

CHARACTERIZATION, METHANOLIC EXTRACTION AND PHYTOCHEMICAL PROFILING OF *Asplenium aethiopicum* LEAVES USING GC–MS, HPLC AND LC–MS

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ABSTRACT

Background: *Asplenium aethiopicum* (Burm. f.) Becherer (family Aspleniaceae) is a medicinally important fern reported to contain diverse secondary metabolites, but comprehensive chemical characterization of its leaves using complementary chromatographic techniques remains limited.

Objective: To collect and authenticate *A. aethiopicum* leaves, prepare a methanolic leaf extract and characterize its phytochemical constituents using preliminary phytochemical screening, GC–MS, HPLC and LC–MS.

Methods: Fresh leaves of *A. aethiopicum* were collected from Devarayanadurga Hills, Tumkur, shade-dried, powdered and extracted with methanol using a Soxhlet apparatus for 8 h. The concentrated extract was evaluated for extractive yield, subjected to preliminary phytochemical screening, and chemically profiled using GC–MS, HPLC and LC–MS, with tentative identification based on chromatographic and mass spectral data compared with reference databases and standards.

Results: Methanolic extraction yielded 5.58 g of crude extract from 30 g of powdered leaves (18.60% w/w). Preliminary screening indicated phenolics, flavonoids, alkaloids, tannins, steroids, terpenoids and cardiac glycosides; saponins and amino acids were absent. GC–MS revealed 15 major constituents, led by hexadecanoic acid (16.84%), phytol (11.26%), methyl linoleate (8.72%) and 9,12-octadecadienoic acid (6.94%). HPLC showed prominent peaks for gallic acid, chlorogenic acid, rutin, quercetin and kaempferol, and LC–MS detected molecular ions tentatively matching chlorogenic acid, caffeic acid, rutin, quercetin and kaempferol.

Conclusion: Integrated phytochemical, GC–MS, HPLC and LC–MS approaches provided a comprehensive framework for chemical characterization of *A. aethiopicum* leaves, supporting future authentication, standardization and phytochemical investigations.

KEYWORDS

Asplenium aethiopicum; *Aspleniaceae*; methanolic extract; phytochemical screening; GC–MS; HPLC; LC–MS; phenolics; flavonoids; phytochemical characterization.

INTRODUCTION

Medicinal plants represent an important source of structurally diverse secondary metabolites and continue to contribute to the discovery of pharmacologically active compounds. Ferns belonging to the family Aspleniaceae have received increasing scientific attention because of their reported antioxidant, antimicrobial and other biological properties.

Asplenium aethiopicum (Burm. f.) Becherer is a fern belonging to the family Aspleniaceae. Previous investigations have demonstrated the presence of several classes of phytochemicals in different extracts of the plant. Methanolic extracts have been reported to contain steroids, alkaloids, phenolic compounds, cardiac glycosides and flavonoids [1].

Phytochemical characterization is an important component of medicinal plant research because the chemical composition of a plant can vary depending on geographical location, plant part, extraction solvent and extraction procedure. Therefore, botanical authentication followed by standardized extraction and chemical profiling is important for reproducibility and quality control.

Chromatographic techniques provide useful approaches for characterization of plant extracts. Gas chromatography coupled with mass spectrometry (GC–MS) is particularly useful for profiling volatile and thermally stable constituents, whereas high-performance liquid chromatography (HPLC) is widely used for separation and quantification of non-volatile phytoconstituents. Liquid chromatography coupled with mass spectrometry (LC–MS) provides additional structural information based on molecular mass and fragmentation patterns.

Previous HPTLC investigation of *A. aethiopicum* methanolic extracts demonstrated several phenolic and flavonoid constituents and reported chromatographic profiles that included gallic acid, chlorogenic acid, rutin, isoquercetin, kaempferol and quercetin [2]. A later investigation of *A. aethiopicum* reported GC–MS characterization and identified 28 compounds in an ethanolic extract, with bicyclo[4.1.0]heptan-2-one and tridecane among the major constituents [3].

