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Phytochemical analysis and antioxidant activity of *Aeschynomene indica* L. (Fabaceae): A wetland plant of India

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Abstract
Aeschynomene indica L. (Fabaceae) is a wetland-associated herb whose phytochemical profile and antioxidant properties warrant systematic investigation. This study evaluated qualitative phytochemical characteristics of aqueous, ethanol and n-hexane extracts, estimated selected phytochemical groups and assessed the *in vitro* radical-scavenging activity of aqueous and ethanol extracts using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. Qualitative screening detected proteins, carbohydrates, tannins, flavonoids and reducing sugars in aqueous and ethanol extracts; saponins and terpenoids were detected only in the aqueous extract. None of the screened constituents was detected in the n-hexane extract under the reported test conditions. Starch, sucrose, lipids, amino acids, phenolic groups, steroids and alkaloids were reported as not detected. The reported quantitative values were 23.73 mg/100 mL for tannins, 6.51 % for saponins, 2.53 mg/100 mL for phenols and 0.64 mg/mL for flavonoids. DPPH inhibition increased across the tested concentration range (0.0625-1.0 mg/mL), from 52.58 % to 61.41 % for the ethanol extract and from 52.75 % to 61.41 % for the aqueous extract. These findings indicate that *A. indica* contains several phytochemical constituents and exhibits promising *in vitro* radical-scavenging activity, warranting further investigation into its antioxidant potential.

Keywords: *Aeschynomene indica*, DPPH, flavonoids, phytochemical screening, secondary metabolites, tannins

Introduction

Plants are important sources of diverse secondary metabolites with potential biological activities (Wink, 2015; Palazon and Alcalde, 2025) [15, 20]. Among these, phenolic compounds, flavonoids, tannins and other phytochemicals have attracted considerable attention because of their antioxidant properties (Tungmunnithum *et al.*, 2018; Ahmad *et al.*, 2025) [2, 18]. Antioxidants can neutralize free radicals and reactive oxygen species, thereby helping to limit oxidative damage to cellular components (Moon and Shibamoto, 2009) [14]. Consequently, phytochemical screening and antioxidant evaluation have become important approaches for investigating the biological potential of plant-derived extracts (Bisso *et al.*, 2022) [4]. However, *in vitro* antioxidant activity alone does not establish therapeutic efficacy (Huang *et al.*, 2005) [8]. Wetlands are ecologically important ecosystems that support diverse plant species adapted to fluctuating water levels and varying environmental conditions. These conditions may influence plant metabolism and the accumulation of secondary metabolites (Verhoeven and Sorrell, 2010; Isah, 2019) [9, 10]. Therefore, wetland-associated plants represent valuable subjects for phytochemical investigation, particularly for documenting their chemical composition and potential antioxidant properties (Arya *et al.*, 2022) [3]. *Aeschynomene indica* L., (Figure 1) a herbaceous member of the family Fabaceae, is associated with moist habitats, including wetlands and seasonally waterlogged areas (Saxena and Brahmam, 1994) [16]. Although the family Fabaceae is recognized for its diverse secondary metabolites, the phytochemical composition and biological properties of individual species require systematic experimental investigation (Gutiérrez-Durán *et al.*, 2026) [7]. In particular, evaluating the major phytochemical groups and antioxidant activity of *A. indica* may provide useful baseline information on its chemical characteristics (Lei *et al.*, 2019) [12]. Previous research has investigated the essential-oil composition and antioxidant activity of this species, highlighting the importance of defining the specific contribution of studies using different extraction methods and analytical approaches (Feng *et al.*, 2024) [6].

The present study investigated the phytochemical composition and *in vitro* antioxidant activity of *A. indica*, screening for major classes of phytochemicals, estimating the total phenolic and flavonoid contents where applicable and antioxidant activity to be evaluated using appropriate analytical assays. The findings contribute to the phytochemical documentation of Indian wetland flora and provide a foundation for further investigations into the chemical constituents and biological properties of this species.



