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Extraction of different plants of family Acanthaceae in various solvents and determination of their phytoconstituents

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Abstract
Plants belonging to the family *Acanthaceae* are well known for their medicinal importance and rich phytochemical diversity. The present study was undertaken to evaluate the extractive efficiency of different solvents and to qualitatively and quantitatively determine primary and secondary metabolites present in various plant parts of *Justicia adhatoda*, *Andrographis paniculata*, and *Barleria prionitis*. Stem, leaves, and flowers were extracted using water, ethanol, and n-hexane representing polar, mid-polar, and non-polar solvents, respectively. Extractive values varied significantly with solvent polarity and plant part, with ethanol yielding the highest extractive values across all species, particularly in leaves. Qualitative phytochemical screening revealed the presence of carbohydrates, proteins, phenols, flavonoids, alkaloids, terpenoids, phytosterols, and lipids with solvent-specific distribution patterns. Quantitative estimation showed maximum carbohydrate content in leaves, while proteins, phenols, flavonoids, alkaloids, and phytosterols varied among species and plant parts. *Andrographis paniculata* exhibited higher levels of phenols, alkaloids, and lipids, whereas *Barleria prionitis* showed greater accumulation of proteins, flavonoids, and phytosterols, especially in leaves and flowers. The study highlights the importance of solvent selection in phytochemical extraction and confirms the therapeutic potential of selected *Acanthaceae* members based on their rich metabolite profiles.

Keywords: Acanthaceae, phytochemicals, solvent extraction, primary metabolites, secondary metabolites, medicinal plants

Introduction

Medicinal plants have been used as a primary source of healthcare since ancient times and continue to play a vital role in traditional and modern medicinal systems. According to the World Health Organization, nearly 80% of the global population relies on plant-based medicines for primary healthcare needs. The therapeutic efficacy of medicinal plants is largely attributed to the presence of diverse bioactive compounds, commonly referred to as phytoconstituents, which include both primary and secondary metabolites (Harborne, 1998; Pandey & Tripathi, 2014) [10, 19].

Primary metabolites such as carbohydrates, proteins, and lipids are essential for plant growth, development, and metabolic functions, whereas secondary metabolites including phenols, flavonoids, alkaloids, terpenoids, and phytosterols play crucial roles in plant defense, ecological interactions, and pharmacological activities. These secondary metabolites exhibit a wide range of biological properties such as antioxidant, antimicrobial, anti-inflammatory, anticancer, and hepatoprotective activities, making them valuable targets for drug discovery and development (Gonfa *et al*., 2020; Iqbal *et al*., 2012) [8, 12].

The family *Acanthaceae* comprises approximately 250 genera and over 4,000 species, many of which are well recognized for their medicinal properties. Several members of this family are extensively used in Ayurveda, Unani, and folk medicine for the treatment of respiratory disorders, infections, fever, inflammation, and gastrointestinal ailments (Khan *et al*., 2017; Sharma *et al*., 2016) [14, 22]. Among them, *Justicia adhatoda* is widely known for its bronchodilatory and expectorant properties, primarily attributed to alkaloids such as vasicine. *Andrographis paniculata*, often referred to as the "King of Bitters," possesses strong anti-inflammatory, hepatoprotective, and immunomodulatory activities due to the presence of diterpenoid lactones and phenolic compounds. *Barleria prionitis* is traditionally used for wound healing, dental disorders, and microbial infections, owing to its rich flavonoid and sterol content (Stephen *et al*., 2020; Siriwardhene *et al*., 2020) [24, 25].

Extraction is a critical step in phytochemical investigation, as the efficiency of extraction and the nature of phytoconstituents obtained largely depend on the solvent used. Polar solvents such as water are effective in extracting hydrophilic compounds like carbohydrates and proteins, while mid-polar solvents like ethanol are known to extract a broad range of bioactive compounds including phenols, flavonoids, and alkaloids. Non-polar solvents such as n-hexane are primarily effective in extracting lipophilic compounds such as lipids and phytosterols (Bae *et al*., 2012; Nezhdbahadori *et al*., 2018) [1, 17]. Therefore, comparative solvent extraction provides valuable insight into the chemical composition and therapeutic potential of medicinal plants.

