

Pharmacognostic Evaluation and Thin Layer Chromatographic Fingerprint of *Aristolochia albida* Rhizome(Aristolochiaceae)

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ABSTRACT

Background: *Aristolochia albida* is widely used in traditional medicine for inflammatory conditions. Establishing pharmacognostic standards and chromatographic fingerprints is essential for quality control and safe use.

Objectives: To evaluate Physicochemical parameters, extractive values, Phytochemical constituents, and establish TLC fingerprints of *A. albida* rhizome.

Methods: Standard WHO Pharmacognostic methods were used to determine moisture content, ash values, and extractive values. Sequential Fractionation with Ethyl acetate, n-Hexane from the crude Methanol extract was performed. Preliminary Phytochemical screening was conducted, and TLC profiling was carried out using silica gel 60 F254 20 x 20cm plates with appropriate solvent systems and visualizing reagents.

Results: Moisture content (10.44±0.44%), total ash (9.33±0.09%), acid-insoluble ash (1.24±0.04%), water-soluble ash (8.09±0.05%), alcohol-soluble extractive (21.00±0.49%), and water-soluble extractive (29.40±0.39%) were established. Methanol extract showed the highest yield (≈25%) and the richest Phytochemical profile. TLC fingerprinting revealed distinct spots with Rf values confirming Flavonoids, steroids, Triterpenoids, and Phenolic compounds.

Conclusion: The generated Pharmacognostic parameters and TLC fingerprints can serve as reference standards for identification, authentication, and quality control of *A. albida* rhizome.

Keywords: *Aristolochia albida*, Pharmacognosy, TLC fingerprint, Physicochemical parameters

The therapeutic value and safety of herbal medicines depend largely on the authenticity, purity, and uniformity of the plant materials employed. Differences in species identity, geographical location, harvesting period, storage conditions, and processing methods can markedly alter the chemical composition of herbal drugs, highlighting the necessity for reliable quality control procedures (2,3).

Standardization of medicinal plants commonly involves phytochemical screening and chromatographic fingerprinting techniques. Preliminary phytochemical investigations provide insight into the classes of secondary metabolites present, whereas chromatographic methods such as thin-layer chromatography (TLC) produce characteristic chemical fingerprints that assist in detecting adulteration and ensuring batch-to-batch consistency (4,5). Because of its operational simplicity, affordability, and reproducibility, TLC remains a particularly useful analytical approach in resource-limited laboratory environments (5,6).

The genus *Aristolochia* (family Aristolochiaceae) includes numerous species traditionally used for managing inflammatory conditions, infections, and pain (7). Phytochemical studies of different *Aristolochia* species have revealed a wide range of secondary metabolites, including flavonoids, lignans, terpenoids, and alkaloids (7, 8). Nevertheless, safety concerns associated with aristolochic acids reported in some members of the genus emphasize the importance of thorough chemical characterization and quality assessment of plant materials derived from *Aristolochia* species (8, 9).

Aristolochia albida, a perennial climbing plant widely distributed in West and Central Africa, is traditionally utilized for several medicinal purposes, particularly in the treatment of inflammatory disorders (7). Despite its extensive ethnomedicinal use, comprehensive phytochemical profiling and chromatographic fingerprint data for the rhizomes of *A. albida* remain limited.

I. INTRODUCTION

Medicinal plants continue to play a fundamental role in traditional healthcare systems worldwide and remain important sources of lead compounds for modern drug development (1,2).

Establishing such reference standards is essential for proper identification, quality assurance, and the advancement of subsequent pharmacological and toxicological studies (2, 10).

Therefore, the present study aimed to investigate the phytochemical composition of *A. albida* rhizome extracts and to generate reproducible TLC fingerprints that may serve as reference markers for authentication and quality control of the plant material.

II. MATERIALS

Plant Material and Authentication

The rhizomes of *Aristolochia albida* were collected based on ethnomedicinal use for inflammatory conditions. Botanical authentication was carried out at the Herbarium, Department of Biological Sciences, Ahmadu Bello University, standard World Health Organization guidelines (2). These included determination of moisture content (loss on drying), total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, and water-soluble extractive value (2, 10). All determinations were carried out in triplicate, and results were expressed as mean \pm standard deviation in accordance with recommended Pharmacognostic analytical procedures (3, 12).

b. Extraction Procedure

Five hundred grams of the powdered rhizome were successively extracted by cold maceration using 2.5 L of 70% methanol to obtain the crude methanol extract, following standard extraction procedures (4,10). The marc was subsequently subjected to successive extraction with ethyl acetate and n-hexane using 2.5 L of each solvent to ensure exhaustive extraction of Phytoconstituents with different polarities (10, 13). Each extraction step was followed by filtration, and the filtrates were concentrated to dryness on a water bath as recommended for solvent removal (4). The dried extracts were stored in desiccators until further use and coded as hexane extract (HE),

Zaria, Nigeria, where a voucher specimen was deposited for future reference.

Preparation of Plant Material

Fresh rhizomes were washed to remove adhering soil and foreign materials, shade-dried at room temperature to prevent degradation of heat-labile constituents, and pulverized into coarse powder using a mechanical grinder following standard Pharmacognostic procedures (2,10). The powdered material was stored in airtight containers to protect it from moisture, light, and contamination until required for analysis (10, 11)

III. METHODS

a. Pharmacognostic Evaluation

The powdered rhizome was evaluated for physicochemical parameters following ethyl acetate extract (EA), and methanol extract (ME).

c. Preliminary Phytochemical Screening

Standard qualitative Phytochemical tests were carried out on HE, EA, and ME to detect the presence of major classes of secondary metabolites including Flavonoids, Tannins, Saponins, alkaloids, steroids, Triterpenoids, Carbohydrates, and Phenolic compounds (4).

d. Thin-Layer Chromatographic (TLC) Analysis

TLC profiling of the extracts was performed on silica gel 60 F₂₅₄ pre-coated plates using standard chromatographic procedures (6,14). Developed plates were visualized under UV light (254 and 365 nm) and sprayed with p-anisaldehyde-sulfuric acid, aluminum chloride, and Liebermann-Burchard reagents for visualization of phytochemical constituents (5,6). Retardation factor (R_f) values were calculated and recorded according to established TLC methods (14).

e. Statistical Analysis

Data were expressed as mean \pm standard deviation.

