Chapter 6

Natural strategies against antibiotic resistance: bioactivity of *Cyperus cuspidatus* Kunth (Cyperaceae)

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ABSTRACT

Plants are rich sources of natural chemicals known as secondary metabolites, many of which have strong medicinal properties. Since ancient times, humans have used plants to treat infections and diseases, and even today, a large part of the world depends on traditional plant-based medicine. Several modern drugs, such as artemisinin, taxol, galantamine, and curcumin, have been developed from plant compounds and are used to treat serious illnesses including malaria, cancer, and neurological disorders. Although only a small percentage of plant-derived compounds are currently approved as antimicrobial agents, their true potential is far greater. *Cyperus cuspidatus* in that context was profiled for its phytochemical richness that revealed the presence of several primary and secondary metabolites including proteins, carbohydrates, starch, tannins, flavonoids, phenolic compounds and more. The antibacterial assay was used to validate its potential against gram negative *Escherichia coli* which showed significant activity. This highlights the importance of studying phytochemicals for their possible role in future drug discovery and development.

Keywords: Multidrug resistance, plant-based therapeutics

INTRODUCTION

Microorganisms are evolving at a rate that scientists all over the world are having difficulty to track down making it one of the leading concerns that affect public health and well-being (Cavicchioli *et al.,* 2019). Deactivation of various drugs by restraint strains is precisely due to the extensive of antibiotics (Jadimurthy *et al.,*

2023). Over years, microorganisms have been exposed to multiple drugs and through genetic change, they've acquired resistance against them leading to the crisis of Multi drug resistance (MDR) (Tanwar *et al.,* 2014). This makes infections and treatments more difficult to handle, failures in surgical healthcare and increased mortality (Salam *et al.,* 2023). While, excessive antibiotic use is globally restricted but the amount of prescription and use of antibiotics around the world remains the same (Jadimurthy *et al.,* 2023). Reports (Van Boeckel 2025) expects a 67% rise in antibiotic consumption by 2030 specially in populated countries like China, India, Brazil, United States and South Africa. In that context, phytochemicals can be used as natural support or alternatives to antibiotics because they work in different ways and microbes are quickly becoming resistant to many common drugs (Khameneh *et al.,* 2021). Compounds such as alkaloids, phenols, coumarins, and terpenes have shown strong ability to stop the growth of drug-resistant bacteria (Khare *et al.,* 2021). They act by damaging bacterial cell membranes, blocking efflux pumps, stopping biofilm formation, and interrupting communication between bacterial cells, processes that normally help bacteria survive and develop resistance (Suganya *et al.,* 2022). Plant extracts contain a wide mixture of these compounds, which makes it harder for bacteria to develop resistance to them (Vaou *et al.,* 2021). Around 80% of people in developing countries still depend on plant based traditional medicine. Even today, about 25–28% of modern medicines come from plants. Some important examples (Jadimurthy *et al.,* 2023) include:

a) Artemisinin from *Artemisia annua* to treat malaria

b) Galantamine from *Galanthus* species for Alzheimer's disease

c) Nitisinone, derived from a plant compound, for hereditary disorders

d) Taxol and Camptothecin for cancer treatment

e) Curcumin from turmeric for skin diseases and antibacterial effects

Although only a small percentage of plant compounds are officially approved as antimicrobials, these numbers do not show their full potential (Cowan, 1999). Many plant chemicals can fight harmful microbes and may also be used for new or repurposed drug development. Overall, phytochemicals show strong and diverse promise in treating many human diseases, including infections (Ndezo Bisso *et al.,* 2022). While medicinal plants have been actively discussed with respect to screening, evaluation, isolations and characterization of phytochemicals; sedges remain widely unexplored in that context (Roy and Devi, 2025). *Cyperus cuspidatus* (Figure 1) is small annual sedge (POWO, 2025); usually frequent in damp, disturbed soils, especially in grasslands, wetlands, and along water bodies was screened in this

chapter for studying its phytochemical richness and preliminary antibacterial screening.



