Research Article

Phytochemical Profiling of An 80% Ethanolic Extract of Hypericum Perforatum L. Aerial Parts By Preliminary Screening and LC-MS

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ABSTRACT

Background: Hypericum perforatum L. (St. John's wort, Hypericaceae) is a widely used medicinal plant whose biological activity is attributed to a complex mixture of flavonoids, phenolic acids, biflavonoids, naphthodianthrones and phloroglucinols. The chemical composition of an extract depends on plant part, solvent and extraction method, so each preparation needs its own characterization.

Objective: To prepare an 80% ethanolic extract of H. perforatum aerial parts and characterize its phytochemical composition by preliminary qualitative screening and liquid chromatography–mass spectrometry (LC-MS).

Methods: Shade-dried, powdered aerial parts collected in November 2025 were extracted by Soxhlet extraction with 80% ethanol for 48 h. Percentage yield was calculated on a dry-powder weight basis. The extract was screened with standard chemical tests for alkaloids, flavonoids, phenolics and tannins, anthraquinones, triterpenoids, sterols, saponins and glycosides, and was analyzed by LC-MS in electrospray negative (ES^-) and positive (ES^+) modes.

Results: The extractive yield was 11.45% w/w. Screening indicated flavonoids, phenolic compounds and tannins, anthraquinones, triterpenoids and phytosterols; saponins were not detected, and alkaloids gave a positive result with only one of three reagent groups. LC-MS showed about 20 peaks in ES^- and about 17 in ES^+ mode. Eight compounds were assigned by retention time and molecular ions: protocatechuic acid, chlorogenic acid, isoquercitrin, rutin, hyperoside, kaempferol-3-O-glucoside, quercetin and amentoflavone. Together these accounted for 95.2% of the total chromatographic peak area. Kaempferol-3-O-glucoside (37.30%), hyperoside (32.24%) and quercetin (14.83%) were the dominant peaks.

Conclusion: The 80% ethanolic extract is a flavonoid-dominated preparation, with flavonoids and the biflavonoid amentoflavone representing 93.2% of the peak area. These data provide a chemical fingerprint for the extract and a basis for standardization and further study.

Keywords: Hypericum perforatum; St. John's wort; ethanolic extract; phytochemical screening; LC-MS; flavonoids; hyperoside; kaempferol-3-O-glucoside.

INTRODUCTION

Hypericum perforatum L., commonly known as St. John's wort, is a herbaceous perennial of the family Hypericaceae. It has a documented history of medicinal use of more than two thousand years, and it remains one of the most widely used herbal medicines worldwide [1,2]. The species is native to Europe and Asia and is now naturalized on every continent. In India it grows in the temperate and sub-alpine Himalayan zone, and it is recognized in the Ayurvedic and Unani systems and in the Indian Herbal Pharmacopoeia [3].

The phytochemistry of the plant is complex. The aerial parts contain naphthodianthrones (hypericin and pseudohypericin), prenylated phloroglucinols (hyperforin and adhyperforin), flavonoids (hyperoside, rutin, quercitrin, isoquercitrin, quercetin and kaempferol), biflavonoids (amentoflavone and biapigenin), phenolic acids (chlorogenic and caffeic acids), xanthones, tannins, procyanidins and essential oils [1,4]. Hypericin is typically present at 0.03–0.3% of the dried herb [1]. Hyperforin is the most abundant lipophilic constituent of hydroalcoholic extracts [5,6]. Amentoflavone has been reported to bind brain benzodiazepine receptors with high affinity in vitro [7], and

rutin is reported to show antioxidant, anti-allergic and anti-inflammatory activity [8].

Because these classes differ widely in polarity and stability, the profile of any given extract depends on the plant part, harvest, solvent and extraction technique [5,9]. Alcoholic extracts of the plant have been characterized as rich in flavonoids and phenolic acids [5]. However, detailed chemical data for extracts prepared in a given laboratory are often not reported alongside the extract, which limits reproducibility and the interpretation of any later biological work.

The present study therefore aimed to (i) collect, authenticate and extract the aerial parts of H. perforatum with 80% ethanol; (ii) determine the extractive yield; and (iii) characterize the major phytoconstituents of the extract by qualitative chemical screening and LC-MS.

MATERIALS AND METHODS

Plant material and authentication

Fresh aerial parts (leaves, stems and flowers) of Hypericum perforatum L. were collected in November 2025 [collection site / supplier to be stated]. The plant was authenticated by Mrs. V. U. Waghamare, Department of Botany, Willingdon College, Sangli. [Voucher specimen number and herbarium deposition to be added.]

Preparation of the extract

The collected material was cleaned to remove dust and surface impurities and dried in the shade. The dried aerial parts were powdered with a mixer grinder. The powder was extracted in a Soxhlet apparatus with 80% ethanol as solvent for 48 h, following previously described solvent-extraction approaches for this species [5,9]. The extract was dried and concentrated at room temperature, weighed, and stored in a tightly closed container until analysis.

