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Original Researcher Article

Sequential Extraction -Driven Phytochemical Mapping of Arisaema Tortuosum Tubers for Secondary Metabolite Screening and Polyphenol Quantification

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Name of Author:

Sourabh Malviya[1], Rashmi Dahima[2], Devashish Rathore[3]

Affiliation: *[1,2,3]School of Pharmacy, Devi Ahilya Vishwavidlaya, Indore, Madhya Pradesh*

Corresponding Author: Sourabh Malviya

Email: srbh19@gmail.com

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Abstracts: Arisaema tortuosum (Wall.) Schott is a medicinally important plant with considerable ethnopharmacological significance, yet its tubers remain inadequately characterized for their phytochemical composition. This study aimed to investigate the phytochemical profile of A. tortuosum tubers by evaluating extractive yield, secondary metabolite composition, and the total phenolic and flavonoid content of different solvent extracts. Sequential solvent extraction with chloroform, ethyl acetate, ethanol, and aqueous solvents produced extractive yields of 2.5%, 2.9%, 6.3%, and 11.2% w/w, respectively. Qualitative phytochemical analysis confirmed the presence of major secondary metabolites such as alkaloids, flavonoids, phenolics, tannins, diterpenes, proteins, carbohydrates, and saponins in different extracts. Among all fractions, the ethanolic extract exhibited the highest total phenolic content (0.878 mg GAE/100 mg) and total flavonoid content (0.805 mg QE/100 mg), suggesting that ethanol is a more suitable solvent for extracting polyphenolic constituents from the tubers. The findings highlight the phytochemical richness of A. tortuosum tubers and suggest their potential importance in future studies aimed at herbal drug standardization and the discovery of bioactive lead molecules, particularly for anticancer applications.

Keywords: Sequential Solvent Extraction, Phytochemical Screening, Total Phenolic Content, Total Flavonoid Content.

1. INTRODUCTION

Medicinal plants continue to be a significant source of medicinal agents, nutraceutical candidates, and chemically varied natural products for contemporary research and traditional medical systems [1]. Their significance in drug research is mostly due to the extensive array of secondary metabolites they produce, many of which exhibit antioxidant, anti-inflammatory, antibacterial, immunomodulatory, and antiproliferative properties [2]. The chemical diversity of medicinal plants renders early phytochemical profiling not only descriptive but also foundational for extract standardization, biological screening, and the subsequent finding of lead molecules [3]. Among

the several medicinal taxa examined for these objectives, Arisaema has garnered increasing interest due to its historical ethnomedicinal significance and extensive phytochemical variety [3]. Reviews of the genus indicate the presence of flavonoids, terpenoids, glycosphingolipids, alkaloids, steroids, lectins, and more elements that may explain its traditional and experimentally reported bioactivities [4]. Different Arisaema species have been used in the past to treat inflammatory diseases, pain, respiratory problems, skin illnesses, and tumors. This has led to further in-depth pharmacognostic and pharmacological research [5]. Arisaema tortuosum (Wall.) Schott is a particularly intriguing medicinal species within this genus since it has transitioned from ethnobotanical significance to experimental validation in many biological models [5]. Research on this plant has demonstrated antiviral, antioxidant, anti-inflammatory, antibacterial, and antiproliferative properties, indicating that the species may possess multiple classes of physiologically relevant phytoconstituents [6]. The increasing evidence renders A. tortuosum an appropriate subject for targeted phytochemical research, particularly for the purpose of establishing baseline data to facilitate standardization and future medication development [7]. One of the most significant finding regarding A. tortuosum is the extraction of a lectin from its tubers, which exhibits anticancer properties against human cancer cell lines.

