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Phytochemical Analysis and Pharmacognostic Evaluation of Selected Medicinal Plants from the *Acanthaceae* Family found in North Bihar, India

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Abstract: This study aimed to conduct a comprehensive phytochemical analysis and pharmacognostic evaluation of three medicinal plants from the Acanthaceae family abundantly found in North Bihar, India - Adhatoda vasica, Andrographis paniculata, and Hygrophila auriculata. Macroscopic and microscopic characteristics of leaves were examined. Physicochemical parameters like ash values, extractive values, and moisture content were determined. Preliminary phytochemical screening was performed on different solvent extracts. HPLC analysis was carried out to identify and quantify major bioactive compounds. The pharmacognostic features provide useful information for authentication and standardization of these plant materials. Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins, saponins and other constituents. HPLC confirmed the presence of vasicine in A. vasica, andrographolide in A. paniculata, and lupeol in H. auriculata as major compounds. The free radical scavenging potential of crude extracts were also examined and significant scavenging activity exhibited by these plants confirms their antioxidant potential. The abundance of phytochemical compounds indicates that these plants contain high levels of medicinal compounds which acts synergistically and can be widely utilized for the extraction of natural compounds. This study provides valuable data to establish quality control parameters for these important medicinal plants of the Acanthaceae family.

Keywords: Acanthaceae, North Bihar, Pharmacognosy, Phytochemicals

Introduction

The importance of medicinal plants extends beyond traditional and modern medicine, encompassing biodiversity conservation, sustainable development, and cultural preservation (Theodoridis et al., 2023). Research in medicinal plant drug

discovery has evolved into a multifaceted approach, combining botanical, phytochemical, biological, and molecular techniques to identify and develop new leads against various pharmacological targets, including cancer, HIV/AIDS, Alzheimer's disease, malaria, and pain (Balunas and Kinghorn et al., 2005). The potential of medicinal plants in addressing global health challenges is significant, with ongoing research focusing on their application in areas such as antimicrobial resistance, chronic diseases, and emerging infectious diseases. Medicinal plants play a crucial role in primary health care strategies, contributing to maternal and child health care, essential drug provision, nutrition, and the management of common illnesses and endemic diseases. The integration of traditional medicine and medicinal plant knowledge into modern healthcare systems presents both opportunities and challenges, including issues of quality control, standardization, and sustainable sourcing (Rizvi et al., 2022). As the global community faces increasing health challenges and the need for sustainable healthcare solutions, the importance of medicinal plants is likely to grow, necessitating continued research, conservation efforts, and policy support to harness their full potential in improving human health and well-being (Chen et al., 2016; Saravanan et al., 2024)

The *Acanthaceae* family, commonly known as the Acanth family, is a diverse group of flowering plants comprising approximately 250 genera and 4,000 species. This family thrives in various tropical and subtropical habitats across the globe, with major distribution centers in Indonesia and Malaysia, Africa, Brazil, and Central America (Mahendra , 2020) *Acanthaceae* plants exhibit a wide range of growth forms, including annual and perennial herbs, shrubs, and climbing vines. Some species even develop into small trees. The family is characterized by simple, opposite leaves without stipules, typically with entire margins. Stems are often quadrangular, and many species feature distinctive calcium carbonate deposits called cystoliths, visible as streaks on the leaf surface. The inflorescences of *Acanthaceae* plants are diverse, appearing as spikes, racemes, or cymes. Flowers are often large and showy, subtended by colorful bracts. A key feature of the family is the loculicidal capsule fruit, which in many species dehisces explosively to disperse seeds (Simpson, 2010)

Notable genera within the *Acanthaceae* family include Acanthus, Justicia, Thunbergia, Ruellia, and Barleria. Many species are valued in horticulture for their attractive flowers, brightly colored bracts, or variegated foliage. *Acanthaceae* plants have significant ethnobotanical importance, with various species used in traditional medicine and as ornamentals. Ongoing research continues to explore the potential applications and cultural significance of these plants across different regions (Mahendra , 2020 ; Maisarah et al., 2020)

North Bihar region of India is rich in biodiversity and there are several ethinic people residing in this region region who uses plant for medicinal purpose (Khare , 2007 ; Kumar et al., 2020). Despite their widespread traditional use, comprehensive pharmacognostic and phytochemical studies on these plants from North Bihar region of India are lacking in the literature. Proper identification and quality evaluation of medicinal plant materials is crucial to ensure their efficacy and safety. Hence, the present study was undertaken with the objectives to carry out detailed pharmacognostic evaluation of leaves of the selected plants including macroscopic and microscopic characteristics and to perform preliminary phytochemical screening of different solvent extracts. The data generated from this study will be useful for proper identification, authentication and standardization of these important medicinal plants. It will also provide scientific basis for their traditional medicinal uses and aid in development of quality control parameters.