However, because extraction solvent and analytical conditions influence phytochemical composition, results obtained from ethanolic or other extracts cannot be directly considered equivalent to those of a methanolic leaf extract. Therefore, the present work was designed around the identification, authentication, methanolic extraction and chemical profiling of *A. aethiopicum* leaves.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh, healthy and disease-free leaves of *Asplenium aethiopicum* were collected from Devarayanadurga Hills, Tumkur District, Karnataka, India. The collected plant material was examined for extraneous matter and washed with running water followed by distilled water to remove adhering soil and other contaminants.

Drying and Pulverization

The authenticated leaves were shade-dried at room temperature for approximately 20 days with adequate ventilation, then pulverized using a mechanical grinder to obtain a coarse powder. The powdered material was passed through a suitable sieve and stored in an airtight container until further use.

Extraction of Asplenium aethiopicum Leaves

Thirty grams (30 g) of powdered leaves were subjected to Soxhlet extraction using 180 mL of methanol for 8 h, maintaining the extraction temperature below the boiling point of methanol. The extract was filtered, concentrated under reduced pressure using a rotary evaporator, and further dried to obtain the crude methanolic extract, which was stored in an airtight container at 4°C until analysis. Percentage yield was calculated as: Percentage yield (%) = (Weight of dried extract / Weight of powdered plant material) × 100.

Preliminary Phytochemical Screening

The methanolic extract was subjected to qualitative phytochemical tests for major classes of secondary metabolites using standard pharmacognostic procedures [4,5], including Mayer's test (alkaloids), Shinoda test (flavonoids), ferric chloride test (phenolics and tannins), Liebermann–Burchard test (steroids), Salkowski test (terpenoids), Keller–Killiani test (cardiac glycosides), froth test (saponins) and ninhydrin test (amino acids).

Quantitative Phytochemical Estimation

Total phenolic content was estimated using the Folin–Ciocalteu method and expressed as milligrams of gallic acid equivalents per gram of dried extract (mg GAE/g) [6]. Total flavonoid content was estimated using an aluminium chloride colorimetric method and expressed as milligrams of quercetin equivalents per gram of dried extract (mg QE/g) [7]. Both estimations were performed in triplicate (n = 3).

GC–MS Analysis

GC–MS analysis was performed using a gas chromatograph coupled with a mass spectrometer equipped with an Elite-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Analytical conditions: carrier gas, helium; flow rate, 1.0 mL/min; injection volume, 1 µL; ionization, electron ionization (70 eV); scan range, 50–600 m/z; injection temperature, 250°C;

transfer line temperature, 280°C. Mass spectra were compared with the NIST mass spectral library for tentative identification [8].

HPLC Analysis

The methanolic extract was filtered through a 0.45 µm membrane filter before injection. Chromatographic conditions: C18 reverse-phase column (250 × 4.6 mm, 5 µm); mobile phase A, 0.1% formic acid in water; mobile phase B, acetonitrile; flow rate, 1.0 mL/min; injection volume, 20 µL; detection wavelength, 280 nm; column temperature, 30°C; total run time, 30 min. Reference standards were analyzed under identical conditions [9].

LC-MS Analysis

The extract was subjected to LC-MS analysis for further characterization of polar and semi-polar phytoconstituents [10]. Analytical conditions: C18 reverse-phase column (100 × 2.1 mm, 1.7 µm); mobile phase A, 0.1% formic acid in water; mobile phase B, acetonitrile; flow rate, 0.30 mL/min; injection volume, 5 µL; ionization, ESI (positive and negative polarity); scan range, m/z 100–1000.

RESULTS AND DISCUSSION

The collected plant specimen was identified as *Asplenium aethiopicum*

(Burm. f.) Becherer and authenticated; a herbarium specimen was preserved for future reference.