The report is significant as it illustrates that the tubers possess not just botanical and traditional value but also the potential to produce compounds with biological significance [6]. Subsequent research further investigated A. tortuosum lectin, detailing its carbohydrate-binding properties and antiproliferative capabilities, so strengthening the notion that this species may represent a significant source of lead biomolecules [6]. Studies of leaf extract also demonstrated that it can fight the herpes simplex virus type 2, which adds to the plant's pharmacological profile and shows that bioactivity in this species isn't limited to one tissue or mechanism [8]. In addition to the chromatographic indication of compounds such as quercetin, rutin, luteolin, and lectin, research on A. tortuosum tubers has also reported antioxidant, anti-inflammatory, and antiproliferative activity in the methanolic extract [7]. The therapeutic potential of the species was further supported by a study that examined crude methanolic extract and organic fractions and reported antibacterial, antifungal, phytotoxic, and antioxidant activities [5]. Collectively, these results indicate that A. tortuosum possesses a significant pharmacological profile; however, they also highlight the necessity for further fundamental research on extraction behaviour and comparative phytochemical content, particularly for the tubers. Extraction is crucial to phytochemical analysis since it dictates the compounds that are transported from raw plant material into a useable and analysable fraction. The use of several solvents is especially pertinent in medicinal plant research, as chemicals exhibit significant variability in polarity, solubility, and chemical stability, indicating that a single solvent seldom provides a comprehensive phytochemical overview [9]. The investigation of A. tortuosum is significant as prior studies have demonstrated biological potential; nonetheless, the plant requires a more robust chemical foundation for its tubers. Bioactivity-based studies are important for showing that herbs may have medicinal value, but they don't provide a strong phytochemical basis for quality control on their own. A systematic examination of tuber extracts prepared in different solvents can therefore help bridge the gap between traditional use, preliminary pharmacology, and future standardization [10]. Building upon this sequentially established foundation, the current study was structured around five interrelated stages: identification and collection of the plant, sequential extraction of the tubers in various solvents, initial phytochemical screening of secondary metabolites, quantification of total flavonoid content, and assessment of total phenolic content. The present study intends to create a defined phytochemical baseline for Arisaema tortuosum tubers, find changes in extract composition that depend on the solvent, and provide relevant data for future herbal standardisation and lead-oriented exploration of this medicinal plant by focusing on these processes. This map will then guide a systematic chemical and biological evaluation of the extract's potential against human cancer cell lines, thereby rigorously translating the traditional legacy of Arisaema tortuosum into a validated contribution to modern medicine.

2. MATERIAL AND METHODS

2.1 Reagents and Standards

All chemicals and solvents used were of high analytical grade and purchased from Merck (Darmstadt, Germany) [2].

2.2 Plant material

Fresh tubers of Arisaema tortuosum (Wall.) Schott were harvested from the Raisen Region of Madhya Pradesh, India, in December 2022. The plant was verified by a Botanist Scientist (क्र./एम.एफ.पी/ब.पी./प.अ/2022/2421) at the Minor Forest Produce Processing and Research Centre in Bhopal, Madhya Pradesh (A PSU under Government of Madhya Pradesh, India) [11].

3. EXPERIMENTAL

The complete experimental workflow is illustrated in Figure 1. After identification and collection, the tubers were processed and subjected to sequential extraction using solvents of different polarity in order to recover a broader range of phytoconstituents. The resulting extracts were then screened by standard qualitative tests for the presence of important secondary metabolites commonly associated with medicinal activity. To strengthen the phytochemical evaluation, total flavonoid content and total phenolic content were estimated by widely accepted methods using suitable reference standards. This systematic procedure was adopted to compare the chemical nature of different solvent extracts and to generate baseline data useful for future phytochemical, pharmacological, and standardization studies on A. tortuosum tubers.

Tubers of Arisaema tortuosum were sequentially extracted using solvents of increasing polarity. The resultant extracts underwent preliminary phytochemical screening followed by spectrophotometric determination of total phenolic and flavonoid content. [10]

