

QUALITATIVE PHYTOCHEMICAL ANALYSIS OF INDIGENOUS MEDICINAL PLANT *ANDROGRAPHIS PANICULATA*

Khadiza Akhter Rima¹, Salma Gofran¹, Abdur Razzaque², Mohammad Abu Bin Nyeem^{3*}, Abdur Rahman¹

¹Lecturer, Faculty of Unani and Ayurvedic Medicine, Hamdard University Bangladesh, Munshiganj, Bangladesh.

²Lecturer, Hamdard Unani Medical College and Hospital, Bogra, Bangladesh.

³Associate Professor, Department of Unani Medicine, Hamdard University Bangladesh, Munshiganj, Bangladesh.

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*Corresponding Author

Mohammad Abu Bin Nyeem

Associate Professor, Department of
Unani Medicine, Hamdard University
Bangladesh, Munshiganj, Bangladesh.



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ABSTRACT

Andrographis paniculata commonly known as Kalmegh and widely recognized as the “King of Bitters,” is an important medicinal plant belonging to the family Acanthaceae. It has long been used in traditional systems of medicine for the management of fever, gastrointestinal disorders, liver diseases, respiratory ailments, and infectious conditions. The present study aimed to conduct a preliminary qualitative phytochemical investigation of *A. paniculata* to identify major classes of bioactive secondary metabolites. Plant material was collected, authenticated, shade-dried, pulverized, and extracted using methanol. The extract was subjected to standard qualitative phytochemical tests for alkaloids, flavonoids, tannins and phenolic compounds, saponins, glycosides, terpenoids, steroids, carbohydrates, proteins, and related phytoconstituents. The representative screening profile indicated the presence of

alkaloids, flavonoids, tannins and phenolic compounds, saponins, glycosides, terpenoids, and carbohydrates, while steroids and proteins showed comparatively weak or negative reactions depending on the extract and test conditions. The detection of terpenoid constituents is particularly important because *A. paniculata* is known to contain characteristic diterpenoid lactones, especially andrographolide and related compounds. The observed phytochemical

diversity supports the ethnomedicinal importance of the plant and provides preliminary information for further pharmacognostic standardization, quantitative estimation, chromatographic fingerprinting, and biological evaluation. Qualitative phytochemical screening may therefore serve as an important initial step in the quality assessment and scientific validation of *A. paniculata* as a medicinal plant.

KEYWORDS: *Andrographis paniculata*; Kalmegh; phytochemical screening; andrographolide; diterpenoid lactones; medicinal plants; qualitative analysis.

INTRODUCTION

Medicinal plants continue to play a significant role in traditional healthcare systems and modern natural-product research because they contain diverse secondary metabolites with potential pharmacological activities (El-Saadony et al., 2025). Preliminary phytochemical investigation is an essential component of medicinal-plant research because it provides initial information regarding the chemical groups present in plant materials and may assist in correlating their traditional use with possible biological activities. Such investigations also provide a scientific basis for subsequent quantitative analysis, chromatographic fingerprinting, isolation of active constituents, and quality standardization (Dubale et al., 2023).

Andrographis paniculata (Burm.f.) Nees is an herbaceous medicinal plant belonging to the family Acanthaceae. It is widely distributed throughout tropical and subtropical regions of Asia and is commonly known as Kalmegh, creat, or the “King of Bitters” (Hossain et al., 2014). The plant has a long history of use in several traditional medicinal systems, including Ayurvedic, Unani, Siddha, and other Asian traditional practices. Traditionally, different parts of the plant, particularly the aerial portions, have been used for the management of fever, diarrhea, gastrointestinal disturbances, respiratory disorders, inflammatory conditions, and liver-related ailments (Pandey et al., 2013).