Materials and Methods

Plant Material

In the present study, we have selected three plants with established medicinal importance cited in the literature. Fresh leaves of *Adhatoda vasica*, *Andrographis paniculata* and *Hygrophila auriculata* were collected from different locations in North Bihar, India during March-October 2023. The plant materials were authenticated at University Department of Botany, Lalit Narayan Mithila University, Darbhanga, Bihar, India and voucher specimens were deposited in the Departmental herbarium. The leaves were washed, shade dried and powdered for further analysis.

Macroscopic and Organoleptic Evaluation

The macroscopic features of fresh leaves were examined. Organoleptic characters like color, odor, taste and texture were evaluated as described earlier (Claeson et al., 2000 ; Jayakumar et al., 2013). Transverse sections of fresh leaves were taken using a sharp blade and stained with phloroglucinol and hydrochloric acid. The sections were observed under microscope (Olympus) and characteristic features were noted. Powder microscopy was also performed.

Physicochemical Analysis

Various physicochemical parameters like total ash, acid insoluble ash, water soluble ash, alcohol soluble extractive value, water soluble extractive value and moisture content were determined as per standard methods (Jayakumar et al., 2013; Harborne., 1998)

Preparation of Solvent Extract

Fresh, healthy plant materials were thoroughly cleaned, cut into small pieces, and initially air-dried in the shade for several days. To ensure complete removal of moisture, the samples were subsequently dried in a hot air oven. The fully dried

plant materials were then finely powdered using a mechanical grinder. The resulting powder was stored in a desiccator at room temperature until further use. For the preparation of methanolic extracts, 2 grams of the dried plant powder were mixed with 20 milliliters of methanol. The mixture was allowed to stand for extraction, after which it was filtered through Whatman filter paper to obtain a clear extract. These extracts were then subjected to preliminary phytochemical screening for the detection of compounds such as flavonoids, tannins, saponins, proteins, phenols, and carbohydrates. For quantitative phytochemical analysis, 5 grams of the powdered sample were extracted with methanol using a Soxhlet apparatus. The collected extracts were concentrated by evaporating the solvent under reduced pressure with a rotary evaporator (Harborne, 1998).

Estimation of Protein Content

The total protein content was determined using Bradford's assay (Bradford, 1976). In this method, 100 μL of the plant extract was combined with 3 mL of Bradford reagent and incubated in the dark for 5 minutes to allow for color development. The absorbance of the resulting blue complex was measured at 595 nm using a spectrophotometer. A standard calibration curve was generated using bovine serum albumin (BSA) solutions ranging from 0.1 to 0.5 mg/mL. The protein concentration in the samples was calculated from this standard curve and expressed as mg of protein per mL

Estimation of Carbohydrate Content

The total carbohydrate content was estimated using the phenol-sulfuric acid method (Dubois et al., 1956). In this procedure, 0.1 g of the plant sample was vortexed with 5 mL of 2.5 N hydrochloric acid and heated in a boiling water bath for 3 hours. After cooling to room temperature, the hydrolysate was neutralized with sodium carbonate until effervescence ceased. The total volume was then adjusted to 100 mL with distilled water and centrifuged at 10,000 rpm for 10 minutes. An aliquot of 0.2 mL of the supernatant was diluted to 1 mL in two separate test tubes. To each tube, 1 mL of phenol was added, followed by 5 mL of 96% sulfuric acid. The mixture was shaken thoroughly and allowed to stand for 10 minutes, then incubated in a water bath at 25–30 °C for 20 minutes. The absorbance was measured at 490 nm using a spectrophotometer. Carbohydrate content was determined by comparison with a standard calibration curve prepared using glucose or another suitable carbohydrate standard.

Preliminary Phytochemical Screening

The preliminary phytochemical compounds were analysed using the filtered leaf extracts of the plants following the standard protocol (Samkeliso et al., 2018) as described below:

Estimation of Tannin Content (TC)

Tannin content was quantified using the Folin-Ciocalteu spectrophotometric method (Singleton et al., 1999). Briefly, 0.2 mL of the plant extract was mixed with 0.5 mL of Folin-Ciocalteu reagent. Subsequently, 2 mL of 20% (w/v) sodium carbonate solution was added gradually with continuous stirring. The reaction mixture was incubated in darkness at 25°C for 2 hours to facilitate color development. Absorbance was measured at 725 nm using a UV-Vis spectrophotometer. For quantification, a standard calibration curve was prepared using tannic acid solutions (concentration range: 10–100 μg/mL). The tannin content in samples was calculated from the linear regression derived from the standard curve. Results were expressed as mg tannic acid equivalents (TAE) per gram of dry plant material.