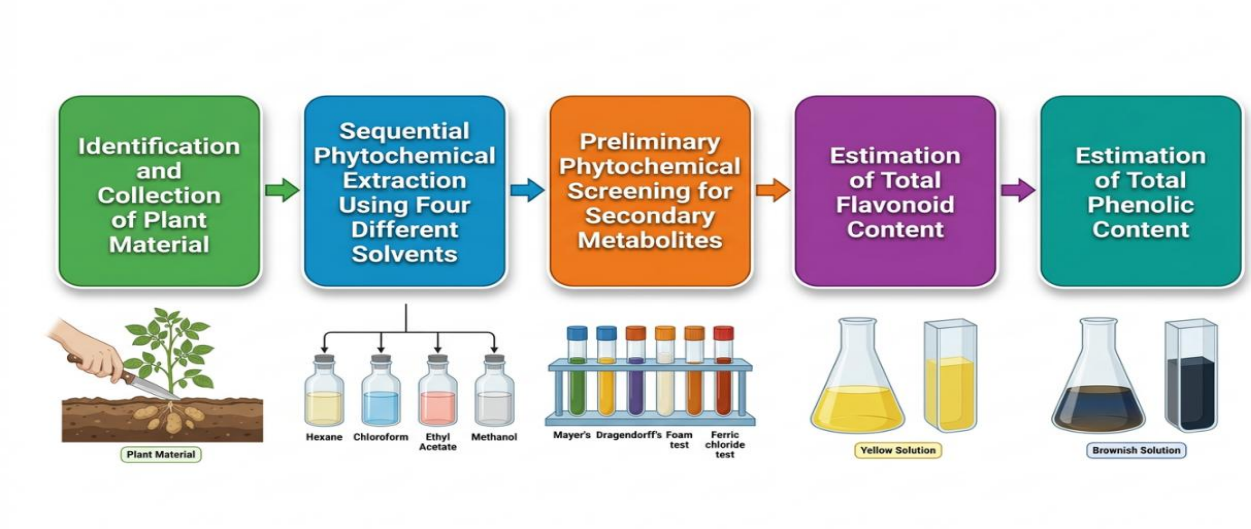

Figure 1: Flowchart illustrating the sequential stages of the research process

3.1 Processing and Preparation of Plant Material

The tubers were thoroughly washed. The cleaning procedure was divided into the following manner: The decayed or deteriorated plant matter was removed first. The samples were then washed with tap and purified water. To extract excess water, the washed plant materials were covered with blotting paper. Plant material was then chopped and shade-dried shortly after washing Figure 2. The primary goal of drying is to extract the water content of the plant for processing. The dried tuber was finely powdered using an electric grinder, sieved and stored in polyethylene bags [12].

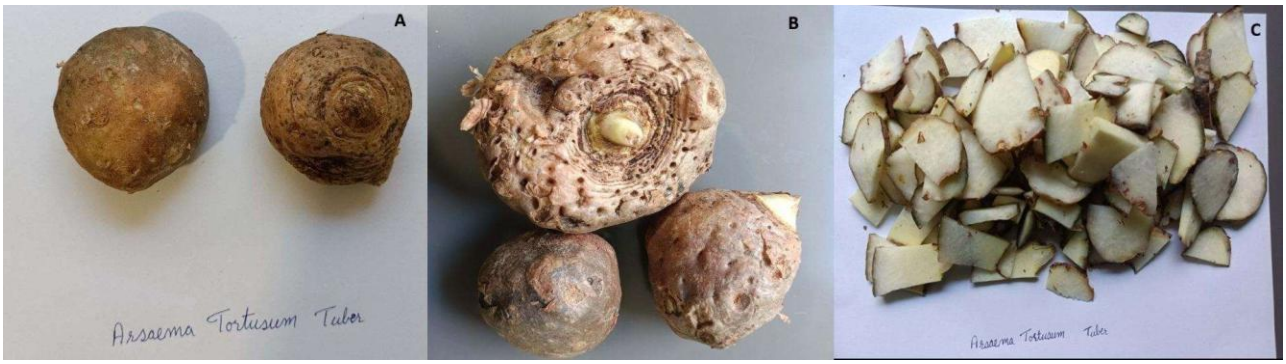

Figure 2: Sequential steps in the preparation of Arisaema tortuosum tubers: (A) Collection of tubers, (B) Cleaning process, and (C) Cutting the tubers into slices for experimental use.

3.2 Extraction by Maceration Process

The Dried powder (40 g) was extracted with different solvents (chloroform, ethyl acetate, ethanol and water) using the sequential maceration technique. The samples were left for 48 hours under sterile environment. The liquid extract was then filtered through Whatman filter paper no. 40. The filtrate was kept in a water bath at 80-90ºC till the extract was dried.

3.3 Biochemical Assays

Preliminary phytochemical screening of all four extracts was conducted to evaluate the presence of various phytochemicals found in plants. The crude extracts were analysed for presence of secondary metabolites, such as alkaloids, phenolic compounds, flavonoids, saponins, tannins, and glycosides.

3.4 Quantitative Analysis of Phytoconstituents

3.4.1 Evaluation of Total Phenol Content

The total phenolic content was determined using the modified Folin Ciocalteu method. 10 mg of Gallic acid was dissolved in 10 ml of methanol, yielding concentration of 10 to 50 µg/ml. 10 mg of dried extract was solubilized in 10 ml of methanol and filtered. 2 ml (1 mg/ml) of this extract was used for the quantification of phenol. The extract and each standard (2 ml) were mixed with 1 ml of Folin Ciocalteu reagent (diluted with distilled water at a 1:10 v/v ratio) and 1 ml (7.5 g/l) of sodium carbonate. The mixture was vortexed for 15 seconds and allowed to stand for 10 minutes to facilitate colour development. Absorbance was taken at 765 nm with a spectrophotometer.