Figure 1: Collection of *C. cuspidatus* from moist grasslands

The morphological traits (Saxena and Brahmam, 1996) show that it has fibrous roots and smooth, triangular stems. The leaves are very narrow and grass-like, usually shorter than the stem, and become slightly rough towards the tip. The leaf bases are thin and membranous with faint reddish-brown streaks. The plant produces a simple flower head, often appearing as a single cluster at the top, with a few fine bracts that extend beyond the flowers. The spikelets are arranged in finger-like clusters and contain many tiny flowers packed closely together. The flower bracts are brownish, with visible veins and a small curved tip. The plant has two to three stamens and three stigmas. After flowering, it produces a small, three-angled, chestnut-coloured nut. It is distributed throughout India upto 1500 m and widely distributed in tropical and subtropical regions across Asia, Africa, Australia and the Americas (Saxena and Brahmam, 1996; POWO, 2025). Its morphological traits closely resemble other members of the Cyperaceae family that can help explain its shared ecological value. Like many sedges, it has a trigonous stem, narrow grass-like leaves, and a fibrous root system, which help it anchor firmly in loose, wet, or disturbed soils. These features allow it to thrive in wetlands and along water margins, where it plays role

in stabilizing soil and reducing erosion (Barrett, 2013). Its compact growth and clustered spikelets support seed dispersal and rapid colonization, similar to other sedges that are considered pioneer species in degraded habitats (Larridon *et al.,* 2021). By growing in dense clumps, it helps retain moisture in the soil and provides microhabitats for small insects and microorganisms (Bertola *et al.,* 2021). These shared characteristics highlight its ecological role in maintaining soil structure, supporting biodiversity, and sustaining wetland ecosystem functions (Simpson and Inglis, 2001). While all these factors and characteristics indicate that *C. cuspidatus* is a prominent member of the family Cyperaceae, information available about its ethnomedicinal, pharamacological and ecological aspect remains scarce. Through this study, we want to document it not only as a common misunderstood weed because of its physical characteristics but from a sustainable, ecological and pharmacological perspective.

METHODOLOGY

Collection and identification of plant material: Plants of *C. cuspidatus* (Figure 1) were collected from the wetlands of the Chandaka areas of Khordha district, Odisha, India. The plant species was taxonomically identified by the authors using standard flora (Saxena and Brahmam, 1996).

Preparation of plant material: The sedge was thoroughly washed under running water to remove adhering soil particles and other surface contaminants. Then, air dried under shade at ambient temperature (25 ± 2 °C) for 7 days to minimize thermal degradation of bioactive constituents. Dried plants (Figure 2) were coarsely powdered and sieved to obtain uniform particle size, and stored in sterile, airtight glass containers under dry conditions to prevent moisture absorption and microbial contamination until further analysis (Devi *et al.,* 2023).

Solvent extraction: The powdered fruit material was subjected to solvent extraction using the Soxhlet method (Jena *et al.,* 2025). Three solvent systems of differing polarity were employed to ensure broad spectrum extraction of phytoconstituents:

a) n-Hexane
b) Ethanol
c) Aqueous

For each extraction, powdered material was placed in a thimble and was subjected to Soxhlet extraction using the above mentioned solvents. The extracted material was collected and was placed in room temperature for evaporation of extra solvent. The remaining extracts were stored in sterile glass vials under refrigerated conditions (4 °C) until further experimental use.



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

Qualitative phytochemical screening: Preliminary phytochemical screenings of the extracts were performed following standard protocols (Jena *et al.,* 2025) to detect the presence of major classes of primary and secondary metabolites.

Determination of antibacterial activity: The antibacterial potential of the hydroethanolic extract (1:1) was evaluated against *Escherichia coli* (MTCC 45) obtained from the Microbial Type Culture Collection, Chandigarh, India. The minimum inhibitory concentration (MIC) was determined using the broth microdilution method as described by Jena et al. (2025) with minor modifications.

