34.33±0.666	09.00±0.577	20.00±0.577	08.33±0.333	16.33±0.333
Inflorescence	13.00±0.577	28.66±0.333	11.33±0.333	24.66±0.333	10.66±0.333	23.33±0.333	04.66±0.333	10.66±0.333

For the investigation of antibacterial, antifungal and anthelmintic activities of different extracts of *Cyperus exaltatus* and *Scirpus maritimus* were prepared by using maceration techniques. Selection of solvents was polarity based (polar/non-polar). Parts of plants were dried, grinded, collected extracts from them of their respective solvents, précised expected results from antibacterial, antifungal and anthelmintic performances and confirmed by literature about these n-hexane, chloroform, ethanol and distilled water solvents.

The antimicrobial activity of crude plant extracts was assessed against the bacterial and fungal strains and measured their zone of inhibition. The water extract of *C. exaltatus* leaves and ethanolic extract of *S. maritimus* inflorescence showed the maximum zone of inhibition against *B. subtilis* while the water extract of *C. exaltatus* inflorescence and ethanolic extract of *S. maritimus* stem presented the significant inhibition zone against *E. coli*. The antifungal activity of the inflorescence of *C. exaltatus* and *S. maritimus* in ethanol extract exhibited the highest inhibition zone against *C. albicans* while highest zone of inhibition was shown by the *S. maritimus* stem in water extract and ethanolic stem extract of *C. exaltatus* against *P. communae*. The standard discs were used for the comparison against the bacterial and fungal strains showed inhibition zones compared to plant extracts.

The anthelmintic activity of crude plant extracts was tested against *Haemonchus contortus* and results were recorded in terms of paralysis and death time of worms. The water extract of *S. maritimus* inflorescence and ethanol rhizomes extract of *C. exaltatus* expressed the promising results against *H. contortus* as compared to the plant extract.

## CONCLUSION

Based on the results thus obtained in the present study, it can be concluded that the crude extract of both sedges shows very good antimicrobial results against bacterial and fungal strains. So, these plant extracts may be used in replacement of the standard drugs to treat various diseases caused by bacteria and fungi. Similarly, the anthelmintic activity of both sedges also showed the significant results as compared to the standard drug. Therefore, the extract obtained from these plants may be used to cure animal helminthiasis is to improve dairy products and meat requirements. So, these sedges may prove helpful in Pakistan dairy industry.

## AUTHORS' CONTRIBUTION

Conceptualization: Zaheer-ud-din Khan, Data curation: Zonarah Arif, Kiran Akhtar, Formal analysis: Muhammad Bilal, Investigation: Muhammad Fahad Shakeel, Methodology: Muhammad Fahad Shakeel, Muhammad Bilal, Project administration: Zaheer-ud-din Khan, Supervision: Zaheer-ud-din Khan, Writing - Original draft: Zonarah Arif, Kiran Akhtar, Writing – review and editing: Mobeen Iqbal, Javeria Naseem

## CONFLICT OF INTEREST

The authors declared no conflict of interest.

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