Despite the extensive medicinal use of *Acanthaceae* plants, comparative studies evaluating solvent-specific extraction efficiency and quantitative variation of phytoconstituents across different plant parts remain limited. The present study was designed to systematically analyze the extractive values and phytochemical profiles of stem, leaves, and flowers of *Justicia adhatoda*, *Andrographis paniculata*, and *Barleria prionitis* using water, ethanol, and n-hexane. The findings aim to establish a scientific basis for their traditional use and to identify plant parts and solvents most suitable for extracting pharmacologically important compounds.

Materials and Methods

Collection of plant materials

Plant material (stem, leaves and flowers) of the selected plants were collected. The collected plant was identified at

Herbarium, Department of Botany, University of Rajasthan, and Jaipur. Plant parts were washed with running tap water and then distilled water to remove dust particles. After that, plant parts were air dried and grinded into mixer grinder to make coarse powder. Powdered form of plant parts was stored for further work.

Extraction of the collected plant parts

Plant materials were extracted in different polar and non-polar solvents. Selected solvents were- were water (highly polar), ethanol (mid-polar) and n-Hexane (Non-polar). For this purpose, 1 gm of each plant part (root, stem, leaves and flowers) were dipped into 10 ml of the solvent individually. Total 12 tubes were prepared for this purpose. Those were kept at shaker at room temperature for overnight. After that, tube was centrifuged at 10000 rpm for 10 minutes to collect supernatant. Supernatant were taken into pre-weighed petri-dishes and were left for evaporation of solvent. After complete drying, petri-plates were weighed again and weight of extract per gram plant material was calculated. Extracts were collected in glass vials for further use.

Qualitative determination of primary and secondary metabolites

Carbohydrates

Fehling Test: Fehling's reagent made up of two solutions: A and B of Fehling's solutions. Aqueous copper sulphate makes up Fehling's solution A, while alkaline sodium potassium tartrate makes up Fehling's solution B (Rochelle salt). The chelating agent in this reaction is provided by the Rochelle salts (sodium potassium tartrate) in the reagent. Before the test, an equal mixture of these two solutions is used. The mixture was heated on a water bath using an equal amount of freshly made Fehling's solution and 2ml of the aliquot. Reddish brown precipitate results from this. The presence of carbohydrates is shown by the red cuprous oxide precipitate that forms (MacLean, 1906) [15].

Proteins

Ninhydrin Test: Proteins interact with Ninhydrin's pyridine solution, changing it from a deep blue to a violet-pink or, on rare instances, a red colour. Ninhydrin 0.1gm is dissolved in approximately 100ml of distilled water to prepare a solution. However, this Ninhydrin solution is unstable and only lasts for two days. The availability of proteins is confirmed by the violet-colored solution's appearance (Dhuner *et al.*, 1960) [5].

Flavonoids

NaOH Test: Add a few drops of sodium hydroxide solution to the test solution; the presence of flavonoids is indicated by the production of an intense yellow colour that turns colorless when a few drops of diluted acetic acid are added (Saxena *et al.*, 2012) [21].

Alkaloids

Iodine Test: In 3 ml of test solution, a few drops of diluted iodine solution were added. Blue colour initially appeared, then vanished upon boiling and then reappeared upon chilling (Joshi *et al.*, 2013) [13].

Phenols

$FeCl_3$ Test: The presence of phenols was determined by adding 0.5 ml of FeCl3 (w/v) solution to 2 ml of the test solution (Joshi *et al.*, 2013) [13].

Phytosterols

Liebermann-Burchard Test: Concentrated sulphuric acid is added along the side of the test tube after the extract has been treated with a few drops of acetic anhydride, boiled and cooled. This causes the upper layer to turn green, indicating the presence of sterols, and a deep red colour to form, indicating the presence of triterpenoids (Cook, 1961) [3].

Terpenoids

Salkowski Test: 2ml of chloroform and 0.5gm of plant extract were combined. Then, 3ml of concentrated H2SO4 was carefully blended to create a layer. At the point of contact, a reddish-brown coloration showed the presence of terpenoids (Rahman *et al.*, 2010) [20].

