

Pharmacological evaluation of *Aristolochia indica* L. (Aristolochiaceae)

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Abstract

Aristolochia indica L. (Aristolochiaceae) is a medicinally important plant. It is traditionally associated with various therapeutic applications. The present study evaluated the primary and secondary metabolites and antioxidant potential of *A. indica* (Whole plant parts) using aqueous, ethanol and n-hexane extracts. Qualitative phytochemical screening revealed the presence of proteins and carbohydrates in the aqueous and ethanol extracts. Among the secondary metabolites, tannins and phenolic groups were detected in both aqueous and ethanol extracts, while saponins and reducing sugars were detected in the aqueous and ethanol extracts. Quantitative analysis recorded tannin at 75.84 mg/100 mL, saponin at 4.77%, phenol at 6.85 mg/100 mL and flavonoids at 0.08 mg/mL. Antioxidant activity was evaluated using the DPPH radical-scavenging assay at concentrations ranging from 0.0625 to 1.0 mg/mL. The ethanol extract showed inhibition ranging from 54.99% to 62.67%, whereas the aqueous extract exhibited inhibition from 52.79% to 66.96%. Both extracts demonstrated increasing DPPH radical-scavenging activity with increasing concentration. The findings indicate that *A. indica* contains several detectable phytochemical constituents and exhibits measurable antioxidant potential, providing preliminary evidence for further phytochemical and pharmacological investigation.

Keywords: Free-radical scavenging, *in-vitro* assay, secondary metabolites

Introduction

Medicinal plants have played an important role in traditional healthcare systems and continue to attract considerable scientific interest in contemporary times (Sofowora *et al.*, 2013; Kanhar *et al.*, 2020; Das *et al.*, 2026) [3, 8, 13]. The growing demand for plant-based healthcare products, natural antioxidants and biologically active compounds has increased the need to scientific evaluation of medicinal plant

resources (El-Saadony *et al.*, 2025) [5]. Phytochemical investigation provides an important preliminary approach for understanding the chemical constituents of medicinal plants and identifying metabolite groups that may contribute to their biological activities (Lulu, 2025) [12]. Such studies are particularly relevant for traditionally used species whose therapeutic potential requires systematic scientific assessment (Tadesse *et al.*, 2025) [15].

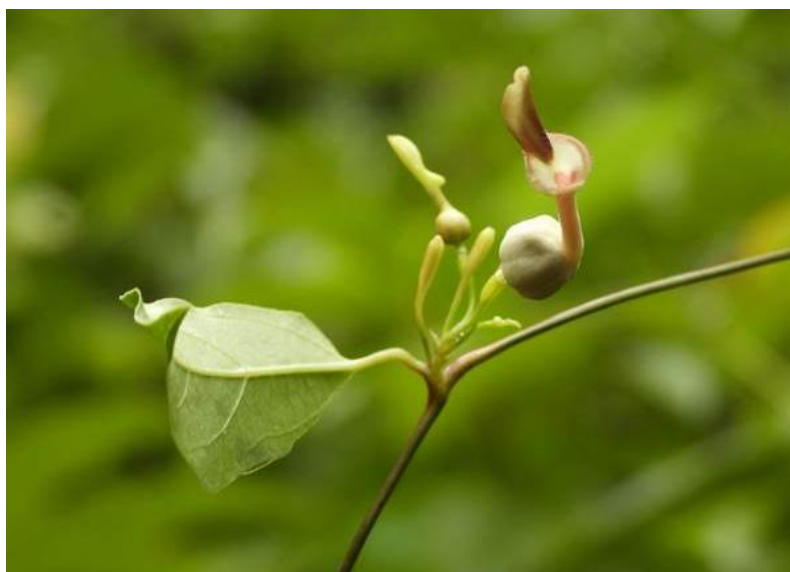


Fig 1: Leaves and flower of *A. indica*

Aristolochia indica L. (Aristolochiaceae) is a perennial medicinal plant (Fig 1) has several medicinal uses (Dey *et al.*, 2021) [4]. Members of the family Aristolochiaceae are recognized for their traditional importance and diverse bioactive compounds (Kuo *et al.*, 2012) [11]. The biological properties of medicinal plants are often associated with secondary metabolites such as tannins, phenolics, saponins, flavonoids,

Terpenoids and alkaloids (Elshafie *et al.*, 2023) [6]. However, the occurrence and detectability of these metabolites may vary depending on the extraction solvent and experimental conditions (Barthwal and Mahar, 2024) [2]. Therefore, comparative phytochemical screening using solvents of different polarities can provide useful baseline information on the chemical profile of a medicinal plant (Khan *et al.*, 2022) [9].