RESULTS AND DISCUSSION

Phytochemical screening: The qualitative phytochemical analysis showed that no primary or secondary metabolites were detected in the non-polar (n-hexane) extract

of *Cyperus cuspidatus*, indicating that most bioactive compounds in the plant are polar in nature. In the polar extracts, both ethanol and aqueous solvents tested positive for primary metabolites such as proteins and carbohydrates, while starch was detected only in the aqueous extract, suggesting better extraction efficiency of highly polar compounds in water (Table 1) For secondary metabolites, the ethanolic extract contained flavonoids and reducing sugars, while the aqueous extract showed a wider range of compounds, including tannins, saponins, flavonoids, phenols, reducing sugars, and moderate levels of terpenoids, reflecting higher phytochemical richness in the aqueous extract (Table 2).

Table 1: Qualitative phytochemical analysis for detection of primary metabolites

Polarity	Extract	Detected primary metabolites	Phytochemical richness
Non-Polar ↓ Polar	n-Hexane	No tested metabolites were detected	◆
	Ethanol	Protein and Carbohydrates	◆
	Aqueous	Protein, Starch and Carbohydrates	◆

(◆= low, ◆= moderate, ◆= high)

Table 2: Qualitative phytochemical analysis for detection of secondary metabolites

Polarity	Extract	Detected secondary metabolites	Phytochemical richness
Non-Polar ↓ Polar	n-Hexane	No tested metabolites were detected	◆
	Ethanol	Flavonoids and Reducing sugars	◆
	Aqueous	Tannin, Saponin, Flavonoids, Terpenoids (moderate), Phenol and Reducing sugars	◆

(◆= low, ◆= moderate, ◆= high)

Antioxidant activity: The antimicrobial potential assessed through Minimum Inhibitory Concentration (MIC) revealed that the hydroethanolic extract of *C. cuspidatus* effectively inhibited microbial growth at a concentration of 12.5 mg/mL, as no growth was observed at this level. Higher concentrations also showed no microbial growth, indicating strong inhibitory activity (Table 3). In contrast, normal microbial broth showed visible growth at all concentrations, while the antibiotic control (Kanamycin) confirmed the reliability of the test. These results suggest that

C. cuspidatus contains polar bioactive compounds with promising antimicrobial potential.

Table 3: Estimation of Minimum Inhibitory Concentration (MIC)

Hydroethanolic Extract	Concentration (mg/mL)				MIC (mg/mL)
	100	50	25	12.5	
C.cuspidatus	NG	NG	NG	NG	12.5
Nutrient broth	NG	NG	NG	NG	-
Microbial broth	G	G	G	G	-
Antibiotics (Kanamycin)	NG	NG	NG	NG	-

(NG: No growth of bacteria; G: Growth)

Ecological link: The presence of many polar compounds like tannins, flavonoids, saponins, and phenols in *C. cuspidatus* helps the plant survive in open, wet, and often disturbed habitats where microbes, pests and environmental stress are common (Divekar *et al.,* 2022). These compounds act as natural chemical defenses, protecting the plant from infection and damage. This chemical protection explains why *C. cuspidatus* may grow successfully as a sedge in challenging environments, spreading easily and competing with other plants.

CONCLUSION

Plants play a vital role in human healthcare by providing a wide range of bioactive compounds with antimicrobial, anticancer, and therapeutic effects. Traditional knowledge, combined with modern scientific research has proven that phytochemicals can be powerful tools in the treatment of both infectious and chronic diseases. Although their full potential is still underexplored, evidence from existing plant based drugs shows that nature remains an important source for new medicines. In this study *C. cuspidatus* showed promising results in the qualitative phytochemical screening which showed that it contained major polar metabolites like flavanoids, tannins, saponins, phenol and terpenoids while, presence of carbohydrate, starch and protein in its aqueous extract highlighted its nutritional aspect. Antibacterial assay validated its potency against *E. coli* that can be used as baseline information for further research regarding the development of natural

therapeutic agents. Therefore, further investigation into sedges and their active compounds is essential for the discovery of novel drugs and the development of alternative, more sustainable healthcare solutions for the future.

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