Extractive yield

The percentage yield was calculated as:

Percentage yield (% w/w) = (weight of extract ÷ weight of dried powder) × 100

Preliminary phytochemical screening

The extract was subjected to standard qualitative chemical tests to detect the following classes. The tests used are given in parentheses.

- Alkaloids (Mayer's, Bertrand's and Valser's tests; Dragendorff's and Kraut's tests; Wagner's test)
- Flavonoids (concentrated H_2SO_4 test, alkaline reagent test, lead acetate test)
- Phenolic compounds and tannins (ferric chloride test, gelatin test)
- Anthraquinones (Borntrager's test, ammonium hydroxide test)
- Triterpenoids (Salkowski's test)
- Sterols/phytosterols (sulphur test, Hesse's reaction)
- Saponins (foam test)
- Glycosides (Keller–Killiani test, bromine water test)

A change characteristic of each test was recorded as positive (+), and its absence as negative (−).

2.5 LC-MS analysis

The 80% ethanolic extract was analyzed at Honeychem Pharma Analytical Services Private Limited, Bangalore 560058, Karnataka, India. A Shimadzu LC-MS system operated with LCsolution software was used. The prepared sample (5 µL) was injected, and data were acquired in both electrospray negative (ES^-) and positive (ES^+) ionization modes over a retention window of about 0.5–15.5 min (data files acquired February 2026). [Column, mobile phase and gradient, flow rate, column temperature, detector channel, ion-source and mass-range settings, sample preparation and concentration to be added from the analytical report.]

Compounds were assigned from retention time and the $[M+H]^+$ (ES^+) and $[M-H]^-$ (ES^-) ions of the corresponding mass spectra. The relative abundance of each compound is expressed as the percentage of the total

chromatographic peak area.

RESULTS

Extractive yield

Soxhlet extraction of the dried aerial parts with 80% ethanol gave a yield of 11.45% w/w.

Preliminary phytochemical screening

The results of the qualitative tests are summarized in Table 1. Flavonoids, phenolic compounds and tannins, anthraquinones, triterpenoids and phytosterols were detected. Saponins were absent. Alkaloids were negative with Mayer's, Bertrand's, Valser's and Wagner's reagents but positive with Dragendorff's and Kraut's reagents. Among the glycoside tests, the Keller–Killiani test was negative and the bromine water test was positive.

Table 1. Preliminary phytochemical screening of the 80% ethanolic extract of H. perforatum aerial parts (+ present; − absent).

Phytoconstituent	Test	Result
Alkaloids	Mayer's / Bertrand's / Valser's	−
	Dragendorff's / Kraut's	+
	Wagner's	−
Flavonoids	Conc. H_2SO_4	+
	Alkaline reagent	+
	Lead acetate	+
Phenolic compounds and tannins	Ferric chloride	+
	Gelatin	+
Anthraquinones	Borntrager's	+
	Ammonium hydroxide	+
Triterpenoids	Salkowski's	+
Sterols / phytosterols	Sulphur test	+
	Hesse's reaction	+
Saponins	Foam test	−
Glycosides	Keller–Killiani	−
	Bromine water	+

LC-MS profile

The chromatograms showed a complex mixture of constituents, with about 20 distinct peaks in ES^- mode and about 17 in ES^+ mode between 0.5 and 15.5 min. The chromatogram of the extract (Figure 1) is dominated by a cluster of peaks between 6 and 7.5 min. Eight peaks were assigned to known constituents of the plant (Table 2). Each gave the pseudomolecular ions expected for its molecular weight, with $[M+H]^+$ in ES^+ mode and $[M-H]^-$ in ES^- mode (for example, m/z 465/463 for the molecular weight of 464.10 Da).

Table 2. Compounds assigned in the 80% ethanolic extract of H. perforatum by LC-MS.

No.	RT (min)	Compound	Class	MW (Da)	ES^+ ion (m/z)	ES^- ion (m/z)	Peak area (%)
1	1.26	Protocatechuic acid	Phenolic acid	154.12	155	153	0.748
2	4.49	Chlorogenic acid	Phenolic acid	354.31	355	353	1.289

No.	RT (min)	Compound	Class	MW (Da)	ES^+ ion (m/z)	ES^- ion (m/z)	Peak area (%)
3	5.61	Isoquercitrin	Flavonol glycoside	464.10	465	463	0.218
4	5.78	Rutin	Flavonol glycoside	610.52	611	609	2.807
5	6.10	Hyperoside	Flavonol glycoside	464.10	465	463	32.236
6	6.32	Kaempferol-3-O-glucoside	Flavonol glycoside	448.38	449	447	37.304
7	6.90	Quercetin	Flavonol aglycone	302.04	303	301	14.830
8	7.46	Amentoflavone	Biflavonoid	538.50	539	537	5.766

Figure 1. LC-MS chromatogram of the extract (retention time in min on the x-axis; detector response in mV on the y-axis).