Quantitative determination of primary and secondary metabolites

Carbohydrates

Procedure for Extraction

a. **Total soluble sugars:** A 50 mg sample of dried plant material was macerated overnight in a mortar and pestle with 20ml of 80% ethanol. Supernatants were collected and concentrated on a water bath following the centrifugation of each homogenate (1200rpm, 15min). Then, each concentration was diluted to 50ml with water and processed (Ext. I).

b. **Starch:** Starch was extracted from the residual pellet made using the aforementioned process by agitating it vigorously for five minutes while it was suspended in 5 ml of 52% perchloric acid and 6.5 ml of water (2500rpm, 20min). After three rounds of the procedure, the sample's supernatant was combined, and the volume was increased to 100 ml with distilled water (Ext. II). To obtain a quantitative estimate of starch, independent tests on 1 ml of the sample were conducted.

Quantification

With the use of the phenol-H2SO4 reagent and the construction of a regression curve for standardization with glucose, aliquots (1 ml) of each test sample (Ext. I and II) were used to calculate the total amounts of carbohydrates.

Lipids

In a mortar and pastel, 10 ml of distilled water was used to homogenise each sample (1 gramme). The pulp was then moved to a 250 ml conical flask and 30 ml of a 1:2, v/v, mixture of methanol and chloroform was added. The aforementioned mixture was kept at room temperature the previous night before 20 ml of distilled water and 20 ml of chloroform were added. Three films—a thick paste interphase, a transparent coloured film of chloroform holding all the lipids, and an upper coloured aqueous methanol film containing all the soluble components—emerged after the mixture had been completely mixed in a separating funnel. The lowest layer was collected after the methanol film was removed. In this instance, the organic layer was carefully concentrated in beakers that had already been weighed. The weight was once more determined after complete evaporation, and the total lipids/gm of dry plant material were computed.

Proteins

Procedure of Extraction

Every dried sample (60 mg) was macerated for 30 minutes in 10 ml of 10% TCA solution, then kept at 40C the next

day before centrifugation. Supernatants were removed from each experiment, and the pellets were then resuspended in 10 ml of 5% TCA and warmed in a water bath for 30 minutes at 800C. The samples were always centrifuged after being chilled, the supernatant was always discarded, and the pellet was always centrifuged after being rinsed with distillate water. Each residual after centrifugation was combined with 10 ml of 1N NaOH and left overnight at room temperature.

Quantification

A 1 ml portion of the extract was used to calculate the total protein levels. A BSA stock solution made in NaOH (1N; 1mg/ml) was pipetted into each test tube in amounts ranging from 0.1 to 0.8 ml, and the volume was then increased to 1 ml by distilled water. Each test tube received a 5 ml alkaline solution, which was prepared fresh by mixing 50 mL of 2% sodium carbonate in 0.1 N NaOH with 1 mL of 0.5% copper sulphate in 1% sodium potassium tartarate. The alkaline solution was kept at room temperature (10 ml). After that, each of these tubes received an immediate addition of 0.5 ml of Folin-Ciocalteau reagent (diluted with distil. Water), and after 30 minutes, ODs were measured at 750 nm using a spectrophotometer against a blank. Plotting the average values of three replicates against the corresponding concentrations produced the regression curve. The total protein level was calculated by comparing the ODs of each sample to a standard curve after the same processing method was applied to all of these samples. Three replicates were looked at for each example, and the mean values were given.

Flavonoids

Procedure of Extraction

Shade-dried leaves were ground into a fine powder (250 g), soxhlet extracted with 70% (v/v) ethanol, and vacuum concentrated to dryness under decreased pressure at 60°±1°C. It was placed in an airtight container and kept in a refrigerator at 5°C after drying in a hot air oven (40–45°C). Hydro-ethanolic extract was assigned to the leftover material. Plants' dried leaves (250 g) were first extracted with benzene, pet-ether, chloroform, ethyl acetate, and ethanol before being macerated with distilled water (non-polar to polar) to obtain the appropriate extracts. Using quercetin as a reference, the method of Harborne (1998) [10] was used to determine the existence and number of flavonoids. Soxhlet extraction and the TLC technique were used to evaluate the extracts.

Phytosterols

Procedure of Extraction

Plant materials are put in a thimble holder and then placed in a distillation flask during the Soxhlet extraction process. The solvent is put into the distillation flask. The solution in the thimble holder is added and syphoned back into the distillation flask when the solvent reaches the overflow level. The extract is introduced into the solvent. Extracted solutes are stored in the distillation flask, and the new solvent is returned to the plant material's thimble container. Up till the extraction is finished, this process is repeated. The Soxhlet extraction technique should be used to extract the desired chemicals, and a suitable solvent should be selected. Due to the use of various extraction solvents, the yield and content of the extract differ. Petroleum ether, methanol, and water were used for extraction.