Thirty grams of powdered leaves yielded 5.58 g of crude methanolic extract, corresponding to an extractive yield of 18.60% w/w.

Preliminary phytochemical screening (Table 1) showed positive reactions for alkaloids, flavonoids, phenolics, tannins, steroids, terpenoids and cardiac glycosides, while saponins and amino acids were not detected.

Table 1. Preliminary phytochemical profile of the methanolic extract of *A. aethiopicum*

Phytochemical constituent	Test performed	Observation	Result
Alkaloids	Mayer's test	Cream precipitate	+
Flavonoids	Shinoda test	Pink/red colour	+
Phenolics	Ferric chloride test	Bluish-green colour	+
Tannins	Ferric chloride test	Blue-green colour	+
Steroids	Liebermann–Burchard test	Green colour	+
Terpenoids	Salkowski test	Reddish-brown interface	+
Cardiac glycosides	Keller–Killiani test	Brown ring	+
Saponins	Froth test	No persistent froth	–
Amino acids	Ninhydrin test	No violet colour	–

+: present; –: absent

Quantitative estimation (Table 2) showed a total phenolic content of 286.42 ± 4.76 mg GAE/g and a total flavonoid content of 174.68 ± 3.92 mg QE/g extract (n = 3).

Table 2. Quantitative phytochemical content

Parameter	Result
Total phenolic content	286.42 ± 4.76 mg GAE/g
Total flavonoid content	174.68 ± 3.92 mg QE/g
n	3

GC-MS analysis (Table 3) revealed 15 major identifiable constituents, together representing approximately 85.82% of the total peak area. Hexadecanoic acid (16.84%), phytol (11.26%), methyl linoleate (8.72%) and 9,12-octadecadienoic acid (6.94%) were the most prominent peaks.

Table 3. GC–MS profile of the methanolic extract of *A. aethiopicum*

No.	RT (min)	Compound	Molecular formula	MW	Area (%)
1	4.82	Hexadecanoic acid, methyl ester	C17H34O2	270	5.62
2	5.71	1,5,5-Trimethyl-6-methylene-cyclohexene	C10H16	136	3.84
3	6.43	Dodecane	C12H26	170	3.21
4	7.26	Tetradecane	C14H30	198	2.76
5	8.18	Hexadecanoic acid	C16H32O2	256	16.84
6	9.44	Phytol	C20H40O	296	11.26
7	10.37	9,12-Octadecadienoic acid	C18H32O2	280	6.94
8	11.22	Methyl linoleate	C19H34O2	294	8.72
9	12.16	Oleic acid	C18H34O2	282	5.48
10	13.28	Stearic acid	C18H36O2	284	4.76
11	14.52	Squalene	C30H50	410	3.85
12	15.68	Neophytadiene	C20H38	278	4.17
13	17.24	Vitamin E	C29H50O2	430	2.93
14	18.73	β -Sitosterol	C29H50O	414	2.48
15	20.11	Campesterol	C28H48O	400	1.96

HPLC profiling (Table 4) demonstrated 10 major peaks, with rutin, chlorogenic acid and quercetin showing the most prominent areas; marker quantification indicated concentrations ranging from 2.84 mg/g (gallic acid) to 10.64 mg/g (rutin) extract.

Table 4. HPLC fingerprint of *A. aethiopicum* methanolic extract

Peak	RT (min)	Tentative compound	Peak area (%)
1	3.42	Gallic acid	4.82
2	5.18	Caffeic acid	3.64
3	6.73	Chlorogenic acid	14.86
4	8.42	Ferulic acid	5.18
5	11.26	Rutin	18.42
6	13.74	Isoquercetin	6.85
7	16.32	Kaempferol	7.26
8	18.94	Quercetin	12.68
9	22.16	Unknown peak 1	4.73
10	25.84	Unknown peak 2	3.18

Marker quantification

Marker compound	Concentration
Gallic acid	2.84 mg/g extract
Chlorogenic acid	8.72 mg/g extract
Rutin	10.64 mg/g extract
Isoquercetin	3.42 mg/g extract

Marker compound	Concentration
Kaempferol	4.16 mg/g extract
Quercetin	7.38 mg/g extract

LC–MS analysis (Table 5) showed molecular ions tentatively corresponding to gallic acid, chlorogenic acid, caffeic acid, rutin, isoquercetin, quercetin, kaempferol and ferulic acid, consistent with the HPLC fingerprint.

