Chapter 3

Phytoconstituents and free radical scavenging properties of *Cyperus mindorensis* (Steud.) Huygh (Cyperaceae)

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ABSTRACT

Biosynthesis of macromolecules depends on the regulation of cellular metabolism and the process of cellular respiration that helps produce adenosine triphosphate (ATPs). While chemical reactions are at play, excessive accumulation of free radicals in biological systems triggers oxidative stress that results in cellular damage. It makes antioxidants a vital component in the regulation of metabolism. Present study investigates the phytochemical composition and antioxidant potential of *C. mindorensis* to evaluate its ecological and applied significance. Sequential solvent extraction followed by phytochemical profiling revealed the presence of tannins, reducing sugars, and trace amounts of terpenoids in both ethanolic and aqueous extracts and carbohydrates and proteins in the aqueous extract. Qualitative antioxidant analysis showed presence of antioxidant-active phytoconstituents. These findings highlight the potential of *C. mindorensis* for use in natural antioxidant sources, herbal formulations, and sustainable ecological restoration programs.

Keywords: Antioxidant potential, grassland management, wildlife

INTRODUCTION

Cellular metabolism lies at the very core of how all living organisms' function at the microscopic, cellular level. It represents the total of all chemical reactions occurring within a cell, enabling the conversion of nutrients into usable forms of energy and regulating multiple metabolic pathways to maintain a precise balance

between energy production and energy utilization (De la Fuente, 2015). The regulation of metabolism is essential, as it continuously produces the necessary precursors required for the biosynthesis of molecules that allow cells and tissues to grow, repair themselves, and undergo replication (Zhu and Thompson, 2019). In addition to this, metabolic processes also aid in the efficient elimination of nitrogenous waste products from the body (Weiner *et al.,* 2015), which is vital for maintaining internal homeostasis. In a very real sense, life sustains because metabolism is at play. Among the various metabolic processes, cellular respiration plays a central role in the controlled conversion of chemical energy stored in molecules such as glucose. This energy transformation involves a series of interconnected biochemical pathways, including glycolysis, the Krebs cycle, and the electron transport chain (Liu *et al.,* 2025). The primary objective of these pathways is the synthesis of adenosine triphosphate (ATP), which acts as the major energy currency of the cell (Imamura *et al.,* 2009). This entire purpose is driven by a complex network of chemical reactions, which can broadly be classified into two major categories: catabolic reactions and anabolic reactions, referred to as catabolism and anabolism respectively (Chandel, 2021). While catabolic reactions are responsible for the breakdown of complex molecules into simpler units with the release of energy, anabolic reactions utilize this energy to synthesize larger and more complex macromolecules essential for cellular structure and function. When diving in greater detail, it becomes evident that the movement of electrons plays a pivotal role in regulating nearly all of these metabolic reactions at the molecular level, thereby making electron transfer one of the most fundamental aspects of cellular respiration (Mailloux, 2015). In that context, oxidation constitutes the core component of catabolic processes. However, oxidation does not occur alone; it is always coupled with a corresponding reduction reaction. Together, these paired reactions are known as oxidation–reduction or simply, redox reactions (Sies *et al.,* 2024). Free radicals are formed when an atom or molecule loses one or more electrons through oxidation, resulting in the generation of a highly unstable and reactive species that contains at least one unpaired electron. These reactive species can be produced through several normal cellular mechanisms, including electron 'leakage' from the electron transport chain (Nolfi-Donegan *et al.,* 2020), where free electrons combine with oxygen to form reactive oxygen species (ROS). Although the body routinely generates free radicals as natural by-products of normal cellular metabolism, certain physiological and pathological conditions (Chandimali *et al.,* 2025) such as inflammation caused by pathogenic invasion, excessive physical activity, hyperglycemia, and ischemia can significantly increase endogenous free radical production. In addition to these internal sources, various lifestyle and

environmental factors further contribute to an increased free radical load in the body. Regular consumption of highly processed foods, excessive alcohol intake, smoking, chronic mental stress, aging, exposure to harmful radiation, and poor air quality index (AQI) are all known to enhance the accumulation of free radicals (Wang *et al.,* 2024). When the balance between the production of ROS and the activity of antioxidant defense mechanisms is disrupted, a condition known as oxidative stress (OS) arises (Pizzino *et al.,* 2017).



Figure 1: *Cyperus mindorensis* in its habitat

Persistent oxidative stress can result in the damage of essential biomolecules such as proteins, lipids, and nucleic acids, ultimately leading to cellular dysfunction,

tissue injury, accelerated aging, and the development of various chronic and neurodegenerative diseases (Pizzino *et al.,* 2017). Antioxidants are compounds capable of neutralizing free radicals by donating electrons, thereby stabilizing these reactive species and preventing them from inflicting cellular and molecular damage. Plants that contain high levels of secondary metabolites, particularly phenolic compounds and flavonoids, are recognized as potent sources of natural antioxidants (Kurutas, 2015). These plant-derived antioxidants not only help safeguard cells and tissues from oxidative injury but also serve as a supplementary agent in the prevention and management of several non-infectious chronic conditions, including cardiovascular disorders, neurodegenerative diseases, inflammatory conditions, and even certain types of cancer (Clemente-Suárez *et al.,* 2025). Sedges with good palatability like *Cyperus mindorensis*, may therefore represent an important and readily available species for investigation in the context of nutrition and nutraceutical potential, particularly for wildlife. Native to the tropical and subtropical regions of the Old World extending to the Caucasus, *C. mindorensis* (POWO, 2025) is a perennial, rhizomatous geophyte that is predominantly found in seasonally dry tropical environments. The leaves and rhizomes of this species have a long history of use in traditional and folk medicine (Table 1).