Alkaloids

Extraction Procedure

The powder is treated with lime water, which releases any free bases that may be present as salts and then reacts with the acids, tannins, and other phenolic compounds. Following that, an appropriate organic solvent, typically chloroform, is agitated with the alkaline extract. Separated and concentrated organic layer (containing free alkaloids and lipophilic impurities). After shaking with aqueous acid, the concentrated organic extract is then allowed to separate. This separates the majority of other contaminants, which are still present in the organic layer, from the alkaloids as their salts (in the aqueous layer) (purification).

Results and Discussion

Extraction of plant parts-

As shown in Table 1, for *J. adhatoda* plant, extractive value of water extract of leaves was highest i.e. 35 mg/g. dw followed by its stem (27 mg/g. dw) and flowers (18 mg/g. dw.). Similar results were recorded for *A. peniculata* as well as *B. prionitis. For A. peniculata*, the values were 65 mg/g. dw (leaves), 38 mg/g. dw (stem), 33 mg/g. dw (flowers) while for *B. prionitis*, the values recorded were 51 mg/g. dw, 19 mg/g. dw and 12 mg/g. dw for leaves, stem and flowers, respectively. The color of extracts varied in plant parts. For *J. adhatoda*, stem extract color was brown, for leaves color was green while for flowers color was recorded as yellow. For *A. peniculata*, light brown color for stem, green for leaves and yellow for flowers, was recorded while for *B. prionitis*, dark brown, dark brown and brown colors were recorded for stem, leaves and flowers, respectively.

Extractive value of ethanolic extract of *J. adhatoda* leaves was highest i.e. 74 mg/g. dw followed by its stem (46 mg/g. dw) and flowers (31 mg/g. dw.). Similar results were recorded for *A. peniculata* as well as *B. prionitis. For A. peniculata*, the values were 93 mg/g. dw (leaves), 82 mg/g. dw (stem), 45 mg/g. dw (flowers) while for *B. prionitis*, the values recorded were 47 mg/g. dw, 31 mg/g. dw and 27 mg/g. dw for leaves, stem and flowers, respectively. The color of extracts varied in plant parts. For *J. adhatoda*, stem extract color was brown, for leaves color was dark green while for flowers color was recorded as light brown.

For *A. peniculata*, dark brown color for stem, brown for leaves and yellow for flowers, was recorded while for *B. prionitis*, brown, dark brown and yellow colors were recorded for stem, leaves and flowers, respectively.

For *J. adhatoda* plant, extractive value of hexane extract of leaves was highest i.e. 12 mg/g. dw followed by its stem (8 mg/g. dw) and flowers (7 mg/g. dw.). In case of *A. peniculata*, the highest value was recorded for leaves i.e. 16 mg/g. dw preceded by flowers (12 mg/g. dw) and stem (11 mg/g. dw). For *B. prionitis*, the values recorded were 16 mg/g. dw (highest), 14 mg/g. dw and 9 mg/g. dw for stem, leaves and flowers, respectively.

The color of extracts recorded different in different plant parts. For *J. adhatoda*, stem and flower extracts were found to be transparent while leaf extract was white in color. All three extracts were transparent for *A. peniculata* while for *B. prionitis*, stem and flowers extracts were found to be white in color and leaf extracts was recorded as transparent.

Extraction with ethanol resulted in higher yields across all plant parts, with *A. paniculata* showing the highest ethanol-extracted values, particularly from the leaves. The extracts from this plant were relatively darker, suggesting presence of ethanol-soluble compounds in plant extracts such as polyphenols and terpenoids. Furthermore, extraction with hexane yielded the lowest values, indicating the extraction of non-polar compounds, such as lipids. Extracts isolated using hexane were mostly transparent or white in colour. Based on this, it can be concluded that ethanol is the most effective for extraction of phytocompounds while hexane is the least effective, extracting only non-polar solvents. (Nezhdbahadori *et al.*, 2018; Bae *et al.*, 2012; Simorangkir *et al.*, 2019; Ghosh *et al.*, 2020; McGaw *et al.*, 2013; Danlami *et al.*, 2015) [1, 4, 7, 16, 17, 23].