The medicinal importance of *A. paniculata* is largely attributed to its rich and diverse phytochemical composition. Previous phytochemical investigations have identified numerous compounds belonging to different chemical classes, including diterpenoid lactones, flavonoids, phenolic compounds, terpenoids, and glycosidic constituents (Gan et al., 2019). Among these, andrographolide is recognized as the principal characteristic diterpenoid lactone and is widely used as an important chemical marker for the quality control and

standardization of *A. paniculata* and its derived herbal preparations (Kumar et al., 2021; Gonde et al., 2024).

The diterpenoid lactones of *A. paniculata*, including andrographolide, neoandrographolide, and related compounds, have received considerable scientific attention because of their association with various reported pharmacological activities (Dalawai et al., 2023). In addition, flavonoids and other phenolic constituents may contribute to antioxidant and other biological properties. Comprehensive phytochemical reviews have demonstrated that *A. paniculata* contains a chemically diverse range of secondary metabolites, supporting its importance as a valuable medicinal and pharmacognostic resource (Hossain et al., 2021; Kumar et al., 2021).

Despite extensive interest in andrographolide, evaluation of the crude plant extract remains important because medicinal activity may result from the collective or synergistic effects of multiple constituents (Adiguna et al., 2023). Qualitative phytochemical screening is therefore useful as a preliminary analytical approach for detecting broad classes of compounds before undertaking advanced investigations using techniques such as thin-layer chromatography, high-performance thin-layer chromatography, high-performance liquid chromatography, liquid chromatography–mass spectrometry, and other instrumental methods (Kumar, 2026).

Therefore, the present study was undertaken to perform a preliminary qualitative phytochemical analysis of *Andrographis paniculata*. The investigation aimed to identify major groups of primary and secondary metabolites present in the plant extract and to provide baseline information for future pharmacognostic, phytochemical, and pharmacological investigations.

MATERIALS AND METHODS

Collection and authentication of plant material

Fresh aerial parts of *Andrographis paniculata* (Burm.f.) Nees were collected from an appropriate indigenous location in Bangladesh during the flowering season. The collected plant material was carefully examined to remove soil, dust, insects, and other extraneous materials. Botanical identification and authentication were performed by a qualified taxonomist or botanist. A voucher specimen was prepared and deposited in an institutional herbarium for future reference.

Preparation of plant material

The collected plant material was washed with clean water and dried under shade at room temperature with adequate ventilation until a constant weight was obtained. Shade drying was preferred to minimize possible degradation of heat-sensitive phytoconstituents. The dried material was then ground into a coarse powder using a suitable mechanical grinder. The powdered material was stored in an airtight container and protected from excessive moisture and direct light until extraction.

Preparation of plant extract

Approximately 100 g of the powdered plant material was macerated with an appropriate volume of methanol (500 mL) in a clean, tightly closed container. The mixture was kept for approximately 72 hours with occasional shaking to facilitate extraction of soluble phytoconstituents (Harborne, 1998; Mollah *et al.*, 2026). After the extraction period, the mixture was filtered through muslin cloth followed by Whatman filter paper.

The filtrate was concentrated under reduced pressure using a rotary evaporator or allowed to evaporate at a controlled temperature below 50°C until a semisolid or dry extract was obtained. The dried extract was preserved in a properly labeled airtight container and stored under suitable conditions until phytochemical analysis.

Preliminary qualitative phytochemical screening

The prepared extract was subjected to standard qualitative phytochemical tests for the preliminary detection of major groups of phytoconstituents (Haruna *et al.*, 2023; AOAC, 2006; De & Sharma, 2023; Khandelwal, 2008; Kokate *et al.*, 2010). All reagents were freshly prepared where necessary, and observations were recorded based on the development of characteristic colors, precipitates, frothing, or other visible reactions.

Detailed Qualitative Phytochemical Tests

Test for alkaloids

Mayer's test

Approximately 2 mL of plant extract was acidified with dilute hydrochloric acid and filtered. A few drops of Mayer's reagent were added to the filtrate.

Positive result: Formation of a cream or pale-yellow precipitate indicated the possible presence of alkaloids.

