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Phytochemical analysis and antioxidant potential of *Arisaema tortuosum* (Wall.) Schott (Araceae)

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Abstract
Arisaema tortuosum was evaluated for its primary and secondary metabolites and antioxidant potential using aqueous, ethanol and n-hexane extracts of the whole plant. Qualitative phytochemical screening revealed the presence of proteins and carbohydrates. Among the secondary metabolites, tannins, terpenoids, phenolic groups, reducing sugars and steroids were detected. Quantitative analysis recorded tannin at 75.84 mg/100 mL, saponin at 13.3%, total phenol at 7.39 mg/100 mL and flavonoids at 0.05 mg/mL. Antioxidant activity was evaluated using the DPPH radical-scavenging assay. The aqueous extract showed inhibition ranging from 57.09% to 61.32%, while ethanol and n-hexane extracts showed ranges of 54.27–60.99% and 55.68–61.41%, respectively, at concentrations of 0.0625–1.0 mg/mL. The results demonstrated measurable DPPH radical-scavenging activity in all three extracts, with activity generally increasing with increasing extract concentration. The findings indicate that *A. tortuosum* contains diverse phytochemical constituents and possesses appreciable antioxidant potential, supporting its potential for further phytochemical and pharmacological investigation.

Keywords: Bioactive constituents, free-radical scavenging, plant extracts

Introduction
Plants are important sources of primary and secondary metabolites (Sharma *et al.,* 2025; Hossain *et al.,* 2025 [6, 21]; Das *et al.,* 2026a). Phytochemical investigation of plants provides useful preliminary information regarding their chemical composition and potential biological properties (Mishra *et al.,* 2025 [13]; Das *et al.,* 2026b). Members of the family Araceae are represented by numerous herbaceous species occurring in tropical and subtropical regions (Sahu *et al.,* 2025) [19]. Several species belonging to this family have attracted attention because of their ethnobotanical, nutritional and medicinal significance (Kumar *et al.,* 2022) [9]. However, the phytochemical characteristics and antioxidant potential of several less-studied Araceae members remain insufficiently documented. *Arisaema tortuosum* (Figure 1) is an herbaceous member of the family Araceae (Rittà *et al.,* 2020) [17]. Like other wild plant species, it may contain a range of primary and secondary metabolites that contribute to its biological activities (Kant *et al.,* 2020 [8]; Qi *et al.,* 2021; Ali and Yaqoob, 2021) [2]. Phytochemical screening can help determine the occurrence of these compounds in extracts prepared using solvents of different polarities (Sasidharan *et al.,* 2011) [20].

Fig 1: Habitat of *A. tortuosum*

The present study was therefore undertaken to evaluate the primary and secondary metabolites of *A. tortuosum* through qualitative phytochemical screening, quantify selected secondary metabolites and assess the antioxidant potential of aqueous, ethanol and n-hexane extracts using the DPPH radical-scavenging assay. The study provides preliminary information on the phytochemical composition and antioxidant potential of *A. tortuosum* and may serve as a basis for further phytochemical and pharmacological investigations.

Methodology

Plant parts of *A. tortuosum* was collected from Mahanadi River areas, Cuttack, Odisha, India. Whole plant material of *A. tortuosum* was extracted with aqueous, ethanol and n-hexane solvents (Jena *et al.,* 2024) [7]. Primary metabolites (proteins, carbohydrates, starch, sucrose, lipids and amino acids) and secondary metabolites (tannins, saponins, flavonoids, terpenoids, phenolics, reducing sugars, steroids and alkaloids) were qualitatively screened using standard phytochemical tests (Kumar *et al.,* 2013; Marndi *et al.,* 2024) [10, 12]. Selected secondary metabolites, including tannins, saponins, total phenols and flavonoids, were quantitatively estimated using standard colorimetric methods (Swain and Hillis, 1959; Sadasivam and Manickam, 1996; Obadoni and Ochuko, 2001 [14, 18, 23]; Chang *et al.,* 2002; Shirazi *et al.,* 2014; Raina and Misra, 2023) [16, 22]. Antioxidant activity was evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging assay at 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/mL concentrations. Absorbance was measured at 517 nm, and activity was expressed as percentage inhibition (Abubakar and Haque, 2020; Baliyan *et al.,* 2022; Majhi *et al.,* 2026) [1, 3, 11].

