

Chapter 4

Adaptive chemical ecology of *Cyperus polystachyos* Rottb. (Cyperaceae): linking wetland stress to cytotoxic activity

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ABSTRACT

Plants growing in natural ecosystems often produce bioactive compounds to protect themselves from environmental stress. *Cyperus polystachyos* Rottb., a wetland sedge belonging to the family Cyperaceae, is a widely distributed species with limited scientific study despite its ecological importance. Present study aimed to evaluate its phytochemical composition and cytotoxic potential. Different extracts were screened for primary and secondary metabolites using standard qualitative tests. The ethanol and aqueous extracts showed the presence of carbohydrates, proteins, flavonoids, tannins, and saponins, while the n-hexane extract tested negative for most compounds. Cytotoxic activity was assessed through the brine shrimp lethality assay using hydroethanolic extract at varying concentrations which showed a concentration-dependent increase in mortality of brine shrimp nauplii, reaching 100 % mortality at higher concentrations in the cytotoxicity assay. These findings suggest that *C. polystachyos* contains biologically active compounds with potential pharmaceutical significance. This study provides baseline data and supports the need for further isolation and characterization of its bioactive constituents.

Keywords: Brine shrimp, bioactivity, environmental stress

INTRODUCTION

Plants are an essential source of medicinal compounds and have been used in traditional healthcare systems for centuries (Palombo, 2011). Many plants

synthesize a wide range of bioactive compounds that help them survive under harsh environmental conditions and protect them from herbivores, microbes, and other stress factors (Al-Khayri *et al.*, 2023). These metabolites are known to possess various biological activities and are increasingly being studied for their pharmaceutical and therapeutic potential (Seca and Pinto, 2019). Wetland plants experience fluctuating water levels, microbial pressure, and competition which can stimulate the production of unique chemical compounds (Ramakrishna and Ravishankar, 2011). *Cyperus polystachyos* Rottb., a member of the Cyperaceae family (POWO, 2025), is commonly found in marshy areas, riverbanks and seasonally flooded habitats. Traditionally, decoction of this species has been documented in treatment of diarrhea and dysentery (Khan *et al.*, 2018). Ecologically, this sedge contributes to soil stabilization, nutrient cycling and microhabitat formation (Janyszek-Sołtysiak *et al.*, 2025); however, its chemical and bioactive properties are not well explored. Limited research exists on the phytochemical profile and cytotoxic properties of *C. polystachyos*. Therefore, the present study was designed to investigate the presence of primary and secondary metabolites in different solvent extracts and to evaluate the cytotoxic potential of its hydroethanolic extract using the brine shrimp lethality bioassay. This study aims to provide preliminary scientific evidence that may support future research into its medicinal applications.



Figure 1: inflorescence of *Cyperus polystachyos*

METHODOLOGY

Collection and authentication of plant material: whole plants of *Cyperus polystachyos* (Figure 1) were collected from the swampy areas of Cuttack district, Odisha, India and taxonomically identified on the basis of their morphological characters using standard regional flora (Saxena and Brahmam, 1996).

Cleaning, drying and powder preparation: The collected plant specimens were washed thoroughly under running tap water to eliminate adhered soil particles, dust, and other extraneous materials. The cleaned samples were then shade-dried at ambient laboratory temperature (25 ± 2 °C) for approximately one week. After complete drying, the plant material (Figure 2) was crushed into a coarse powder using an electric mechanical grinder and preserved in sterile, air-tight containers until further use.

Solvent extraction: The powdered plant material was subjected to solvent extraction using solvents of increasing polarity, namely n-hexane (non-polar), ethanol (polar), and distilled water (highly polar). For each solvent, 10 g of the powdered sample was soaked in 100 mL of the respective solvent and kept for 48 hours, during which the mixture was intermittently agitated to enhance the extraction efficiency (Devi *et al.*, 2023). Following this, the extracts were filtered through Whatman No. 1 filter paper and the filtrates were allowed to evaporate at room temperature to obtain the crude residues. The dried extracts were transferred to sterilized glass vials and stored at 4 °C for subsequent analyses.

Preliminary phytochemical screening: *C. polystachyos* extracts were used to determine the presence of both primary metabolites (such as proteins, carbohydrates, starch, sucrose, lipids, and amino acids) and secondary metabolites (including tannins, saponins, flavonoids, terpenoids, phenolic compounds, reducing sugars, steroids, and alkaloids). Standard qualitative phytochemical protocols were followed (Jena *et al.*, 2025) and the development of characteristic colour changes or precipitates in each test was recorded as an indication of the presence (+) or absence (–) of respective metabolites.

Brine shrimp hatching and maintenance: the cytotoxic activity of the hydroethanolic extract of *C. polystachyos* was assessed using the brine shrimp lethality bioassay method (Jena *et al.*, 2025). *Artemia salina* eggs were incubated in artificial seawater prepared with 3.5% saline solution under continuous aeration and natural light for 48 hours to obtain actively swimming nauplii.