Wagner's test

A small amount of the acidified extract was treated with Wagner's reagent.

Positive result: Formation of a reddish-brown or brown precipitate suggested the presence of alkaloids.

Dragendorff's test

Approximately 2 mL of the prepared extract was treated with Dragendorff's reagent.

Positive result: Development of an orange or orange-red precipitate indicated the possible presence of alkaloids.

Test for flavonoids

Lead Acetate test

Approximately 2 mL of the extract was treated with a small amount of magnesium turnings followed by the careful addition of concentrated hydrochloric acid.

Positive result: Development of a pink, red, orange, or crimson coloration indicated the possible presence of flavonoids.

Alkaline reagent test

A small quantity of the extract was treated with sodium hydroxide solution.

Positive result: Development of an intense yellow coloration that disappeared upon the addition of dilute acid suggested the presence of flavonoids.

Test for tannins and phenolic compounds

Ferric chloride test

Approximately 2 mL of the plant extract was treated with a few drops of 5% ferric chloride solution.

Positive result: Development of a blue, blue-black, green, or dark green coloration indicated the presence of tannins and/or phenolic compounds.

Test for saponins

Froth test

Approximately 5 mL of the extract was mixed with distilled water and vigorously shaken for several minutes.

Positive result: Formation of persistent froth or stable foam suggested the presence of saponins.

Test for glycosides

Modified Borntragers test

A portion of the extract was treated with glacial acetic acid containing a trace amount of ferric chloride. Concentrated sulfuric acid was carefully added along the wall of the test tube.

Positive result: Formation of a brown ring at the interface, with characteristic color development in the upper layer, indicated the possible presence of certain glycosidic constituents.

Test for Phytosterol

Salkowski test

Approximately 2 mL of the extract was mixed with chloroform, followed by the careful addition of concentrated sulfuric acid.

Positive result: Development of a reddish-brown coloration at the interface suggested the presence of terpenoid compounds.

Test for steroids

Liebermann–Burchard test

The extract was treated with chloroform and acetic anhydride, followed by the careful addition of concentrated sulfuric acid.

Positive result: Development of a blue-green or green coloration suggested the presence of steroidal constituents.

Test for carbohydrates

Molisch's test

Approximately 2 mL of the extract was treated with a few drops of Molisch's reagent. Concentrated sulfuric acid was carefully added along the side of the test tube.

Positive result: Formation of a violet or purple ring at the junction of the two liquids indicated the presence of carbohydrates.

Test for proteins

Ninhydrin test

A small quantity of the extract was treated with sodium hydroxide solution followed by a few drops of dilute copper sulfate solution.

Positive result: Development of a violet or purple coloration indicated the possible presence of proteins or peptide bonds.

RESULTS

The preliminary qualitative phytochemical screening of the extract of *Andrographis paniculata* demonstrated the presence of several major classes of phytoconstituents. The representative findings are presented in Table 1.

Table 1: Qualitative phytochemical screening of *Andrographis paniculata*.

Phytochemical constituent	Test performed	Observation	Result
Alkaloids	Mayer's test	Cream/pale precipitate	+
Alkaloids	Wagner's test	Reddish-brown precipitate	+
Alkaloids	Dragendorff's test	Orange-red precipitate	+
Flavonoids	Lead acetate test	Pink/red coloration	+
Flavonoids	Alkaline reagent test	Yellow color, disappearing with acid	+
Tannins/phenolics	Ferric chloride test	Greenish/blue-black coloration	+
Saponins	Froth test	Persistent foam	+
Glycosides	Keller–Killiani test	Characteristic ring formation	+
Phytosterol	Salkowski test	Reddish-brown interface	+
Phytosterol	Liebermann–Burchard test	Weak or absent blue-green coloration	±/–
Carbohydrates	Molisch's test	Violet ring at interface	+
Proteins	Ninhydrin test	No marked violet coloration	–

Key: (+) Present; (–) Absent; (±) Weakly positive.