Estimation of Phenol Content

The total phenolic content was estimated using a modified Folin-Ciocalteu method (Singleton et al., 1999). In this procedure, 200 μL of the plant extract was transferred to a test tube and the volume was adjusted to 3 mL with distilled water. Subsequently, 0.5 mL of Folin-Ciocalteu reagent and 2 mL of 20% (w/v) sodium carbonate solution were added to the mixture. The contents were mixed thoroughly and heated in a boiling water bath for 1 minute to facilitate color development. After cooling to room temperature, the absorbance of the resulting blue-colored solution was measured at 638 nm using a spectrophotometer. A standard calibration curve was prepared using catechol solutions of known concentrations. The phenolic content in the samples was calculated from this curve and expressed as mg catechol equivalents per mL of extract.

Estimation of Flavonoid Content

The flavonoid content was quantified using a colorimetric method based on aluminum complex formation (Chang et al., 2002). In this procedure, 100 μL of plant extract was combined with 1.5 mL of ethanol and mixed thoroughly. Subsequently, 100 μL of 1 M potassium acetate and 2 mL of distilled water were added to the mixture. The reaction tubes were incubated at ambient temperature (25 ± 2°C) for 40 minutes to allow complete chromophore development. Absorbance was measured at 415 nm using a UV-Vis spectrophotometer. Quercetin served as the reference standard, with calibration solutions prepared in the concentration range of 2–10 μg/mL. The flavonoid content was calculated from the standard curve and expressed as milligram quercetin equivalents (QE) per gram of dry plant material.

DPPH Radical Scavenging Assay

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method (Brand-Williams et al., 1995). Plant extracts were prepared as stock solutions (1 mg/mL in methanol) and serially diluted to

concentrations of 100–250 μg/mL. For analysis, 1 mL of each dilution was mixed with 3 mL of methanolic DPPH solution (0.1 mM) in test tubes wrapped in aluminum foil. After thorough vortexing, the mixtures were incubated at 37°C in darkness for 30 minutes. Control samples contained 1 mL methanol and 3 mL DPPH solution. Absorbance was measured at 517 nm against a methanol blank. Radical scavenging activity was calculated as:

Scavenging (%) = $[(Abs_{control} - Abs_{sample})/ Abs_{control}] \times 100$

Where, $Abs_{control}$ is the absorbance of the DPPH control and Abs_{sample} is the absorbance of the sample.

HPLC Analysis

HPLC analysis was carried out to identify and quantify major bioactive compounds in the methanolic extracts (Harborne, 1998). The analysis was performed on a Shimadzu HPLC system using a C18 column. The mobile phase consisted of acetonitrile and water. Detection was done at 254 nm. Standard compounds - vasicine for *A. vasica*, andrographolide for *A. paniculata* and lupeol for *H. auriculata* were used for quantification.

Results and Discussion

Pharmacognostic Evaluation

Macroscopic and Organoleptic Characters

The macroscopic and organoleptic features of the leaves of the three plants are summarized in **Table 1** which provides a comprehensive description of the leaf characteristics, which is crucial for plant identification and taxonomic classification. The combination of macroscopic features (shape, size, venation) and organoleptic properties (color, odor, taste) offers a multi-faceted approach to leaf analysis. The data suggests that these leaves belong to a plant species with moderately sized, smooth leaves that have a distinctive bitter taste and characteristic odor. The pinnate venation and color difference between upper and lower surfaces are typical of many dicotyledonous plants.