About the variation in colour of extracts, the colors of extracts (using water as a solvent) was observed to be (brown, green, yellow), indicating the presence of specific water-soluble pigments or compounds. For instance, the green color of the leaves may be due to chlorophyll, whereas yellow could indicate presence of flavonoids or carotenoids. In case of ethanol as a solvent, the colour of extracts was found to range from brown to dark green and dark brown, indicating the presence of polyphenolic compounds such as tannins and flavonoids, as well as terpenoids, all of which are more soluble in ethanol. Also, the dark green color from the leaves suggests the presence of chlorophyll or other plant pigments that are ethanol-soluble. In case of hexane, which is non-polar solvent that mainly extracts lipophilic (fat-soluble) compounds, such as lipids, waxes, and essential oils. Plants generally contain low amounts of these compounds, hence, yield of extractives was the lowest in case of hexane. The transparent or white color of hexane extracts indicates the presence of non-polar, colorless compounds like lipids or waxes. These compounds do not impart much color to the extract compared to the water- and ethanol-soluble compounds (Tan *et al.*, 2013; Oreopoulou *et al.*, 2019; Sulaiman *et al.*, 2011) [18, 26, 27].

Qualitative determination of primary and secondary metabolites in plant extract

According to the results of present study, stem of *J. adhatoda* reported the presence of various primary and secondary metabolites *viz*., carbohydrates, proteins, phenols, flavonoids, alkaloids and terpenoids in water extracts, carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, phytosterols and terpenoids in ethanolic extracts while only lipids and phytosterols in hexane extracts.

For leaves of *J. adhatoda*, presence of carbohydrates, proteins, phenols, flavonoids, alkaloids and terpenoids was reported in water extracts, carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, phytosterols and terpenoids in ethanolic extracts while only lipids and phytosterols in hexane extracts.

Flowers of *J. adhatoda* found to have carbohydrates, proteins, phenols, flavonoids, alkaloids and terpenoids in water extracts, carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, phytosterols and terpenoids in ethanolic extracts while only lipids and phytosterols in hexane extracts.

The qualitative analysis of *Justicia adhatoda* extracts revealed the presence of various primary and secondary metabolites, each with distinct solubility patterns based on the solvent used. For instance, carbohydrates were found to be present in water and ethanol extracts but found to be absent in hexane extracts. This may be attributed to the fact that carbohydrates are polar compounds and therefore more soluble in polar solvents like water as well as ethanol. Thereafter, proteins were detected in water and ethanol extracts from all parts of the plant. Proteins are being polar in nature dissolve well in polar solvents, indicating their widespread presence in plant tissues. Lipids, being non-polar in nature, were found to be present in non-polar solvents, ethanol and hexane but absent in water. Phenols and flavonoids both are polar in nature, and therefore, extremely soluble in polar solvents such as water and ethanol. As a consequence of this, both phenols and flavonoids were found to be present when water and ethanol were used as solvents and absent in case of hexane as solvent. Both phenols and flavonoids play a crucial role in contributing to plant defense and are known for their potent antioxidant activity. Alkaloids are inherently rich in nitrogen and polar in nature, which makes them highly soluble in polar solvents. It is because of this that alkaloids were found to be present when water and ethanol were used as solvents. Alkaloids possess potential medicinal properties and aid in contributing to plant defense. Phytosterols and terpenoids, both are non-polar in nature, as a consequence of which, both phytosterols and terpenoids were found to be present in ethanol and hexane extracts and absent in water. These findings suggest that *Justicia adhatoda* contains a variety of bioactive compounds, with different solubility characteristics, potentially contributing to its medicinal properties (Uzoekwe *et al.*, 2016; Iqbal *et al.*, 2012; Gonfa *et al.*, 2020) [8, 12, 28].

As the results of present study recorded, stem of *A. peniculata* reported the presence of various primary and secondary metabolites *viz*., carbohydrates, proteins, phenols, flavonoids, alkaloids and terpenoids in water extracts, carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, phytosterols and terpenoids in ethanolic extracts while only lipids and phytosterols in hexane extracts.

For leaves of *A. peniculata*, presence of carbohydrates, proteins, phenols, flavonoids and terpenoids was reported in water extracts, carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, and phytosterols in ethanolic extracts while only lipids and phytosterols in hexane extracts.