Results and discussion

Qualitative screening of primary metabolites in the whole plant of *Arisaema tortuosum* revealed differences among the aqueous, ethanol and n-hexane extracts (Table 1). Proteins were detected in the aqueous and ethanol extracts, whereas they were not detected in the n-hexane extract. Carbohydrates showed a strong positive reaction (++) in both aqueous and ethanol extracts but were not detected in the n-hexane extract. Starch was not detected in any of the three extracts. Similarly, sucrose, lipids and amino acids were not detected in the tested extracts. The detection of proteins and carbohydrates in aqueous and ethanol extracts indicates the presence of these important primary metabolites in *A. tortuosum*. The stronger reaction observed for carbohydrates suggests their comparatively detectable presence under the test conditions (Table 1).

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

Primary metabolites	Aqueous extract	Ethanol extract	n-Hexane extract
Protein	+	+	-
Carbohydrates	+ +	+ +	-
Starch	-	-	-
Sucrose	-	-	-
Lipid	-	-	-
Amino acid	-	-	-

(+ = Positive; ++ = Strongly positive; − = Not detected)

In contrast, the absence of these metabolites in the n-hexane extract may be associated with the non-polar nature of n-hexane, which is less effective for extraction of many polar primary metabolites. Similar solvent-dependent differences were observed in the reference study, where proteins, carbohydrates and starch were detected mainly in aqueous and ethanol extracts. Since these are qualitative tests, the results indicate detection or non-detection rather than absolute absence or precise concentration. The qualitative screening of secondary metabolites demonstrated that *A. tortuosum* contains several classes of potentially bioactive constituents (Table 2). Tannins were detected in both aqueous and ethanol extracts but were absent from the n-hexane extract. Saponins were detected only in the aqueous extract. Terpenoids, reducing sugars and steroids were detected in aqueous and ethanol extracts but not in the n-hexane extract. Flavonoids and phenolic groups were not detected in the qualitative screening of any of the three extracts. Alkaloids were also not detected in the tested extracts (Table 2).

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

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

(+ = Positive; − = Not detected)

The aqueous and ethanol extracts exhibited a broader qualitative phytochemical profile than the n-hexane extract. The detection of tannins, saponins, terpenoids, phenolic groups, reducing sugars and steroids indicates the presence of chemically diverse constituents in the whole plant. The absence of several compounds in the n-hexane extract again suggests that solvent polarity plays an important role in the phytochemical profile obtained. The detection of saponins only in the aqueous extract suggests that these constituents were more readily extracted under aqueous conditions. Tannins and terpenoids were detected in both aqueous and ethanol extracts, indicating their extractability in these relatively polar solvents. The absence of alkaloids and flavonoids in the qualitative tests should be interpreted as "not detected under the test conditions" rather than definitive evidence of their complete absence from the plant. Similar caution is appropriate when interpreting qualitative phytochemical screening results. Quantitative analysis of selected secondary metabolites showed that tannin was recorded at 75.84 mg/100 mL, while saponin content was 13.3%. Total phenol was estimated at 7.39 mg/100 mL, and flavonoid content was reported as 0.05 mg/mL (Table 3).

Table 3: Quantitative phytochemical analysis of whole plant parts of *Arisaema tortuosum*

Secondary metabolites	Quantity
Tannin	75.84 mg/100 mL
Saponin	13.3%
Total phenol	7.39 mg/100 mL
Flavonoids	0.05 mg/mL

Among the reported constituents, tannin showed a relatively high measured value of 75.84 mg/100 mL, while saponin was recorded at 13.3%. Total phenol and flavonoid contents were 7.39 mg/100 mL and 0.05 mg/mL, respectively. These findings indicate the presence of several secondary metabolite classes in the whole plant. An important observation is that phenolic groups and flavonoids were recorded as negative during qualitative screening, whereas quantitative analysis produced measurable values for total phenol and flavonoids. This apparent difference may result from differences in detection sensitivity, assay principles or analytical conditions. Therefore, the quantitative results should be retained as measured values, while the qualitative results should be interpreted specifically as the outcome of the preliminary screening tests. The same type of discrepancy was identified and discussed in the uploaded reference paper. The units reported for tannin, saponin, total phenol and flavonoids are different; therefore, their numerical values should not be directly compared as an indication of relative abundance without conversion to a common basis. The DPPH assay demonstrated measurable antioxidant activity in all three extracts of *A. tortuosum* (Table 4). The aqueous extract exhibited inhibition ranging from 57.09% at 0.0625 mg/mL to 61.32% at 1.0 mg/mL. The ethanol extract showed an increase from 54.27% to 60.99%, whereas the n-hexane extract increased from 55.68% to 61.41% over the same concentration range.