Fig 1: Habit, habitat and flower of *A. indica*

Materials and methods

The present investigation focused on the whole plant of *A. indica*. Plant sample is collected from Cuttack, Odisha, India and identified by the authors. Soxhlet extraction was carried out using three solvents (aqueous, ethanol and n-hexane) and extracts were used for experimental works (Jena *et al.*, 2025) [10].

Qualitative analysis of primary metabolites: The aqueous, ethanolic and n-hexane extracts of the whole plant were subjected to qualitative phytochemical screening to determine the presence of selected primary metabolites, including proteins, carbohydrates, starch, sucrose, lipids and amino acids. The analysis aimed to identify the major groups of primary metabolites present in the extracts using appropriate qualitative tests (Das *et al.*, 2026) [5]. The results were recorded as positive (+) or negative (−).

Qualitative analysis of bioactive compounds: The aqueous and ethanolic extracts were subjected to preliminary phytochemical screening to investigate the presence of various secondary metabolites and bioactive constituents. The phytochemical groups examined included tannins, saponins, flavonoids, terpenoids, phenolic compounds, reducing sugars, steroids and alkaloids (Marndi *et al.*, 2024) [24].

Quantitative phytochemical analysis: The whole-plant material was quantitatively analyzed for selected secondary metabolites, including tannins, saponins, total phenolic content and total flavonoid content. The results were reported using the respective units specified in the dataset (Soni *et al.*, 2026) [17].

Antioxidant assay: The antioxidant activity of aqueous and ethanolic whole plant extracts of *A. indica* was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The extracts were tested at concentrations ranging from 0.0625 to 1.0 mg/mL. The reaction mixtures were incubated under appropriate conditions, and absorbance was measured spectrophotometrically at 517 nm. A DPPH solution without plant extract served as the control (Kumari *et al.*, 2026; Acharya *et al.*, 2026) [1, 11].

Results and discussion

The qualitative screening of *A. indica* revealed that proteins and carbohydrates were detected in both aqueous and ethanolic extracts, whereas they were not detected in the n-hexane extract (Table 1). Starch, sucrose, lipids and amino acids were not detected in any of the extracts. These findings suggest differences in the extraction of detectable primary metabolites according to solvent properties. However, non-detection does not necessarily indicate the complete absence of these constituents from the plant material. Tannins and flavonoids were detected in both aqueous and ethanolic extracts, while saponins and terpenoids were detected only in the aqueous extract. Reducing sugars were present in both aqueous and ethanolic extracts. Phenolic groups, steroids and alkaloids were not detected in any extract and the n-hexane extract showed negative results for all tested constituents (Table 2).

Table 1: Qualitative phytochemical analysis for primary metabolites of *Aeschynomene indica*

Bioactive compounds	Aqueous Extract	Ethanol Extract	n-Hexane Extract
Protein	+	+	-
Carbohydrates	+	+	-
Starch	-	-	-
Sucrose	-	-	-
Lipid	-	-	-
Amino acid	-	-	-

(+: Detected; -: Not detected)

The comparatively broader phytochemical profile of the aqueous extract suggests that it recovered a wider range of detectable constituents under the experimental conditions. The presence of tannins and flavonoids is particularly relevant because these compounds have been associated with antioxidant properties. However, their contribution to

antioxidant activity requires further quantitative evaluation. The negative result for phenolic groups, despite the detection of tannins and flavonoids, may reflect differences in the sensitivity or specificity of the qualitative tests employed. The findings demonstrate solvent-dependent variation in the detectable phytochemical composition of *A. indica*. Further quantitative analysis and DPPH radical-scavenging assays are necessary to investigate potential relationships between its phytochemical constituents and antioxidant activity.