3.4.2 Evaluation of Total Flavonoid Content

The total flavonoid content was evaluated utilizing the aluminium chloride method. 10 mg of Quercetin was dissolved in 10 ml of methanol, and several aliquots ranging from 5 to 25 µg/ml were produced in methanol. Ten milligrams of the desiccated extract was solubilized in 10 millilitres of methanol and filtered.

3 ml (1 mg/ml) of the extract was utilized for quantification of flavonoids. One millilitre of 2% AlCl3 solution was combined with three millilitres of extract or each standard and permitted to stand for 15 minutes at ambient temperature, and absorbance was recorded at 420 nm**.**

4. RESULTS AND DISCUSSION

4.1 Solvent-Based Extraction Yields of Plant Components

The present study provides an organised phytochemical analysis of Arisaema tortuosum tubers and demonstrates that solvent polarity has a significant impact on chemical composition and extractive yield. The aqueous extract provided the largest yield (11.2% w/w), followed by ethanol (6.3% w/w). In contrast, ethyl acetate and chloroform gave substantially lower yields, suggesting that a significant amount of tuber contents are polar in nature. The percentage yield obtained from each solvent extract is summarized in Table 1. However, a higher extractive yield did not necessarily correspond to a higher concentration of phenolic and flavonoid constituents. Although aqueous extract contains the highest total mass but the ethanolic fraction contained the highest total phenolic content (0.878 mg GAE/100 mg) and total flavonoid content (0.805 mg QE/100 mg), suggesting that ethanol fraction was more effective in concentrating polyphenolic compounds of probable biological relevance.

Table 1: Percentage yield of extract of tubers of Arisaema tortuosum

S. No.	Extract	% Yield (W/W)
1.	Chloroform	2.5%
2.	Ethyl acetate	2.9%
3.	Ethanol	6.3%
4.	Aqueous	11.2%

4.2 Biochemical Profiling of Secondary Metabolites in Plant Extracts

A series of qualitative biochemical assay was conducted to ascertain the presence of principal categories of secondary metabolites in various solvent extracts as shown in Figure 3 .

Figure 3: Qualitative phytochemical screening of different solvent extracts of Arisaema tortuosum tubers showing colour reactions in standard biochemical tests for secondary metabolites: (A) Aqueous extract, (B) Ethanolic extract, (C) Chloroform extract, and (D) Ethyl acetate extract

The evaluation included the detection of alkaloids, glycosides, flavonoids, diterpenes, phenolic compounds, proteins, carbohydrates, saponins, and tannins by established phytochemical methodologies. The patterns of positive and negative reactions varied across the extracts, reflecting differences in the solubility and distribution of these metabolites. A complete summary of the outcomes for each test and extract is provided in Table 2.

Table 2: Result of phytochemical screening of extract of Arisaema tortuosum

S.No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous Extract
1.	**Alkaloids** Wagner's Test: Hager's Test:	 -ve -ve	 +ve -ve	 +ve +ve	 -ve +ve
2.	**Glycosides** Legal's Test:	 -ve	 -ve	 -ve	 -ve
3.	**Flavonoids**	 -ve	 -ve	 -ve	 -ve

	Alkaline Reagent Test: Lead acetate Test:	+ve	+ve	+ve	+ve
4.	**Diterpenes** Copper acetate Test:	-ve	-ve	+ve	+ve
5.	**Phenol** Ferric Chloride Test: Folin Ciocalteu Test:	-ve +ve	-ve +ve	+ve +ve	+ve +ve
6.	**Proteins** Xanthoproteic Test:	+ve	-ve	+ve	+ve
7.	**Carbohydrate** Fehling's Test: Benedict's Test:	-ve -ve	-ve -ve	+ve +ve	-ve -ve
8.	**Saponins** Froth Test:	-ve	-ve	+ve	-ve
9.	**Tannins** Gelatin test:	+ve	+ve	+ve	+ve

The qualitative phytochemical screening strengthens this interpretation. Ethanol exhibited the most extensive and significant phytochemical profile, yielding positive results for alkaloids, flavonoids, diterpenes, phenolics, proteins, carbohydrates, saponins, and tannins. In contrast, the aqueous extract retains several significant polar constituents, including phenolics, alkaloids, diterpenes, proteins, and tannins.

The comparatively lower yields in chloroform and ethyl acetate fractions indicate that non-polar and moderately polar components are present in lesser amounts in the tubers. However, these extracts were not chemically inert, since certain categories, such as tannins, phenolic compounds, and specific alkaloidal or protein-related responses, had been recognised, demonstrating that the phytochemical content of the tuber is varied and dispersed across different solvent systems.