Table 4: Antioxidant assay (DPPH) of whole plant parts of *A. tortuosum*

Extract	Concentration (mg/mL)	Absorbance (517 nm)	Inhibition Percentage (%)
Aqueous	0.0625	0.517	57.09%
	0.125	0.504	57.95%
	0.25	0.495	58.92%
	0.5	0.471	60.91%
	1.0	0.466	61.32%
Ethanol	0.0625	0.551	54.27%
	0.125	0.526	56.34%
	0.25	0.503	58.25%
	0.5	0.492	59.17%
	1.0	0.470	60.99%
n-Hexane	0.0625	0.534	55.68%
	0.125	0.504	58.17%
	0.25	0.497	58.75%
	0.5	0.481	60.08%
	1.0	0.465	61.41%

The aqueous extract showed the highest inhibition at the first four concentrations, with values of 57.09, 57.95, 58.92 and 60.91%, respectively. At 1.0 mg/mL, however, the n-hexane extract showed the highest inhibition (61.41%), followed closely by the aqueous extract (61.32%) and ethanol extract (60.99%). Thus, it would be more accurate to state that the aqueous extract showed the strongest activity across most of the tested concentration range, whereas the n-hexane extract showed the highest activity at the maximum concentration. The gradual increase in inhibition with increasing extract concentration indicates a concentration-dependent response within the tested range.

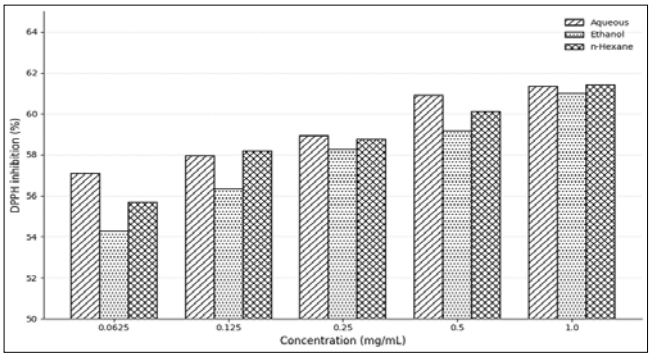

Fig 2: DPPH radical-scavenging activity of *A. tortuosum*

The aqueous extract increased from 57.09% to 61.32%, ethanol from 54.27% to 60.99%, and n-hexane from 55.68% to 61.41%. Such concentration-associated increases suggest that increasing the amount of extract resulted in greater DPPH radical-scavenging activity under the experimental conditions (Figure 2). The antioxidant response may be associated with the presence of phenolic, tannin, saponin, terpenoid and other phytochemical constituents detected in the extracts. However, a direct causal relationship between any individual metabolite and DPPH activity cannot be established from the present data alone. The reference paper similarly emphasizes that stronger DPPH activity can be associated with a broader phytochemical profile but does not establish a direct causal relationship.

Conclusion

The present study demonstrates that *Arisaema tortuosum* possesses a diverse phytochemical profile comprising both primary and secondary metabolites. Proteins and carbohydrates were detected mainly in aqueous and ethanol extracts, while tannins, terpenoids, phenolic groups, reducing sugars and steroids were detected in the aqueous and ethanol extracts, with saponins detected only in the aqueous extract. Quantitative analysis recorded tannin at 75.84 mg/100 mL, saponin at 13.3 %, total phenol at 7.39 mg/100 mL and flavonoids at 0.05 mg/mL. The DPPH assay demonstrated measurable antioxidant activity in aqueous, ethanol and n-hexane extracts, with activity generally increasing with increasing concentration. The aqueous extract showed the highest inhibition at most tested concentrations, while the n-hexane extract showed the highest inhibition at 1.0 mg/mL. These findings indicate that *A. tortuosum* contains potentially bioactive constituents with measurable free-radical-scavenging activity. The study provides useful baseline information for further phytochemical characterization, antioxidant studies and pharmacological investigations of this species.

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