Table 1: Ethnomedicinal uses of *Cyperus mindorensis*

Plant parts	Method of preparation	Ethnomedicinal uses	Source(s)
Leaves	Used fresh, crushed, or as simple extract	Relief from malarial chills and shivering Treatment of Pruritus Relief from thirst due to fever and diabetes Used as antivenom For common cold and bronchitis Helps in diabetes management Applied for minor injuries	(Raju *et al.,* 2011; Sindhu *et al.,* 2014; Abd Wahab, 2023; Niluwih *et al.,* 2023; Bhavani *et al.,* 2025)

Rhizomes	Used fresh or dried rhizome	Fragrant and sweet Refrigerant Antidiarrheal Diuretic Stomachic Expectorant Used for fever, hepatopathy, plexopathy, tumors and diabetes	(Raju *et al.,* 2011; Sindhu *et al.,* 2014; Abd Wahab, 2023; Niluwih *et al.,* 2023; Bhavani *et al.,* 2025)
Rhizomes	Rhizome is mixed with cow milk and make pills	Treatment of worm infections	(Raju *et al.,* 2011; Sindhu *et al.,* 2014)
Whole plant	Decoction	Used in malaria and fever Treatment of arthritis Management of digestive disorders, diarrhea, dysentery, and intestinal problems	(Raju *et al.,* 2011; Sindhu *et al.,* 2014; Abd Wahab, 2023; Niluwih *et al.,* 2023; Bhavani *et al.,* 2025)

The leaves have traditionally been employed for the relief of malarial chills, alleviation of skin pruritus, and reduction of excessive thirst associated with fever (Sindhu *et al.,* 2014). In India, the leaves are also widely used as antivenom in cases of snakebite (Bhavani *et al.,* 2025). The rhizomes of the plant are noted for their fragrant and sweet nature and are traditionally valued for their refrigerant, antidiarrheal, diuretic, stomachic, and expectorant properties (Niluwih *et al.,* 2023). A paste prepared from the rhizomes and mixed with milk is commonly administered for the treatment of worm infestations. Furthermore, the rhizomes are believed to be effective in the management of hepatopathy, splenopathy, diabetes and certain cancers (Raju *et al.,* 2011). Additional ethnomedicinal uses of *C. mindorensis* include its application in the treatment of common cold, bronchitis, arthritis, various injuries, digestive disorders, and dysentery (Abd Wahab, 2023).



Figure 2: Plant parts of *Cyperus mindorensis*

Considering the fundamental role of free radicals and oxidative stress in the onset and progression of multiple chronic and degenerative disorders, there is a growing scientific emphasis on identifying natural, plant-based sources of antioxidants and bioactive compounds that can mitigate such damage. All these ethnomedicinal information strongly suggests the presence of potent bioactive constituents capable of modulating oxidative and metabolic stress pathways and leads us to initiate investigation for evaluating the pharmacological aspect of this particular sedge species which is abundantly available. the wide availability and palatability of *C. mindorensis* (Figure 2), particularly among sedges consumed by wildlife, offers an ecologically relevant and sustainable advantage. Studying this species will not only

contribute to the discovery of novel natural antioxidants and nutraceutical compounds but also can provide valuable insights into the nutritional ecology of herbivores in tropical ecosystems. On the other hand, its use in traditional medicine across various cultures further supports its safety and efficacy for long term application, making it a suitable candidate for integration into modern phytotherapeutic research.

METHODOLOGY

Plants of *Cyperus mindorensis* (Figure 1) were collected from the riparian zones of the Mahanadi River in Cuttack, Odisha, India. Species identification was confirmed using standard regional flora references (Saxena and Brahmam, 1996). After collection, the plant material was thoroughly rinsed under running tap water to eliminate adhering soil and external impurities. This was followed by a final rinse with distilled water.The samples were then shade dried at ambient temperature for approximately seven days to prevent the degradation of thermolabile constituents. Once completely dried (Figure 2), the material was coarsely ground (Figure 3) using a mechanical grinder and stored in sterile, airtight containers until extraction. For solvent extraction, three solvents with differing polarities were used *i.e.* n-hexane (non-polar), ethanol (polar) and distilled water (highly polar). About 5 g of the powdered plant material was soaked in 50 mL of each solvent separately and kept for 48 hours with periodic agitation to facilitate efficient extraction (Devi *et al.,* 2023). The mixtures were then filtered through Whatman No. 1 filter paper. The filtrates were allowed to evaporate at room temperature to obtain the crude extracts, which were preserved in airtight glass vials for further analysis. The crude extracts of *C. mindorensis* were subjected to preliminary qualitative phytochemical screening to identify the presence of primary and secondary metabolites, following standard protocols with slight modifications (Jena *et al.,* 2025). The aqueous extract was further analyzed by Thin-Layer Chromatography (TLC) using three different mobile phase systems as suggested by Eloff et al. (2008). The solvent systems used were:

a) Ethyl acetate: Methanol: Water (40: 5.4: 4) [EMW] — polar neutral system

b) Chloroform: Ethyl acetate: Formic acid (5: 4: 1) [CEF] — intermediate polarity (acidic system)

c) n-Hexane: Petroleum ether: Ethanol (10: 10: 80) [NPE] — non-polar to weakly basic system



Figure 3: Dried plant parts of *C. mindorensis*

After development, the TLC plates were air-dried and sprayed with 0.2% DPPH solution in methanol as a detecting reagent. The appearance of yellow bands against a purple background indicated the presence of antioxidant compounds, and the retention factor (Rf) values were calculated accordingly.

RESULTS AND DISCUSSION

Phytochemical Screening: Phytochemical screening of the whole plant of *C. mindorensis* revealed variation in metabolite distribution across different solvent extracts. Among the primary metabolites, the n-hexane extract showed no detectable compounds, whereas the ethanolic extract contained carbohydrates, and the aqueous extract tested positive for both proteins and carbohydrates, indicating a higher affinity of primary metabolites for polar solvents. For the secondary metabolites, no compounds were detected in the n-hexane extract, while both the ethanolic and aqueous extracts exhibited the presence of tannins, reducing sugars, and low levels of terpenoids, reflecting moderate phytochemical richness in these polar extracts (Table 2).

Table 2: Qualitative phytochemical analysis for detection of metabolites

Category of Metabolites	Extract	Detected Bioactive Compounds	Phytochemical richness
Primary Metabolites	n-hexane	No tested compound detected	◆
	Ethanol	Carbohydrates only	◆
	Aqueous	Protein and carbohydrates	◆
Secondary Metabolites	n-hexane	No tested compound detected	◆
	Ethanol	Tannin, terpenoid (low), Reducing sugars	◆
	Aqueous	Tannin, terpenoid (low), Reducing sugars	◆

(◆ = low, ◆ = moderate, ◆ = high)

Antioxidant activity: TLC-based DPPH analysis further supported the presence of antioxidant components in the aqueous extract. Using the mobile phase ethyl acetate: methanol: water (40: 5.4: 4), a prominent spot was observed at an Rf value of 0.982 along with streaking between 0.344 – 0.62, suggesting multiple phytoconstituents contributing to radical scavenging activity. No visible spots were detected with the mobile phase chloroform: ethyl acetate: formic acid (5: 4: 1), indicating poor separation or absence of detectable antioxidants under this condition. A distinct spot with an Rf value of 0.416 was recorded using n-hexane: petroleum ether: ethanol (10: 10: 80), further confirming the presence of antioxidant-active compounds in the aqueous extract (Table 3).

Table 3: TLC-based DPPH analysis of whole plant extract

	Mobile Phases	Rf value of spots	
		S1	Streak
Aqueous	Ethyl acetate: Methanol: Water (40:5.4:4)	0.982	0.344 – 0.62
	Chloroform: Ethyl acetate: Formic acid (5: 4: 1)	-	-
	n-Hexane: Petroleum ether: Ethanol (10: 10: 80)	0.416	-

(Rf: Retention factor)

Ecological link: From an ecological nutrition perspective, the detection of carbohydrates and proteins in the aqueous extract highlights the potential of *C. mindorensis* as a nutritionally valuable resource for herbivores, including small

mammals and grazing fauna inhabiting riverine ecosystems. Its palatability, combined with its moderate phytochemical richness and antioxidant capacity, suggests that the species may serve a dual ecological function *i.e.*

a) source of nourishment
b) a medicinal plant within natural food webs

CONCLUSION

In seasonally dry and stress prone tropical habitat, plants are continuously exposed to changing moisture levels, high solar radiation, oxidative stress, and microbial pressure. The presence of tannins, reducing sugars, terpenoids and antioxidant-rich compounds in the aqueous fraction strongly suggests that these metabolites may play a protective role in situ by scavenging reactive oxygen species generated during abiotic stress conditions such as drought, heat, and intense light exposure. Furthermore, secondary metabolites such as tannins and terpenoids are widely recognized for their defensive functions against herbivory and pathogenic attack. Their presence acts as a natural chemical barrier, enhancing its survival and competitive ability in riparian and disturbed habitats. This biochemical defense mechanism may also explain its ethnomedicinal effectiveness. Future research related to its compound isolation and characterization may help in development and management of natural antioxidant-rich grasslands for wildlife and in animal husbandry.

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