Flowers of *A. peniculata* found to have carbohydrates, proteins, flavonoids, alkaloids and terpenoids in water extracts, carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, phytosterols and terpenoids in ethanolic extracts while only lipids and phytosterols in hexane extracts.

According to the results of present study, stem of *B. prionitis* reported the presence of various primary and secondary metabolites *viz*., carbohydrates, proteins, phenols, flavonoids, alkaloids and terpenoids in water extracts, carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, and phytosterols in ethanolic extracts while only lipids and phytosterols in hexane extracts.

For leaves of *B. prionitis*, presence of carbohydrates, proteins, phenols, flavonoids, alkaloids and terpenoids was reported in water extracts, carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, phytosterols and terpenoids in ethanolic extracts while only lipids and phytosterols in hexane extracts.

Flowers of *B. prionitis* found to have carbohydrates, proteins, phenols, flavonoids, and alkaloids in water

extracts, carbohydrates, proteins, lipids, phenols, flavonoids, alkaloids, phytosterols and terpenoids in ethanolic extracts while only lipids and phytosterols in hexane extracts.

These findings suggest that *Andrographis paniculata* and *Barleria prionitis* contains a variety of bioactive compounds, with distinct solubility characteristics, potentially contributing to its medicinal properties (Dirar *et al.,* 2019; Hikmawanti *et al.,* 2021) [6, 11].

Quantitative determination of primary and secondary metabolites-

According to Table 2, values of primary and secondary metabolites varied in different parts of selected plants.

For *J. adhatoda*, stem extract was found to have highest value of proteins i.e. 18.55±0.12 mg/g. dw followed by carbohydrates (14.50±0.14 mg/g. dw), lipids (8.64±0.41 mg/g. dw), phenols (4.68±0.44 mg/g. dw), flavonoids (1.10±0.50 mg/g. dw), alkaloids (0.34±0.012 mg/g. dw) and phytosterols with least value; 0.15±0.11 mg/g. dw. Leaf extracts were reported to have highest value for carbohydrates (24.28±0.11 mg/g. dw) followed by proteins (17.84±0.72 mg/g. dw), lipids (7.28±1.18 mg/g. dw), phenols (4.07±0.37 mg/g. dw), flavonoids (1.08±0.87 mg/g. dw), alkaloids (0.31±0.053 mg/g. dw) and phytosterols with least value; 0.13±0.68 mg/g. dw. Highest value for flower extracts was recorded for carbohydrate (13.08±1.24 mg/g. dw) followed by lipids (7.67±1.03 mg/g. dw), proteins (4.67±0.62 mg/g. dw), phenols (2.04±0.10 mg/g. dw), phytosterols (0.60±001 mg/g. dw), alkaloids (0.56±0.053 mg/g. dw) and the least value was noted for flavonoids (0.29±0.52 mg/g. dw).

For *A. peniculata*, stem extract was found to have highest value of carbohydrates i.e. 12.97±0.85 mg/g. dw followed by lipids (6.55±0.68 mg/g. dw), proteins (4.11±0.10 mg/g. dw), phenols (2.10±0.28 mg/g. dw), phytosterols (0.47±0.11 mg/g. dw), alkaloids (0.39±0.027 mg/g. dw) and flavonoids with least value; 0.26±0.14 mg/g. dw. Leaf extracts were reported to have highest value for carbohydrates (34.80±1.33 mg/g. dw) followed by lipids (19.10±3.61 mg/g. dw), proteins (12.41±2.57 mg/g. dw), phenols (8.59±0.59 mg/g. dw), alkaloids (1.36±0.034 mg/g. dw), phytosterols (0.86±0.18 mg/g. dw) and flavonoids with least value; 0.44±0.05 mg/g. dw. Highest value for flower extracts was recorded for lipids (18.38±1.17 mg/g. dw) followed by carbohydrates (14.68±0.78 mg/g. dw), proteins (12.38±1.489 mg/g. dw), phenols (8.17±0.36 mg/g. dw), alkaloids (1.12±0.033 mg/g. dw), phytosterols (0.77±0.56 mg/g. dw) and then flavonoids (0.42±0.10 mg/g. dw).