Physicochemical Analysis

The results of physicochemical analysis are presented in **Table 2**. The analysis of the physicochemical parameters of *A. vasica*, *A. paniculata* and *H. auriculata* leaf powders reveals significant differences in their nutritional compositions. This detailed examination provides insights into the potential uses and benefits of each leaf powder. The ash values help in determining the quality and purity of the crude drug. The extractive values are useful for evaluation of phytoconstituents. The moisture content was within the acceptable limits for all three plants. Out of these, *A.*

paniculata leaf powder exhibits the lowest moisture content at 7.20 ± 0.20%, followed by *A. vasica* at 8.70 ± 0.30%, and *H. auriculata* at 9.50 ± 0.40%. The low moisture content in *A. paniculata* suggests better preservation potential and longer shelf life, as lower moisture typically inhibits microbial growth and enzymatic reactions. Ash content, an indicator of mineral content, is highest in *A. vasica* at 15.20 ± 0.30%, compared to 12.50 ± 0.40% in *H. auriculata* and 9.80 ± 0.20% in *A. paniculata*. This suggests that *A. vasica* leaf powder may be a superior source of essential minerals, potentially offering greater nutritional benefits in this aspect.

Microscopic Characters

The transverse section of *A. vasica* leaf showed dorsiventral structure with prominent midrib. The upper and lower epidermis was single layered with thick cuticle. Stomata were present on both surfaces. The mesophyll was differentiated into palisade and spongy parenchyma. Vascular bundles were collateral type. *A. paniculata* leaf showed single layered epidermis on both surfaces with diacytic stomata. The mesophyll was differentiated into 1-2 layers of palisade cells and 3-4 layers of spongy parenchyma. Calcium oxalate crystals were observed. *H. auriculata* leaf showed single layered epidermis with thin cuticle. Anomocytic stomata were present on both surfaces. The mesophyll was undifferentiated. Cystoliths were observed in the epidermal cells.

Estimation of total protein and Carhohydrate

The total protein present in the plants selected was measured at 595 nm absorbance. The highest amount of protein was present in *H. auriculata* of 12.94 ± 0.21 mg / g of plant sample while the lowest amount of protein was found in *A. paniculata* with 7.13 ± 0.08 mg/ g of plant sample **(Table 3).** Estimation of total carbohydrates was done for different plant samples and its standard graph was prepared using pure glucose at 490 nm absorbance. The highest amount of carbohydrates were present in *A. vasica* with 132 ± 0.27 mg in 1 g of plant sample. *H. auriculata* exhibited less amount of carbohydrate (105 ± 0.15 mg/g of plant sample taken **(Table 3).**

Antioxidant activity analysis using DPPH

Antioxidant activity of the crude plant exracts were evaluated as he percentages of DPPH scavenging against the concentration of samples. The concentration of the samples required to decrease the DPPH concentration by 50% was estimated by the interpolation from linear regression analysis and denoted as IC50 value (μg/ml). *A. paniculata* (78..83%) showed the highest antioxidant potential while , *A. vasica* showed 72.81% activity and *H. auriculata* indicates 89.20% activity of DPPH scavenging at 100 μg/ml of methanol extract **(Table 3).**

Preliminary Phytochemical Screening

The results of preliminary phytochemical screening of different extracts are summarized in **Table 4**. The phytochemical screening revealed the presence of various bioactive constituents like alkaloids, flavonoids, tannins, steroids, terpenoids etc. in the plants. These phytochemicals contribute to the medicinal properties of the plants.

HPLC Analysis

HPLC analysis confirmed the presence of major bioactive compounds in the plants. The chromatograms (**Figure 1**) showed prominent peaks corresponding to the standard compounds. In *A. vasica*, vasicine was detected as the major alkaloid with a retention time of 3.8 min. The vasicine content was found to be 0.85% w/w (**Figure 1A**). Andrographolide was identified as the major diterpenoid lactone in *A. paniculata* with a retention time of 5.2 min. The andrographolide content was 2.1% w/w (**Figure 1B**). In *H. auriculata*, lupeol was detected as the major triterpenoid with a retention time of 12.5 min. The lupeol content was 0.32% w/w (**Figure 1C**). The quantification of these marker compounds can serve as a tool for quality control and standardization of the plant materials.

Conclusion:

The present study provides a comprehensive pharmacognostic and phytochemical profile of selected Acanthaceae species from North Bihar, emphasizing their significance in traditional medicine and potential for future pharmacological applications. Detailed macroscopic and microscopic analyses have established diagnostic features such as leaf arrangement, venation patterns, presence of cystoliths, and unique stomatal types, which are crucial for the accurate identification and authentication of these medicinal plants (Evans, 2009; Kirtikar & Basu, 2001). These characteristics not only help distinguish *Acanthaceae* species from other plant families but also serve as essential quality control parameters for crude drug standardization, thereby minimizing adulteration and ensuring therapeutic efficacy (World Health Organization, 2011).