4.3 Estimation of Total Flavonoid Content and Total Phenolic Content

Total flavonoid content and phenolic content expressed as quercetin equivalent and gallic acid equivalent, respectively, arrived at from the standard calibration line Eqns. $y = 0.036x + 0.015$; $R^2 = 0.999$ and $y = 0.014x - 0.013$; $R^2 = 0.999$ represented in Figure 4.

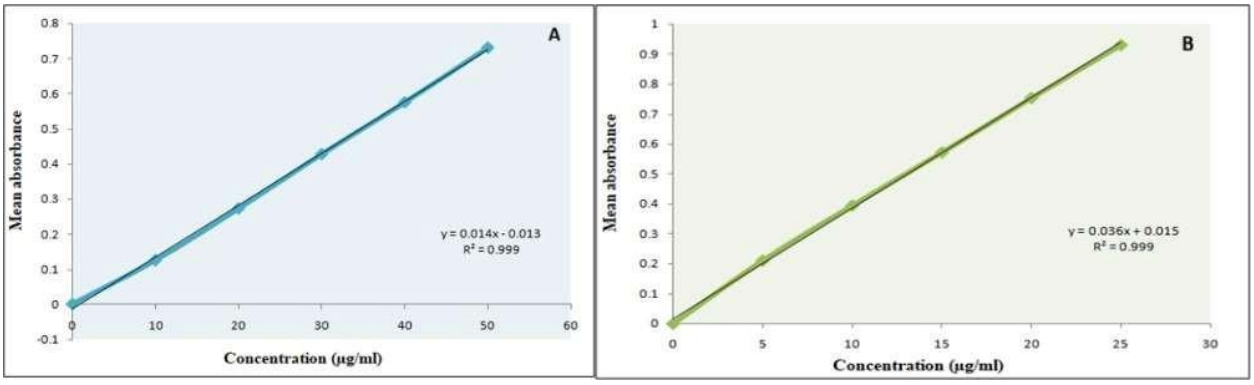

Figure 4 :Calibration curve of Gallic acid and Quercetin.

The ethanolic extract of Arisaema Tortuosum contained the highest total flavonoid content (0.805mg/100 mg) and total phenolic content (0.878mg/100 mg) as compare to other extract as shown in Table 3.

Table 3: Total Phenolic and Total flavonoid content of different extract of Arisaema Tortuosum

S.NO	Extract	Total phenolic content (mg/100 mg)	Total flavonoid content (mg/100 mg)
1.	Chloroform	0.392	0.552
2.	Ethyl acetate	0.485	0.422
3.	Ethanol	0.878	0.805
4.	Aqueous	0.671	0.594

A significant discovery of the study is that ethanol offered an improved proportion between extraction efficiency and the enrichment of bioactive phytoconstituents. The significance of this is highlighted by the frequent use of phenolic and flavonoid compounds as preliminary markers in herbal standardisation studies; their elevated concentrations in the ethanolic extract indicate that this fraction more appropriate for subsequent chromatographic profiling, marker-based standardisation, and biological screening.

The findings further enhance the therapeutic significance of A. tortuosum tubers by establishing a foundational chemical profile that corresponds with the plant's previously acknowledged pharmacological potential outlined in the study. The current data indicate that future research should focus on the ethanolic extract for fractionation, extraction of active compounds, and connection of phytochemical richness with models of antioxidant, antiproliferative, or other bioactivities.

CONCLUSION

The present study shows that Arisaema tortuosum tubers contain a diverse range of phytoconstituents, and that their extraction is strongly influenced by the polarity of the solvent used. Although the aqueous extract produced the highest overall yield, the ethanolic extract demonstrated greater phytochemical significance by showing higher total phenolic and total flavonoid content along with a broader and more promising phytochemical profile. These findings suggest that ethanol is a more suitable solvent for obtaining a phytochemically enriched fraction from the tubers and may serve as the preferred extract for further investigation.

The findings of the present investigation establish a preliminary phytochemical baseline for A. tortuosum and support its relevance for future standardization and biological evaluation. However, the study is limited by its reliance on preliminary screening methods and nonspecific spectrophotometric estimations. Advanced studies based on chromatographic analysis, compound isolation, and bioactivity-guided assessment will be essential to translate these initial findings into stronger evidence for therapeutic application.

Declaration of Financial Support

No financial support was received from governmental/non-governmental body for this study.

Declaration of Competing Interest

No conflict of interest is declared by the authors.

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