Figure 2: Dried plant parts of *C. polystachyos*

Cytotoxicity assessment using Brine Shrimp lethality bioassay: a series of extract concentrations (32, 63, 94, 125 and 156 mg/mL) were prepared using the same saline solution as the diluent. Ten nauplii were transferred into each vial containing different concentrations of the extract. A negative control containing only 3.5% saline and a positive control treated with vincristine sulphate were

maintained alongside the test groups. The number of dead nauplii was recorded at regular intervals of 1, 2, 3, and 4 hours and the percentage mortality was calculated to evaluate the cytotoxic potential of the plant extract (Jena *et al.*, 2025).

RESULTS AND DISCUSSION

Primary metabolite screening: The qualitative analysis showed that ethanol and aqueous extracts of *C. polystachyos* contained some primary metabolites, while the n-hexane extract did not show the presence of any tested compounds (Table 1).

- Proteins and carbohydrates were present in both ethanolic and aqueous extracts.
- Starch was detected only in the ethanolic extract.
- Sucrose, lipids and amino acids were not detected in any of the extracts.

This indicates that, polar solvents (ethanol and water) were more effective in extracting primary metabolites from the plant.

Table 1: Qualitative phytochemical analysis (Primary metabolites)

Primary metabolites	n-hexane	Ethanol	Aqueous
Proteins	-	+	+
Carbohydrates	-	+	+
Starch	-	+	-
Sucrose	-	-	-
Lipid	-	-	-
Amino acid	-	-	-

Present = (+); Absent = (-)

Table 2: Qualitative phytochemical analysis (Secondary metabolites)

Secondary metabolites	n-hexane	Ethanol	Aqueous
Tannin	-	-	+
Saponin	-	-	+
Flavonoids	-	+	+
Terpenoids	-	-	-
Phenolic compounds	-	-	-
Reducing sugars	-	+	-
Steroids	-	-	-
Alkaloids	-	-	-

Present = (+); Absent = (-)

Secondary metabolite screening: The qualitative analysis showed the presence of bioactive compounds in the polar solvents (ethanol and water) while, non-polar (n-hexane) detected no active compounds (Table 2).

- The aqueous extract showed the presence of tannins, saponins and flavonoids.
- The ethanolic extract contained flavonoids and reducing sugars.
- Terpenoids, phenolic compounds, steroids and alkaloids were not detected in any of the extracts.

These findings suggest that *C. polystachyos* mainly contained polar secondary compounds, especially flavonoids, tannins and saponins, which are known for their biological activity.

Cytotoxicity analysis: The hydroethanolic extract of *C. polystachyos* showed strong cytotoxic activity against brine shrimp nauplii and the effect increased with increasing concentration (Table 3).

- At 32 mg/mL, mortality was 80% after 4 hours.
- At 63 mg/mL, mortality increased to 90%.
- At 94 mg/mL and above, 100% mortality was observed.
- The negative control (3.5% saline) showed no mortality, confirming that the deaths were caused by the plant extract.
- The positive control (vincristine sulphate) showed 100% mortality, validating the reliability of the test.

This dose dependent increase in mortality indicates that the plant extract has significant cytotoxic potential, supporting its possible use in future pharmacological and anticancer studies.

Table 3: Cytotoxicity analysis of *C. polystachyos* using Brine shrimp nauplii

Extract	Concentration (in mg/mL)	Initial number of Nauplii	Number of deaths (after hours)				Mortality (%)
			1	2	3	4	
Hydro-ethanolic	32	10	0	6	7	8	80
	63	10	0	7	8	9	90
	94	10	0	9	10	10	100
	125	10	9	10	10	10	100
	156	10	10	10	10	10	100
Negative control	3.5% Saline	10	0	0	0	0	0

Positive control	Vincristine sulphate	10	10	10	10	10	100
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Ecological link: the aqueous and ethanolic extracts of *C. polystachyos* were found to contain flavonoids, tannins and saponins. These compounds are known in ecology to (Huang *et al.*, 2018; Riddick, 2024):

- a) Protect plants from microbial infection
- b) Deter insect feeding and herbivory
- c) Attracts pollination
- d) Protection from environmental stress

Ecologically, this preliminary tested toxicity may also help the plant limit small aquatic organisms around its roots and reduce competition for nutrients and space in wetland ecosystems reflecting its survival strategy (Ayaz *et al.*, 2018).

CONCLUSION

The results of this study confirmed that *Cyperus polystachyos* contains important phytochemicals, particularly in the ethanol and aqueous extracts. The presence of compounds such as flavonoids, tannins, and saponins indicates its potential as a source of biologically active substances. The hydroethanolic extract showed strong cytotoxic activity in the brine shrimp lethality assay with increasing mortality observed at higher concentrations. These findings suggest that *C. polystachyos* possesses promising bioactive potential and may serve as a valuable candidate for further pharmacological and toxicological investigations. In addition to its ecological importance as a wetland species, this sedge may also have unexplored medicinal value. Future studies should focus on isolating specific compounds, determining their mechanisms of action, and evaluating their effects in more advanced biological models.

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