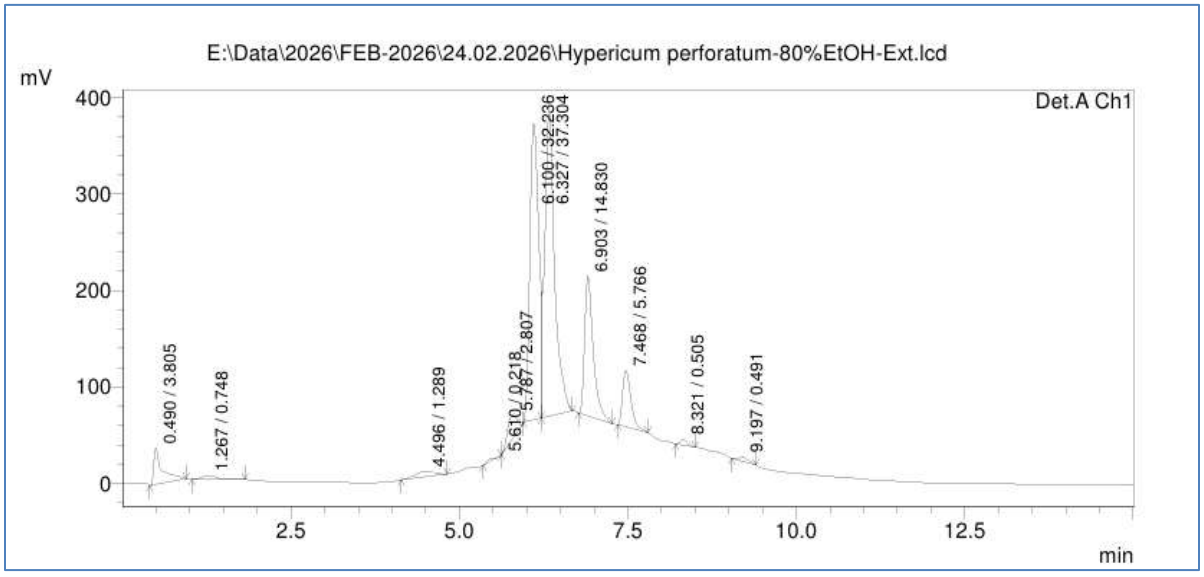


Figure 2. LC-MS Chromatogram of Hypericum perforatum L. extract

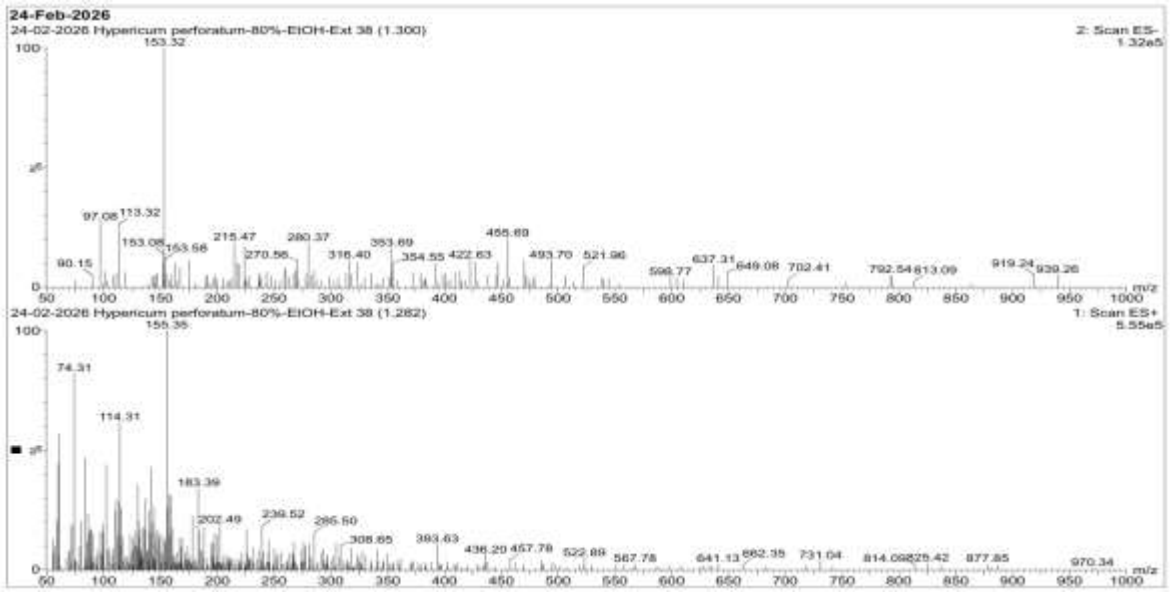


Figure 3. Protocatechuic acid (RT-1.26) mass spectrum

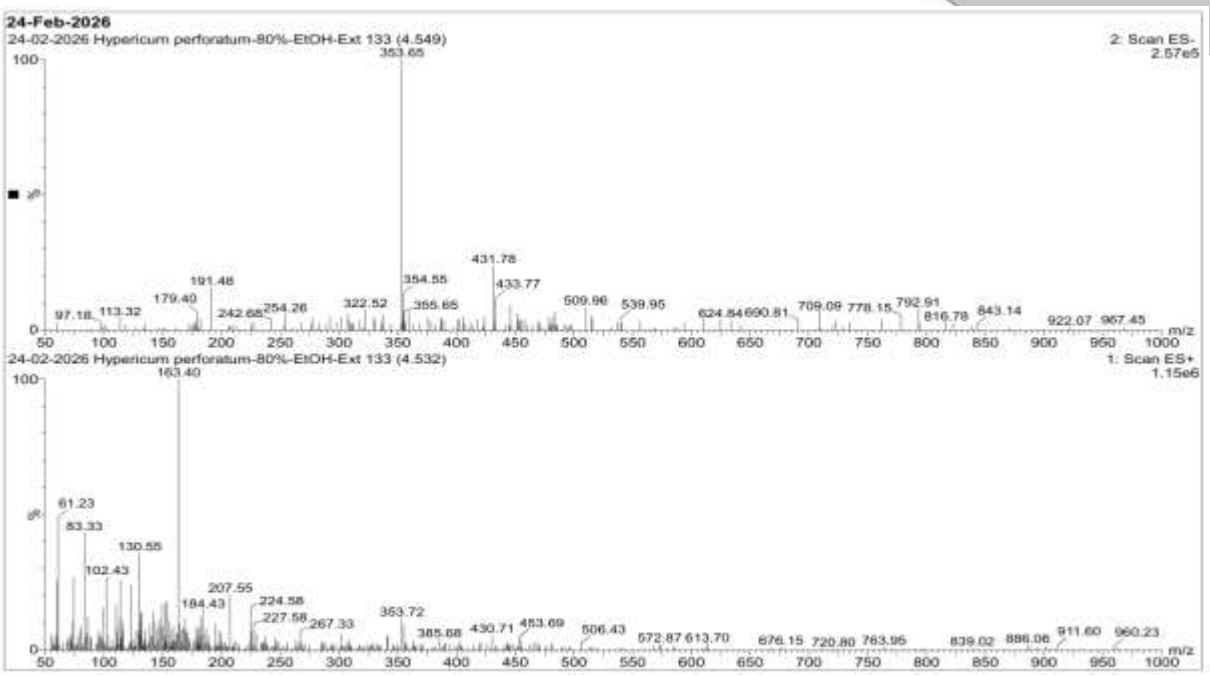