For *B. prionitis*, stem extract was found to have highest value of carbohydrates i.e. 19.78±0.27 mg/g. dw followed by lipids (9.56±1.80 mg/g. dw), proteins (9.367±0.34 mg/g. dw), phenols (3.17±0.07 mg/g. dw), flavonoids (0.97±0.05 mg/g. dw), phytosterols (0.36±0.02 mg/g. dw) and alkaloids with least value; 0.11±0.003 mg/g. dw. Leaf extracts were reported to have highest value for carbohydrates (31.55±1.37 mg/g. dw) followed by proteins (28.56±0.85 mg/g. dw), lipids (12.43±1.27 mg/g. dw), phenols (2.17±0.83 mg/g. dw), flavonoids (1.18±0.03 mg/g. dw), phytosterols (0.81±0.07 mg/g. dw) and alkaloids with least value; 0.51±0.01 mg/g. dw. Highest value for flower extracts was recorded for carbohydrates (15.03±3.11 mg/g. dw) followed by proteins (12.66±0.38 mg/g. dw), lipids (8.36±2.12 mg/g. dw), phenols (4.11±0.47 mg/g. dw), flavonoids (2.15±0.03 mg/g. dw), phytosterols (1.03±0.05 mg/g. dw) and the least value was noted for alkaloids (0.33±0.003 mg/g. dw).

The highest amount of carbohydrates was reported in leaves of *Andrographis paniculata,* followed by leaves of *Barleria prionitis* and *Justicia adhatoda*. This maybe attributed to the fact that leaves, being the primary site for photosynthesis, *Andrographis paniculata* showcases a higher photosynthetic capacity, leading to greater carbohydrate storage. Thereafter, *Barleria prionitis* has the highest protein content in its leaves, while *Andrographis paniculata* and *Justicia adhatoda* have lower values. The higher protein content in leaves of the plant *Barleria prionitis* may indicate increased metabolic activity or growth, especially in leaves involved in photosynthesis and defense. As far as the lipids are concerned, the highest lipid content was observed in leaves and flowers of *Andrographis paniculata*, followed by *Barleria prionitis* and *Justicia adhatoda*. Lipids are essential for cell membrane integrity and energy storage; the higher lipid levels in *Andrographis paniculata* suggest a greater need for these compounds in metabolically active or reproductive tissues. Thereafter, researchers performed quantitative analysis for secondary metabolites, namely, phenols, flavonoids, alkaloids and phytosterols in stem, leaves and flowers of *Andrographis paniculata, Justicia adhatoda* and *Barleria prionitis.* The results showed highest amount of phenols in leaves of *Andrographis paniculata,* followed by *Justicia adhatoda* and *Barleria prionitis*. Phenols are important for plant defense, and their higher concentration in *Andrographis paniculata* is suggestive of stronger protective mechanisms against stressors like UV radiation or herbivores. Thereafter, the researchers evaluated the flavonoid content of plants and found the highest flavonoid content in flowers of *Barleria* followed by its leaves and stems. Flavonoids serve as UV protectants and defense compounds, with *Barleria prionitis* concentrating them in its flowers, likely due to the need for reproductive protection. The highest number of alkaloids were found to be present in leaves and flowers of *Andrographis paniculata, followed by Barleria prionitis and Justicia adhatoda.* Alkaloids are used for defense of plants against herbivores and pathogens. *Andrographis paniculata* likely uses alkaloids more extensively for these purposes. The study further showed that highest amount of phytosterols were found in flowers of *Barleria prionitis.* Followed by *Andrographis paniculata* and *Justicia adhatoda.* Phytosterols play a crucial role in membrane stability, and their accumulation in flowers may support the structural needs of reproductive tissues, especially in *Barleria prionitis.* Based on these results, it can be concluded that *Andrographis paniculata* appears to prioritize plant defense and metabolic efficiency, especially with higher levels of phenols, alkaloids, and lipids. Another plant, *Barleria prionitis* focuses on growth as well as reproduction, as evident from its high protein, flavonoid, and phytosterol levels. In comparison to the above two plants, *Justicia adhatoda* has relatively lower metabolite concentrations, possibly reflecting a more structural role, particularly in its stems.

A number of previously published studies have shown presence of primary and secondary metabolites in all the three-plant species, namely, *Andrographis paniculata, followed by Barleria prionitis and Justicia adhatoda* (Khan *et al.,* 2017; Sharma *et al.,* 2016; Gurjar *et al.,* 2023; Chaudhary *et al.,* 2023; Stephen *et al.,* 2020; Siriwardhene *et al.,* 2020) [2, 9, 14, 22, 24, 25].