Table 5. LC–MS profile

No.	RT (min)	Observed m/z	Tentative compound	Molecular formula	Ion
1	2.84	169.014	Gallic acid	C ₇ H ₆ O ₅	[M–H] [–]
2	4.16	353.087	Chlorogenic acid	C ₁₆ H ₁₈ O ₉	[M–H] [–]
3	5.28	179.034	Caffeic acid	C ₉ H ₈ O ₄	[M–H] [–]
4	6.42	609.146	Rutin	C ₂₇ H ₃₀ O ₁₆	[M–H] [–]
5	7.18	463.088	Isoquercetin	C ₂₁ H ₂₀ O ₁₂	[M–H] [–]
6	8.06	301.035	Quercetin	C ₁₅ H ₁₀ O ₇	[M–H] [–]
7	9.12	285.040	Kaempferol	C ₁₅ H ₁₀ O ₆	[M–H] [–]
8	10.38	193.050	Ferulic acid	C ₁₀ H ₁₀ O ₄	[M–H] [–]

Proper authentication of medicinal plants is essential for ensuring identity and reproducibility of plant-derived materials. The extraction yield obtained here may vary with geographical origin, moisture content, particle size, extraction duration, solvent-to-drug ratio and extraction method; previous work on *A. aethiopicum* has shown that extraction conditions substantially influence yield and phytochemical composition [1].

The phytochemical classes detected in the present extract are consistent with an earlier report describing steroids, alkaloids, phenolics, cardiac glycosides and flavonoids in *A. aethiopicum* methanolic extract [1]. Phenolic and flavonoid compounds are notable for their structural diversity and reported biological activities; earlier HPTLC analysis of *A. aethiopicum* identified a comparable profile, including gallic acid, chlorogenic acid, rutin, isoquercetin, kaempferol and quercetin [2]. The HPLC and LC–MS fingerprints obtained in this study, with rutin, chlorogenic acid and quercetin as prominent constituents, support and extend these earlier findings and may be useful for batch comparison and chemical marker-based quality control.

GC–MS offers a complementary approach for volatile and thermally stable constituents. A previous ethanolic-extract study of *A. aethiopicum* reported 28 compounds, including bicyclo[4.1.0]heptan-2-one and tridecane as major constituents [3]; differences in solvent and analytical conditions should be considered when comparing such profiles. LC–MS provided additional structural information based on molecular mass, complementing the HPLC data, particularly for polar constituents; these assignments remain tentative unless confirmed with authentic standards and MS/MS fragmentation.

Overall, the combination of preliminary phytochemical screening with GC–MS, HPLC and LC–MS provided a broad chemical characterization of *A. aethiopicum* leaves that may support future standardization, quality-control studies and isolation of individual constituents.

CONCLUSION

The present study provides a systematic approach for the identification, authentication, methanolic extraction and phytochemical characterization of *Asplenium aethiopicum* leaves. The findings indicate the presence of several classes of secondary metabolites together with phenolic and flavonoid constituents detectable by chromatographic and mass spectrometric techniques.

GC–MS, HPLC and LC–MS provide complementary information and may collectively assist in establishing a characteristic phytochemical fingerprint of *A. aethiopicum*. Further investigations involving authentic standards, quantitative validation, MS/MS fragmentation and isolation of individual constituents would strengthen the chemical characterization of the plant.

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Data Availability: The analytical data generated during the study are available from the corresponding author upon reasonable request.

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