Fig 2: Dried leaves of *A. indica*

Therefore, there is a need to generate scientific baseline data on *A. indica* to support further phytochemical and pharmacological properties. The present study aimed to investigate the primary and secondary metabolites of *A. indica* using aqueous, ethanol and *n*-hexane extracts, quantitatively estimate selected phytochemical constituents, and evaluate its antioxidant potential through the DPPH radical-scavenging assay. The study was intended to provide preliminary scientific information that may serve as a baseline for further phytochemical characterization, pharmacological evaluation and toxicological investigation of the species.

Methodology

Whole plant material of *A. indica* (Fig 1) was collected from Mahanadi River areas, Cuttack, Odisha and used for present experimental works. The plant material was processed to obtain aqueous, ethanol and *n*-hexane extracts (Fig 2). The extracts were screened for selected primary metabolites and secondary metabolites (Soni *et al.*, 2026) [14]. Selected secondary metabolites were quantitatively estimated (Jena *et al.*, 2025) [7]. Antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay (Acharya *et al.*, 2026; Kumari *et al.*, 2026) [1].

Results and Discussion

Qualitative screening of primary metabolites of *Aristolochia indica* showed clear differences among the aqueous, ethanol and *n*-hexane extracts (Table 1; Fig 3). Proteins were detected in both aqueous and ethanol extracts but were not

detected in the *n*-hexane extract. Similarly, carbohydrates and starch were detected in the aqueous and ethanol extracts, whereas they were not detected in the *n*-hexane extract. The occurrence of proteins, carbohydrates and starch predominantly in aqueous and ethanol extracts suggests that these constituents were more readily detectable in relatively polar extraction systems.

Table 1: Qualitative phytochemical analysis of primary metabolites of *A. indica*

Bioactive compounds	Aqueous extract	Ethanol extract	<i>n</i> -Hexane extract
Protein	+	+	-
Carbohydrates	+	+	-
Starch	+	+	-
Sucrose	-	-	-
Lipid	-	-	-
Amino acid	-	-	-

(Note: + = Positive; - = Not detected)

The qualitative secondary metabolite screening revealed the presence of several phytochemical classes in *A. indica* (Table 2; Fig 4). Tannins were detected in both aqueous and ethanol extracts but were not detected in the *n*-hexane extract. Saponins were detected in the aqueous extract (Fig 4) but not in ethanol or *n*-hexane extracts. Phenolic groups were detected in both aqueous and ethanol extracts and were absent from the *n*-hexane extract.



Fig 3: Qualitative phytochemical analysis of primary metabolites of *A. indica* L. using aqueous extract



Fig 4: Qualitative phytochemical analysis of secondary metabolites of *A. indica* L. using aqueous extract

Table 2: Qualitative phytochemical analysis of secondary metabolites of *A. indica*

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

(Note: + = Positive; - = Not detected)

Quantitative analysis of selected secondary metabolites showed that tannin content was 75.84 mg/100 mL, while saponin content was 4.77%. Phenol content was 6.85 mg/100 mL, and flavonoid content was 0.08 mg/mL (Table 3).

Table 3: Quantitative phytochemical analysis of *A. indica*

Secondary metabolites	Quantity
Tannin	75.84 mg/100 mL
Saponin	4.77 %
Phenol	6.85 mg/ 100mL
Flavonoids	0.08 mg/mL

Among the measured constituents, tannin showed the highest numerical value within its reported unit, followed by phenol. Saponin and flavonoid contents were reported using different units; therefore, their numerical values should not be directly compared as relative abundance without conversion to a common basis. The quantitative results provide evidence that measurable levels of selected

phytochemical constituents were present in the investigated plant material. An important observation is that the quantitative analysis reports flavonoids at 0.08 mg/mL despite their negative qualitative screening result. Such differences can occur because qualitative and quantitative assays use different analytical principles, detection sensitivities and experimental conditions. Therefore, the quantitative value should be retained as the measured result, while the qualitative result should specifically be interpreted as the outcome of the preliminary screening test. The same type of discrepancy was discussed in the reference study. The DPPH assay demonstrated measurable radical-scavenging activity in both aqueous and ethanol extracts of *A. indica* (Table 4). The ethanol extract exhibited inhibition of 54.99% at 0.0625 mg/mL, which gradually increased to 62.67% at 1.0 mg/mL. The aqueous extract showed 52.79% inhibition at 0.0625 mg/mL, increasing to 66.96% at 1.0 mg/mL.

Both extracts showed a concentration-associated increase in DPPH inhibition. In the ethanol extract, inhibition increased from 54.99% to 62.67%, representing an increase of 7.68 percentage points. In the aqueous extract, inhibition increased from 52.79% to 66.96%, representing an increase of 14.17 percentage points. At the lowest concentration tested (0.0625 mg/mL), the ethanol extract showed slightly higher inhibition than the aqueous extract. However, from 0.5 mg/mL onward, the aqueous extract demonstrated greater inhibition. At the maximum concentration of 1.0 mg/mL, the aqueous extract showed the highest activity (66.96%) compared with the ethanol extract (62.67%). The concentration-dependent increase suggests that increasing extract concentration resulted in greater DPPH radical-scavenging activity under the experimental conditions (Table 4; Fig 5).