Figure 4. Chlorogenic acid (RT-4.49) Mass Spectrum

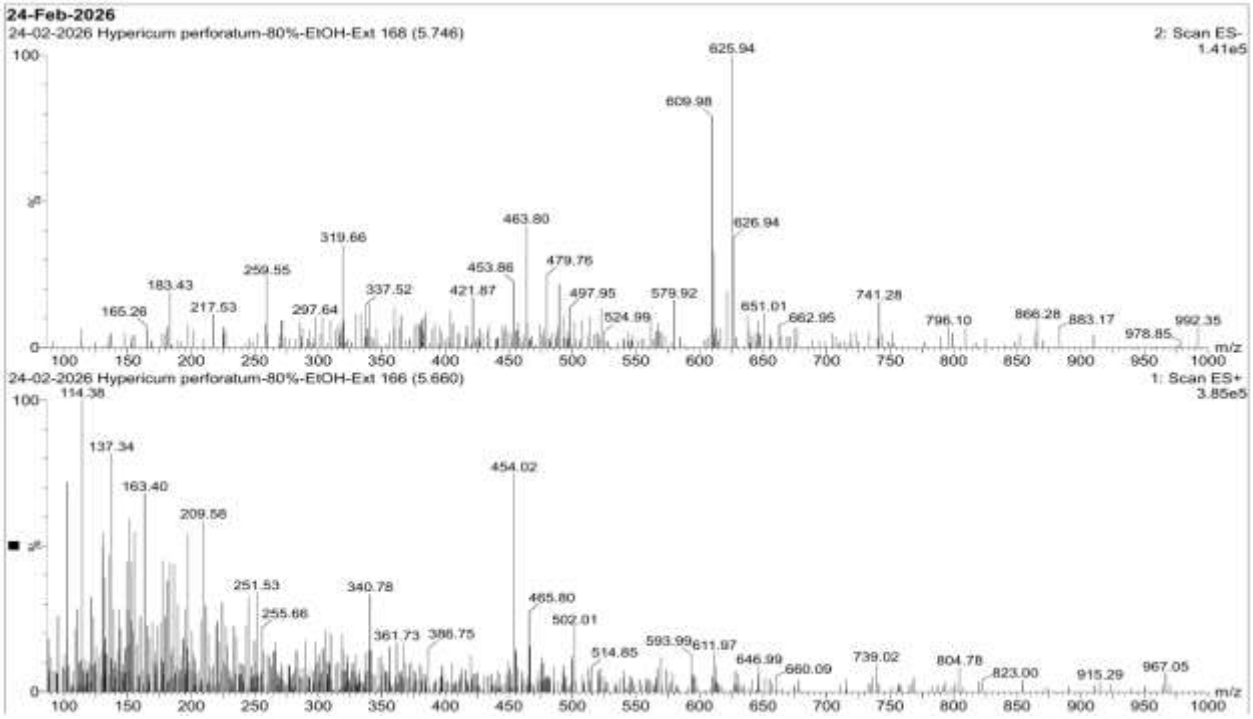


Figure 5. Isoquercitrin acid (RT-4.49) Mass Spectrum

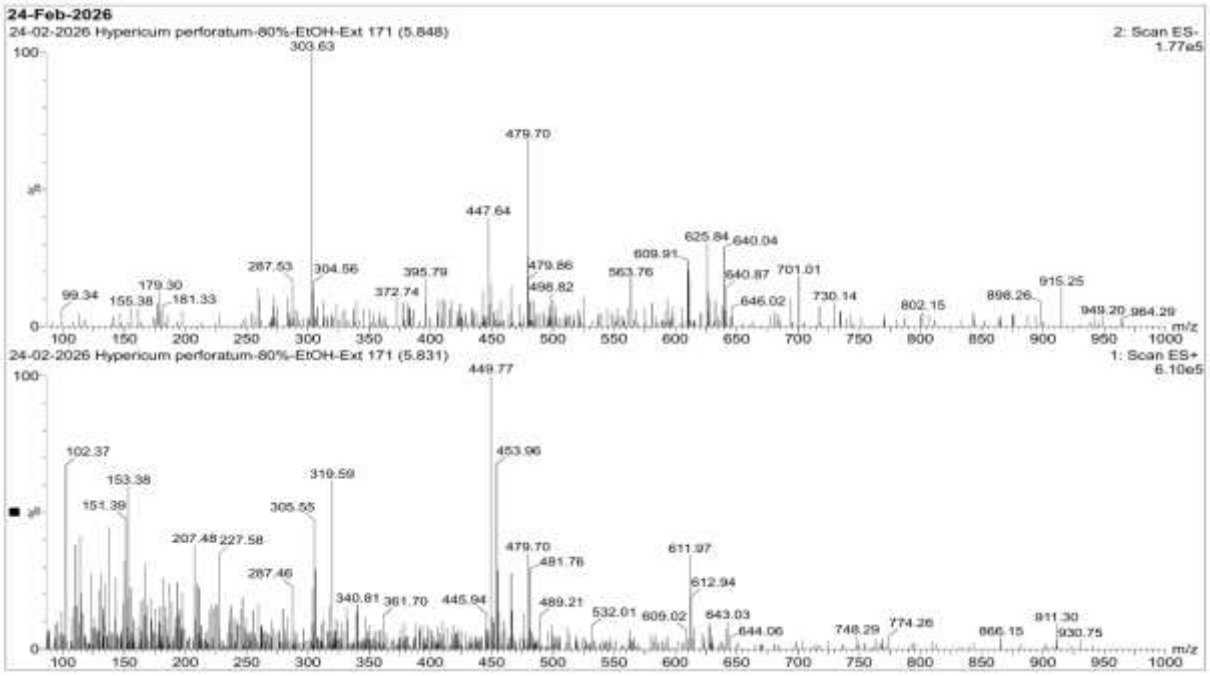


Figure 6. Rutin (RT-5.78) Mass Spectrum

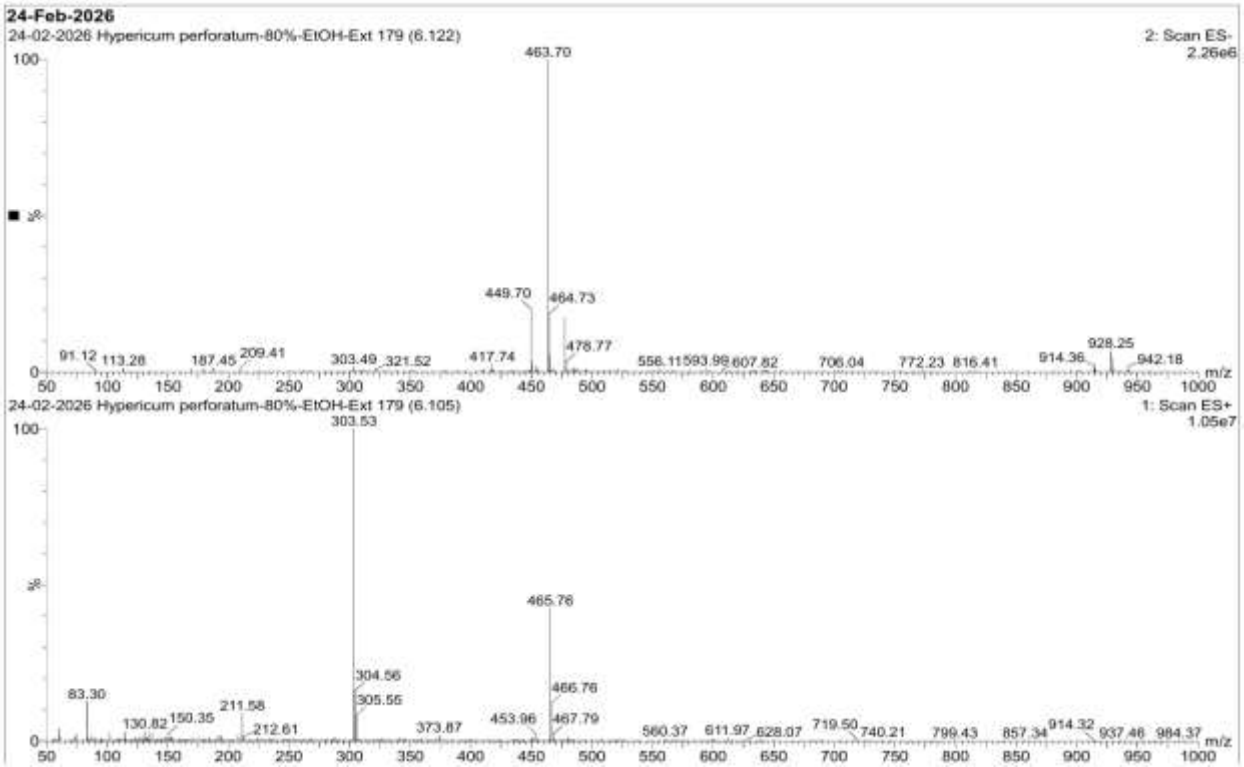