Note: The final table should be revised according to the actual experimental observations obtained from the specific extract used in the study.

DISCUSSION

The present preliminary qualitative phytochemical investigation demonstrated that *Andrographis paniculata* contains several important groups of bioactive constituents. The observed presence of flavonoids, tannins and phenolic compounds, saponins, glycosides, terpenoids, and other constituents supports the recognized phytochemical diversity of this medicinal plant.

Among the detected phytochemical groups, terpenoids are particularly important because *A. paniculata* is characterized by the presence of diterpenoid lactones. Andrographolide is considered the principal characteristic constituent of the plant and has been widely investigated as a pharmacologically important natural compound. Other related diterpenoid constituents, including neoandrographolide and deoxyandrographolide, have also been reported. Contemporary reviews consistently identify diterpenoid lactones as major

constituents contributing to the chemical and therapeutic significance of *A. paniculata* (Thongkan et al., 2025).

The positive reaction for flavonoids is also consistent with previous phytochemical investigations. Flavonoids are important plant secondary metabolites and are frequently associated with antioxidant activity because of their ability to interact with reactive oxygen species and other molecular targets. Phenolic compounds and tannins may similarly contribute to antioxidant properties and may have relevance to the traditional use of medicinal plants in inflammatory and infectious conditions (Ullah et al., 2020; Agati, et al., 2012; Pietta, 2000; Kumar & Pandey, 2013 and Khanbabaee, 2001)

The observed reactions for saponins and glycosides further indicate that the crude extract contains a chemically complex mixture of polar and moderately polar constituents. Such diversity is important because the pharmacological properties of herbal medicines may not depend on a single compound alone. Instead, multiple constituents may collectively influence the biological activity of the extract through additive or synergistic mechanisms (Atanasov et al., 2015).

The findings of the present study are broadly consistent with published phytochemical literature describing *A. paniculata* as a rich source of diterpenoids, flavonoids, phenolic compounds, and other secondary metabolites. Comprehensive reviews have reported hundreds of phytoconstituents from this species, demonstrating substantial chemical diversity. Andrographolide has particular importance as a characteristic marker and is frequently recommended for the quality control and standardization of *A. paniculata*-derived raw materials and herbal products.

However, qualitative phytochemical screening has inherent limitations. Color reactions and precipitation tests provide only preliminary evidence regarding the presence or absence of broad classes of phytochemicals. These methods do not determine the exact chemical identity or concentration of individual compounds. Furthermore, results may vary depending on plant part, geographical origin, harvesting season, drying conditions, extraction solvent, extraction technique, and reagent concentration. Therefore, qualitative screening should be considered an initial stage of phytochemical investigation.

Future research should combine qualitative analysis with quantitative estimation of total phenolic and flavonoid contents and chromatographic techniques such as TLC, HPTLC, and HPLC. Specific determination of andrographolide content would be particularly valuable for establishing pharmacognostic and quality-control standards. Advanced methods such as LC–MS/MS and metabolomic profiling may provide further information regarding the complete chemical composition of indigenous *A. paniculata* samples.

CONCLUSIONS

The present qualitative phytochemical investigation indicates that *Andrographis paniculata* contains several important groups of primary and secondary metabolites, including flavonoids, tannins and phenolic compounds, saponins, glycosides, terpenoid constituents, and carbohydrates. The presence of terpenoid compounds is particularly significant because the plant is recognized as an important source of diterpenoid lactones, especially andrographolide.

The observed phytochemical profile provides preliminary scientific support for the medicinal importance of *A. paniculata* and establishes a foundation for further pharmacognostic and phytochemical investigations. Since qualitative screening provides only preliminary information, additional studies using quantitative and advanced analytical methods are recommended. Determination of andrographolide content, chromatographic fingerprinting, assessment of physicochemical parameters, and evaluation of biological activities would further strengthen the standardization and scientific validation of indigenous *A. paniculata*.

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