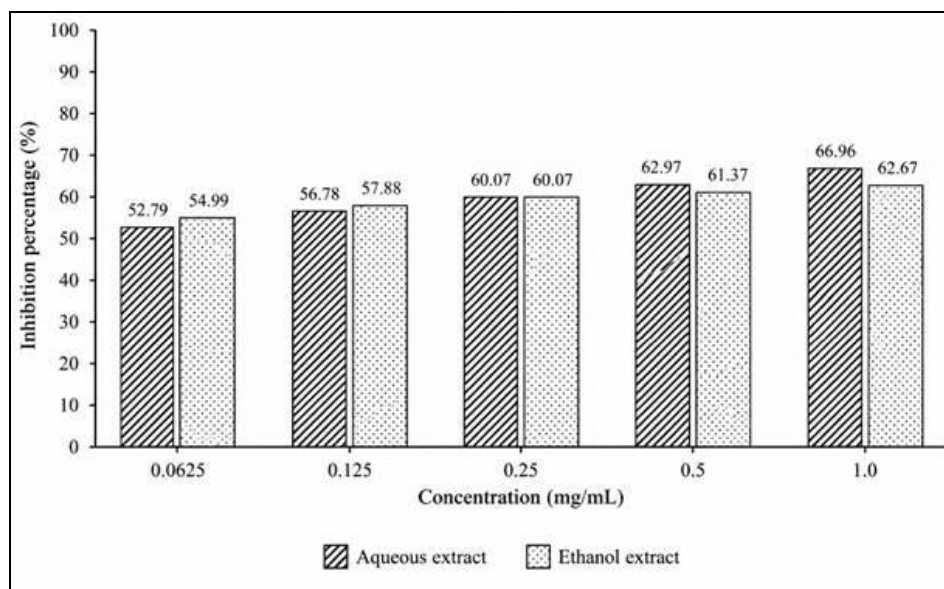


Fig 5: DPPH radical-scavenging activity

Table 4: Antioxidant assay (DPPH) of *A. indica*

Extract	Concentration (mg/mL)	Inhibition Percentage (%)
Ethanol	0.0625	54.99%
	0.125	57.88%
	0.25	60.07%
	0.5	61.37%
	1.0	62.67%
Aqueous	0.0625	52.79%
	0.125	56.78%
	0.25	60.07%
	0.5	62.97%
	1.0	66.96%

Pharmacological significance

The phytochemical profile and DPPH radical-scavenging activity observed in *A. indica* provide preliminary evidence of its biological potential. The detection of tannins, phenolic groups and saponins, together with measurable antioxidant activity, supports further investigation of the species. The aqueous extract showed particularly notable activity at higher concentrations, reaching 66.96% inhibition at 1.0 mg/mL. However, the findings should be regarded as preliminary antioxidant evidence rather than proof of therapeutic efficacy. DPPH activity is an *in-vitro* chemical assay and does not by itself establish biological effectiveness in living systems. Further investigations involving antioxidant assays based on different mechanisms, isolation and characterization of active constituents, antimicrobial or enzyme-inhibition assays, cytotoxicity evaluation, and appropriate *in-vivo* studies would be necessary to substantiate the pharmacological potential of *A. indica*. Importantly, *Aristolochia* species require careful toxicological consideration because some members of the genus are associated with aristolochic acids and related safety concerns. Therefore, any discussion of medicinal or pharmacological applications of *A. indica* should distinguish phytochemical/experimental potential from demonstrated safety and therapeutic usefulness.

Conclusion

The present study demonstrates that *Aristolochia indica* possesses a detectable diversity of primary and secondary

metabolites and measurable antioxidant activity. Proteins, carbohydrates and starch were detected in aqueous and ethanol extracts, whereas sucrose, lipids and amino acids were not detected in any tested extract. Among the secondary metabolites, tannins and phenolic groups were detected in both aqueous and ethanol extracts, while saponins and reducing sugars showed solvent-dependent detection. Quantitative analysis recorded tannin at 75.84 mg/100 mL, saponin at 4.77%, phenol at 6.85 mg/100 mL and flavonoids at 0.08 mg/mL. The DPPH assay demonstrated concentration-associated increases in radical-scavenging activity in both aqueous and ethanol extracts. The aqueous extract showed the highest activity at 1.0 mg/mL (66.96%), whereas the ethanol extract showed 62.67% inhibition at the same concentration. These findings indicate that *A. indica* contains phytochemical constituents associated with measurable antioxidant potential. The present study provides preliminary information supporting further phytochemical and pharmacological investigation of *A. indica*. However, the present results should not be interpreted as evidence of therapeutic efficacy or safety. Detailed chemical characterization, mechanism-based pharmacological studies and toxicological evaluation are required before any medicinal application can be established.

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