Figure 7. Hyperoside (RT-6.10) Mass Spectrum

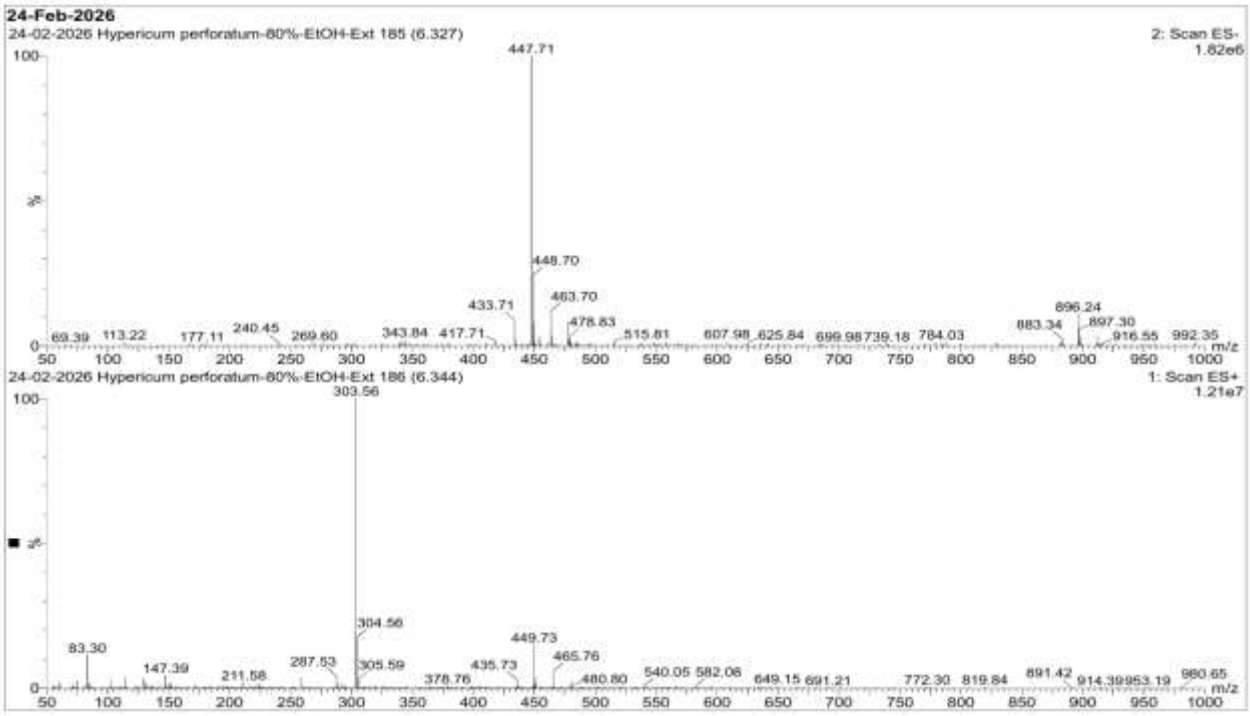


Figure 8. kaempferol-3-O-glucoside (RT-6.32) Mass Spectrum

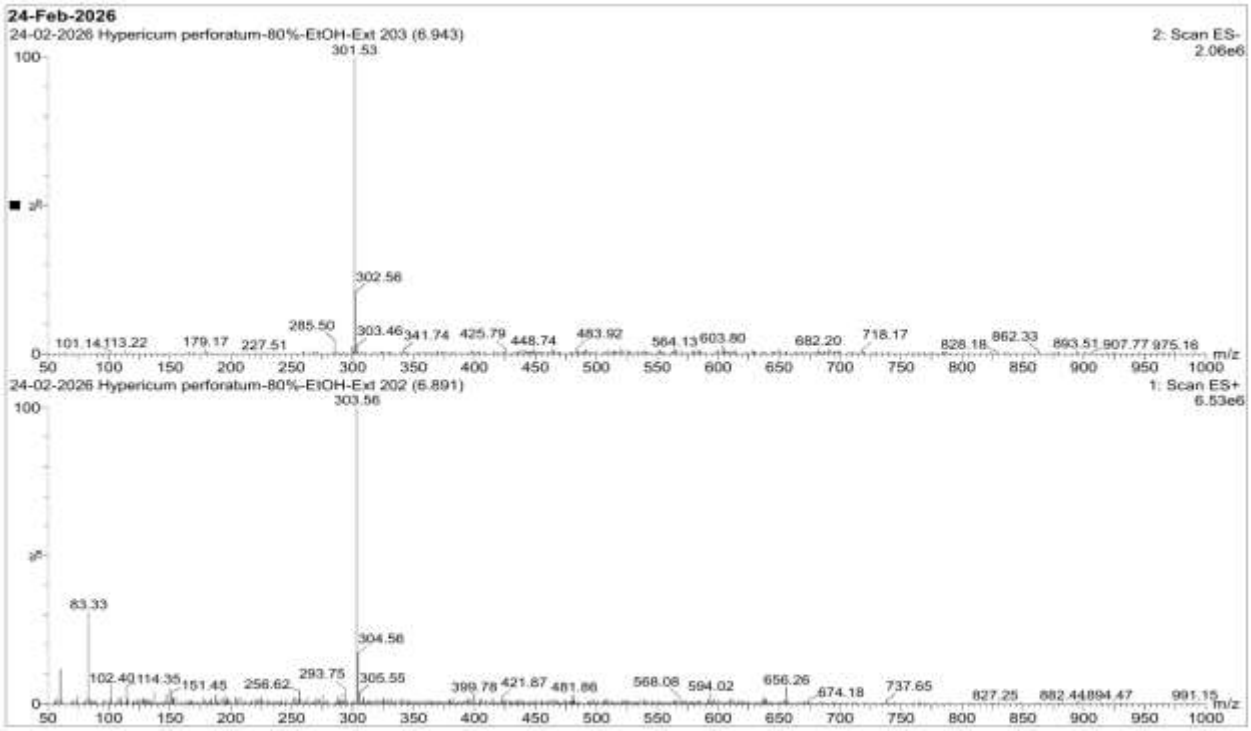


Figure 9. Quercetin (RT-6.90) Mass Spectrum

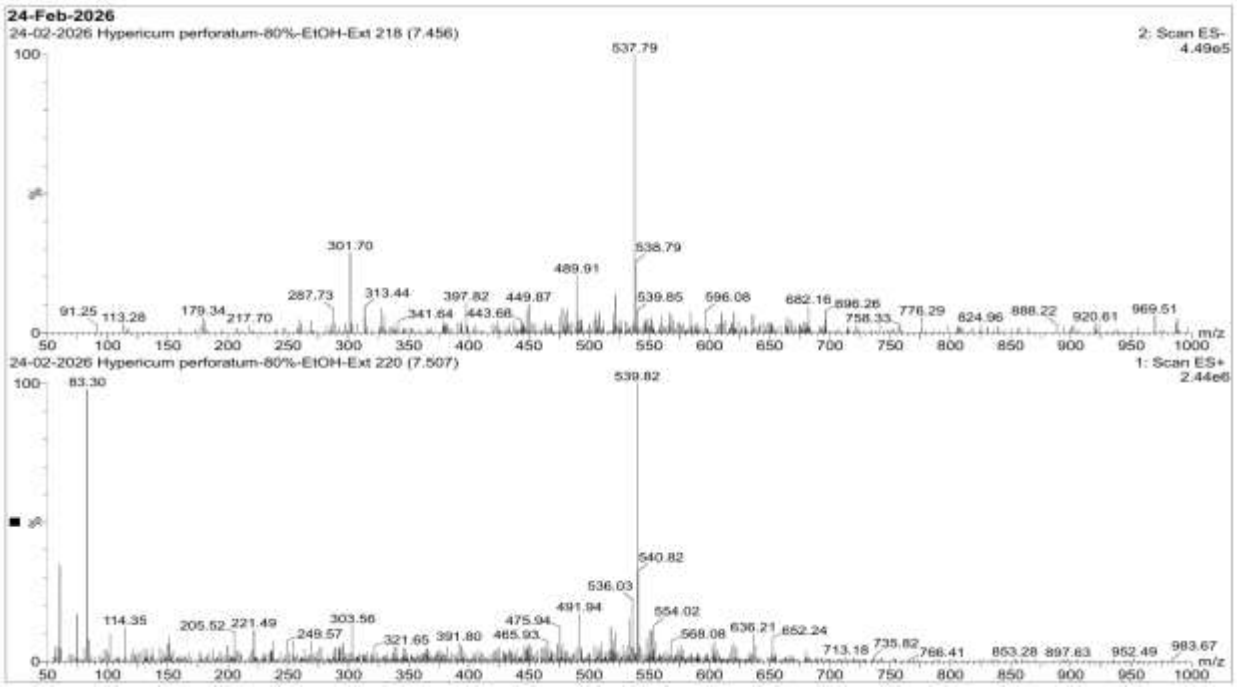


Figure 10. Amentoflavone (RT-7.46) Mass Spectrum

The eight assigned compounds accounted for 95.20% of the total peak area (Table 3). Flavonoids and amentoflavone together represented 93.16%, and the two phenolic acids represented 2.04%. Kaempferol-3-O-glucoside and hyperoside alone made up 69.54%. The quercetin-based constituents (quercetin, hyperoside, isoquercitrin and rutin) totalled 50.09%. Three minor peaks, at about 0.43, 8.32 and 9.20 min (3.81%, 0.51% and 0.49% respectively), were not assigned.