Table 1: Extractive values of the selected plant parts in various solvents

Solvent name	Plant name	Extractive values (mg/g. dw)			Extract colour and nature		
		Stem	Leaves	Flowers	Stem	Leaves	Flowers
Water	*J. adhatoda*	27	35	18	Brown	Green	Yellow
	A. peniculata	38	65	33	Light brown	Green	Yellow
	B. prionitis	19	51	12	Dark brown	Dark brown	Brown
Ethanol	*J. adhatoda*	46	74	31	Brown	Dark green	Lighy brown
	A. peniculata	82	93	45	Dark brown	Brown	Yellow
	B. prionitis	31	47	27	Brown	Dark brown	Yellow
Hexane	*J. adhatoda*	8	12	7	Transparent	White	Transparent
	A. peniculata	11	16	12	Transparent	Transparent	Transparent
	B. prionitis	16	14	9	White	Transparent	White

Table 2: Quantitative determination of primary and secondary metabolites in various parts of the selected plants.

	Values of metabolites in (mg/g. dw)								
	Justicia Adhatoda			*Andographis Peniculata*			*Barleria prionitis*		
	Stem	Leaves	Flowers	Stem	Leaves	Flowers	Stem	Leaves	Flowers
Carbohydrates	14.50±0.14	24.28±0.11	13.08±1.24	12.97±0.85	34.80±1.33	14.68±0.78	19.78±0.27	31.55±1.37	15.03±3.11
Proteins	18.55±0.12	17.84±0.72	4.67±0.62	4.11±0.10	12.41±2.57	12.38±1.489	9.367±0.34	28.56±0.85	12.66±0.38
Lipids	8.64±0.41	7.28±1.18	7.67±1.03	6.55±0.68	19.10±3.61	18.38±1.17	9.56±1.80	12.43±1.27	8.36±2.12
Phenols	4.68±0.44	4.07±0.37	2.04±0.10	2.10±0.28	8.59±0.59	8.17±0.36	3.17±0.07	2.17±0.83	4.11±0.47
Flavonoids	1.10±0.50	1.08±0.87	0.29±0.52	0.26±0.14	0.44±0.05	0.42±0.10	0.97±0.05	1.18±0.03	2.15±0.03
Alkaloids	0.34±0.012	0.31±0.053	0.56±0.053	0.39±0.027	1.36±0.034	1.12±0.033	0.11±0.003	0.51±0.01	0.33±0.003
Phytosterols	0.15±0.11	0.13±0.68	0.60±001	0.47±0.11	0.86±0.18	0.77±0.56	0.36±0.02	0.81±0.07	1.03±0.05

Conclusion

The present investigation provides a comprehensive evaluation of extractive efficiency and phytochemical composition of selected *Acanthaceae* plants using solvents of varying polarity. The results clearly demonstrate that solvent selection plays a crucial role in determining both the yield and nature of phytoconstituents extracted. Ethanol proved to be the most effective solvent, yielding higher extractive values and a broader spectrum of primary and secondary metabolites across all plant parts, particularly leaves. Water extracts were rich in polar compounds such as carbohydrates, proteins, phenols, and flavonoids, whereas n-hexane selectively extracted non-polar compounds like lipids and phytosterols with comparatively lower yields.

Quantitative analysis revealed significant variation in metabolite distribution among species and plant parts. *Andrographis paniculata* exhibited higher concentrations of phenols, alkaloids, and lipids, suggesting its strong defense mechanisms and pharmacological potential. *Barleria prionitis* showed elevated levels of proteins, flavonoids, and phytosterols, particularly in leaves and flowers, indicating its role in growth and reproductive protection. In contrast, *Justicia adhatoda* displayed comparatively moderate metabolite levels, supporting its traditional medicinal applications, especially in respiratory disorders.

Overall, the study confirms that members of the family *Acanthaceae* are rich sources of bioactive compounds and validates their traditional medicinal use. The findings emphasize the importance of selecting appropriate plant parts and extraction solvents for maximizing phytochemical recovery. Further studies involving isolation, characterization, and bioactivity evaluation of these compounds are recommended to explore their potential in pharmaceutical and nutraceutical applications.

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