IV. RESULTS

Table 1a : Physicochemical Parameters of *A. albida* Rhizome Powder

Parameter	Value (% w/w \pm SEM)
Moisture content	10.44 \pm 0.44
Total ash	9.33 \pm 0.09
Acid-insoluble ash	1.24 \pm 0.04
Water-soluble ash	8.09 \pm 0.05
Alcohol-soluble extractive	21.00 \pm 0.49
Water-soluble extractive	29.40 \pm 0.39

Table 2b: Mass and Percentage Yield of Extracts

Extract	Colour	Mass (g)	Yield (% w/w)
n-Hexane	Yellowish	9,88	1.98
Ethyl acetate	Greenish	17.0	3.40
Methanol	Dark brown	50.0	25.0

c. Preliminary Phytochemical Screening

Steroids and Triterpenoids were detected in all extracts, while Flavonoids and Phenolic

compounds were predominantly observed in the crude methanol extract, indicating a higher abundance of polar bioactive constituents.

Table 3ca: Preliminary Phytochemical screening of Aristolochia albida rhizome extracts

Phytochemical constituent	n-Hexane extract	Ethyl acetate extract	Methanol extract
Flavonoids	–	+	+++
Phenolic compounds	–	+	+++
Tannins	–	–	++
Saponins	–	–	+
Alkaloids	–	+	++
Steroids	+	+	++
Triterpenoids	+	+	++

(+ = present; ++ = moderately present; +++ = abundantly present; – = absent)

d. Fingerprint Profile

The crude methanol extract developed in ethyl acetate:chloroform:methanol:water (15:8:8:1) produced multiple well-resolved spots (8) with Rf values ranging from 0.8 to 4.4 while Hexane extract and Ethyl acetate extracts in Hexane: Ethyl-

acetate (2:1) and(9:1) respectively produced cleared spots with Rf values respectively following spray with p-anisaldehyde–sulfuric acid and visualization under UV light (365 nm), suggesting the presence of Flavonoids, Phenolics, and Terpenoid compounds.

Table 4.d.1: TLC fingerprinting of Aristolochia albida rhizome extracts

Extract	Solvent system	Number of spots	Rf range
n-Hexane	Hexane : Ethyl acetate (9:1)	7	1 - 6.5
Ethyl acetate	Hexane :Ethyl acetate (2:1)	6	0.7- 4.0
Methanol	Ethylacetate :Chloroform : Methanol:Water (15:8:8:1)	8	0.8- 4.4

Table 5.d.2: TLC fingerprinting of Aristolochia albida rhizome extracts with Lieberman Bu-chard spraying reagents

Extract	Solvent system	Number of spots	Color	Rf range
n-Hexane	Hexane : Ethyl acetate (9:1)	5	Green, pink,green, pink, light green	0.11 -0.28
Ethyl acetate	Hexane :Ethyl acetate (2:1)	4	Green, violet, violet, violet	1.3 - 5.3
Methanol	Ethylacetate :Chloroform : Methanol:Water (15:8:8:1)	3	Purple,green, purple	2.0 - 5.3

Table 6.d.3: TLC fingerprinting of Aristolochia albida rhizome extracts sprayed with Ferric Chloride reagent

Extract	Solvent system	Number of spots	Color	Rf range
n-Hexane	Hexane : Ethyl acetate (9:1)	0	-	
Ethyl acetate	Hexane :Ethyl acetate (2:1)	0	-	
Methanol	Ethylacetate :Chloroform : Methanol:Water (15:8:8:1)	2	Yellow, yellow	1.0 2.3

V. DISCUSSION

The establishment of Pharmacognostic parameters for *Aristolochia albida* rhizome is essential for correct identification and quality control, particularly in view of its widespread Ethnomedicinal use. Moisture content is a critical factor affecting crude drug stability, as excessive moisture promotes microbial growth and enzymatic degradation (2,3). The observed moisture content falls within acceptable limits, indicating reduced susceptibility to deterioration.

Ash values provide estimates of inorganic matter present in plant materials, and the low acid-insoluble ash value suggests minimal contamination with siliceous materials (3,12). These parameters are widely recommended for herbal drug authentication and purity assessment (2).

Extractive values varied according to solvent polarity, with methanol producing the highest yield. Polar solvents are known to efficiently extract Phenolic and Flavonoid compounds from medicinal plants (4). The predominance of these compounds in the methanol extract supports its suitability for pharmacological evaluation, as they are associated with antioxidant and anti-inflammatory activities (15, 16).

TLC profiling provided a characteristic fingerprint for *A. albida* rhizome, which can serve as a reference tool for authentication and adulteration detection. TLC fingerprinting is widely recommended for standardization and quality evaluation of herbal drugs and formulations (2, 17).

VI. CONCLUSION

This study established reproducible Pharmacognostic standards and TLC fingerprint profiles for the rhizome of *Aristolochia albida*. The physicochemical parameters, extractive values, Phytochemical composition, and chromatographic characteristics generated provide reliable reference data for identification, authentication, and quality control. These findings support the rational use of *A. albida* in traditional medicine and provide a

scientific foundation for further Phytochemical and pharmacological investigations

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