The phytochemical screening conducted in this study revealed a rich spectrum of secondary metabolites, including alkaloids, flavonoids, tannins, saponins, terpenoids, and glycosides. These compounds are well-documented for their diverse pharmacological activities, such as anti-inflammatory, antimicrobial, antioxidant, and bronchodilatory effects (Harborne, 1998; Claeson et al., 2000). For instance, the presence of vasicine in *Adhatoda vasica* is closely associated with its traditional use in treating respiratory disorders, while the abundance of flavonoids and saponins in *Ruellia tuberosa* supports its use in folk remedies for coughs and colds (Jayakumar et al., 2013; Patra et al., 2012). The detection of these bioactive

constituents validates the ethnomedicinal claims and provides a scientific rationale for the continued use of these plants in local healthcare systems.

Moreover, the findings underscore the importance of integrating traditional knowledge with modern scientific approaches to unlock the full therapeutic potential of regional flora. North Bihar, with its rich biodiversity and deep-rooted ethnobotanical traditions, represents an invaluable reservoir for bioprospecting and drug discovery (Kumar & Kumari, 2020). By establishing pharmacognostic and phytochemical baselines, this research lays the groundwork for further studies, including isolation, structural elucidation, and pharmacological testing of individual compounds. Such efforts can contribute to the development of novel plant-based therapeutics and promote the sustainable use of medicinal plant resources (Khare, 2007; Mishra et al., 2007).

In conclusion, the systematic evaluation of *Acanthaceae* species from North Bihar not only affirms their ethnomedicinal relevance but also highlights their potential as sources of bioactive compounds for pharmaceutical development. Future research should focus on advanced analytical techniques, in vivo pharmacological assessments, and clinical trials to fully harness the medicinal value of these plants. Additionally, conservation strategies and cultivation practices should be encouraged to preserve this botanical heritage for future generations (WHO, 2011; Idu et al., 2010). The integration of traditional knowledge, scientific validation, and sustainable management will ensure that the medicinal wealth of the Acanthaceae family continues to benefit both local communities and the broader field of natural product research.

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Table 1: Macroscopic and organoleptic characters of leaves

Character	*Adhatoda vasica*	*Andrographis paniculata*	*Hygrophila auriculata*
Shape	Elliptic-lanceolate	Lanceolate	Oblong-lanceolate
Size	10-30 cm long	5-10 cm long	5-15 cm long
Apex	Acuminate	Acute	Acute
Base	Tapering	Attenuate	Attenuate
Margin	Entire	Entire	Serrate
Color	Dark green	Green	Green
Odor	Characteristic	Odorless	Odorless
Taste	Bitter	Very bitter	Mucilaginous
Texture	Smooth	Smooth	Rough

Table 2: Physicochemical parameters of leaf powders

Parameter	***A. vasica***	***A. paniculata***	***H. auriculata***
Total ash (% w/w)	15.2 ± 0.3	9.8 ± 0.2	12.5 ± 0.4
Acid insoluble ash (% w/w)	1.8 ± 0.1	1.2 ± 0.1	2.1 ± 0.2
Water soluble ash (% w/w)	6.5 ± 0.2	4.3 ± 0.3	5.7 ± 0.3
Alcohol soluble extractive (% w/w)	12.3 ± 0.4	15.6 ± 0.5	9.8 ± 0.3
Water soluble extractive (% w/w)	22.6 ± 0.6	18.9 ± 0.4	16.4 ± 0.5
Moisture content (% w/w)	8.7 ± 0.3	7.2 ± 0.2	9.5 ± 0.4

Table 3: Total Protein, Carhohydrate and Antioxidant activity of Crude leaf extracts

Parameter	***A. vasica***	***A. paniculata***	***H. auriculata***
Total Protein (mg / g)	10.2 ± 0.23	7.13 ± 0.08	12.94 ± 0.21
Total Carhohydrate (mg / g)	132 ± 0.27	124 ± 0.2	105 ± 0.15
DPPH scavenging activity (100 μg/ml) (Equivalent to DPPH Scavenging in %)	72.81	78.83	89.20

Table 3: Phytochemical screening of leaf extracts

Phytoconstituent	***A. vasica***	***A. paniculata***	***H. auriculata***
Alkaloids	+++	++	++
Flavonoids	+	+	++
Tannins	+	++	+
Saponins	++	-	+
Steroids	+	+	+++
Terpenoids	+	+	+
Glycosides	++	+++	++
Phenols	+	++	+

+: Present, -: Absent

Figure 2: HPLC analysis of major bioactive compounds (A) Vasicine (B) Andrographolide (C) Lupeol