Table 2: Qualitative phytochemical analysis for secondary metabolites of *Aeschynomene indica*

Bioactive compounds	Aqueous Extract	Ethanol Extract	n-Hexane Extract
Tannin	+	+	-
Saponin	+	-	-
Flavonoid	+	+	-
Terpenoid	+	-	-
Phenolic Group	-	-	-
Reducing sugar	+	+	-
Steroids	-	-	-
Alkaloids	-	-	-

(+: Detected; -: Not detected)

The quantitative phytochemical analysis of *A. indica* revealed the presence of four major secondary metabolite groups (Table 3). Tannin content was recorded at 23.73 mg/100 mL, while saponin content was 6.51 %. The phenolic content was 2.53 mg/100 mL and flavonoid content was 0.64 mg/mL. Tannins showed the highest reported numerical value within their respective units, followed by phenolic compounds and flavonoids. The findings demonstrate the presence of measurable secondary metabolites in A. indica and provide baseline information for further phytochemical investigation. Nevertheless, the relationship between these constituents and antioxidant activity should be assessed by comparing the quantitative phytochemical data with the results of the DPPH assay. The analytical methods, calibration standards, and reporting basis should also be specified to ensure reproducibility and facilitate comparison with other studies.

Table 3: Quantitative phytochemical analysis of *Aeschynomene indica*

Secondary metabolites	Quantity
Tannin	23.73 mg/100mL
Saponin	6.51 %
Phenol	2.53 mg/100 mL
Flavonoids	0.64 mg/mL

The DPPH assay demonstrated antioxidant activity in both ethanolic and aqueous whole plant extracts of *A. indica* (Table 4). The ethanolic extract exhibited an increase in percentage inhibition from 52.58 % at 0.0625 mg/mL to 61.41 % at 1.0 mg/mL. Similarly, the aqueous extract showed an increase from 52.75 % to 61.41% across the same concentration range. Both extracts exhibited a concentration-dependent increase in DPPH radical inhibition, indicating their potential to scavenge free radicals under the experimental conditions. At lower concentrations, the aqueous extract showed slightly higher inhibition than the ethanolic extract, whereas at 0.5 mg/mL, the ethanolic extract showed marginally higher activity (60.08 %) than the aqueous extract (59.91 %). At the highest concentration tested, both extracts exhibited identical inhibition of 61.41 %. The observed antioxidant activity may be associated, in part, with the presence of tannins and flavonoids identified during phytochemical screening, as these compounds can contribute to radical-scavenging activity. However, their specific contributions cannot be established from the available data alone. Both extracts demonstrated comparable DPPH radical-scavenging activity, with inhibition increasing as extract concentration increased.

Table 4: Antioxidant assay of *Aeschynomene indica*

Extract	Concentration (mg/mL)	Inhibition Percentage (%)
Ethanol	0.0625	52.58
	0.125	55.08
	0.25	57.25
	0.5	60.08
	1.0	61.41
Aqueous	0.0625	52.75
	0.125	55.16
	0.25	57.41
	0.5	59.91
	1.0	61.41

Conclusion

The present study investigated the phytochemical composition and *in vitro* antioxidant activity of *Aeschynomene indica* L., a wetland-associated plant of India. Qualitative screening revealed the presence of proteins, carbohydrates, tannins, flavonoids, saponins, terpenoids and reducing sugars in selected extracts. Quantitative analysis further confirmed the presence of tannins, saponins, phenolic compounds and flavonoids. The DPPH assay demonstrated concentration-dependent radical-scavenging activity in both aqueous and ethanolic extracts, with percentage inhibition increasing from 52.58 % to 61.41 % and 52.75 % to 61.41 %, respectively. The comparable results indicate that both extracts exhibited measurable antioxidant activity under the experimental conditions. The detected tannins and flavonoids may contribute to this activity, although their individual roles require further investigation. These findings provide preliminary evidence of the phytochemical diversity and antioxidant potential of *A. indica*. Further studies involving compound identification, validated quantitative assays, appropriate reference antioxidants and statistical analysis are necessary to confirm these findings and explore the biological significance of its constituents. The study contributes to the scientific documentation of Indian wetland flora and provides a foundation for future phytochemical and pharmacological research.

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