Table 3. Distribution of the chromatographic peak area by compound class.

Class	Compounds	Peak area (%)
Flavonol glycosides	Kaempferol-3-O-glucoside, hyperoside, rutin, isoquercitrin	72.565
Flavonol aglycone	Quercetin	14.830
Biflavonoid	Amentoflavone	5.766
Phenolic acids	Chlorogenic acid, protocatechuic acid	2.037
Unassigned peaks	Peaks at 0.43, 8.32 and 9.20 min	4.806
Total		**100.004**

Values are rounded as reported for the individual peaks; the total differs slightly from 100 because of rounding.

DISCUSSION

The 80% ethanolic extract of H. perforatum aerial parts was obtained in a yield of 11.45% w/w, which indicates that the hydroalcoholic solvent extracted a substantial proportion of the polar and moderately polar constituents of the dried herb. The qualitative tests and the LC-MS data agree on the main chemical character of the extract: it is rich in flavonoids and phenolic compounds.

The LC-MS profile is dominated by flavonol glycosides. Kaempferol-3-O-glucoside, hyperoside and quercetin were the three largest peaks. This matches earlier descriptions of alcoholic Hypericum extracts as rich in flavonoids and phenolic acids [5] and the flavonoid list reported for the aerial parts [1,4]. Amentoflavone, the biflavonoid found

here at 5.77% of the peak area, is a recognized constituent of the genus [1,4] and has been reported to bind benzodiazepine receptors in vitro [7]. The phenolic acids chlorogenic and protocatechuic acid were minor components. Both are reported to have antioxidant and neuroprotective properties in experimental systems [10,11].
The positive screening results for anthraquinones, triterpenoids and phytosterols indicate further compound classes that were not covered by the LC-MS assignments. A positive anthraquinone test may reflect the quinonoid naphthodianthrones of the plant, although the present data cannot establish this. Likewise, alkaloids were detected only with Dragendorff's and Kraut's reagents and were negative with four other reagents. This is best regarded as an equivocal result, since these reagents can also react with non-alkaloidal substances such as phenolics, and alkaloids are not typical major constituents of this species. The negative saponin and Keller–Killiani results indicate the absence of foaming saponins and of cardiac deoxysugar glycosides under the test conditions.
The flavonoid profile also fits the polarity of the solvent. A solvent of intermediate polarity such as 80% ethanol extracts glycosylated flavonoids efficiently, and the choice of extraction solvent is known to determine the proportions of the major constituents recovered from this species [9].

Limitations

Several limitations apply to these data.

- **Relative, not absolute, content.** The percentages in Table 2 are shares of the total chromatographic peak area. They are not concentrations (for example mg/g of extract), because no calibration with reference standards was applied. The values also depend on the detector response of each compound and should be read as a semi-quantitative fingerprint.
- **Identification by retention time and molecular ion.** Compounds were assigned from retention time and $[M+H]^+$ and $[M-H]^-$ ions. Hyperoside and isoquercitrin are isomers (the 3-O-galactoside and 3-O-glucoside of quercetin) with the same molecular weight of 464.10 Da, so mass alone cannot distinguish them; their assignment rests on retention time and should be confirmed with authentic standards or by MS/MS fragmentation.
- **Marker compounds not reported.** Hypericin, pseudohypericin and hyperforin, the best-known markers of H. perforatum, were not among the assigned compounds. Their absence from this profile does not show that they are absent from the extract. It may reflect the analytical conditions, the sample preparation, or the instability of hyperforin and light-sensitivity of hypericin, and a targeted method is needed to address it.
- **Single sample.** One extract from a single collection was analyzed, so batch-to-batch variability was not assessed.

CONCLUSION

The 80% ethanolic Soxhlet extract of Hypericum perforatum aerial parts (yield 11.45% w/w) contains flavonoids, phenolic compounds and tannins, anthraquinones, triterpenoids and phytosterols. LC-MS profiling assigned eight constituents covering 95.2% of the chromatographic peak area. The extract is dominated by the flavonol glycosides kaempferol-3-O-glucoside and hyperoside and by quercetin, with amentoflavone, rutin, chlorogenic acid, protocatechuic acid and isoquercitrin as minor components. This fingerprint characterizes the extract used and can serve as a reference for standardization. Confirmation with reference standards, quantification in absolute units, and targeted analysis of hypericin and hyperforin are the next steps.

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Conflict of interest
No

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