Research Square

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Class-Specific HPTLC Fingerprinting of Triterpenoid Saponins and Protoberberine Alkaloids: A Comparative Standardization Model for Gymnema sylvestre and Berberis aristata Hydroalcoholic Extracts

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Abstract

Gymnema sylvestre R.Br. and *Berberis aristata* DC. are two widely used Ayurvedic antihyperglycemic herbs that are frequently co-formulated in polyherbal preparations, yet their therapeutic reliability depends on consistent phytochemical quality. High-performance thin-layer chromatography (HPTLC) was employed as a preliminary qualitative fingerprinting tool for standardization of hydroalcoholic extracts of both plants. For *Gymnema sylvestre* extract, chromatography was performed on precoated silica gel 60 F_{254} plates using toluene:ethyl acetate:formic acid:methanol as mobile phase, with post-derivatization visualization using 10% sulphuric acid in methanol; three resolved bands at Rf 0.52, 0.33 and 0.19 developed light blue, dark blue and light blue colouration respectively, consistent with an oleanane-type triterpenoid saponin class and corresponding tentatively to gymnemagenin, gymnemic acid IV and deacyl gymnemic acid. For *Berberis aristata* extract, the mobile phase butanol:ethyl acetate:formic acid:water resolved two bands at Rf 0.58 and 0.47, both showing characteristic yellowish-green fluorescence under UV light (254 nm/366 nm) without the need for derivatization, consistent with the protoberberine alkaloid chromophore and tentatively corresponding to berberine and palmatine, with jatrorrhizine co-migrating near the berberine band. The HPTLC fingerprints obtained were reproducible, mobile-phase and detection-mode-appropriate for the respective phytoconstituent classes and provide validated qualitative fingerprints suitable for batch-to-batch identity confirmation of both extracts, forming the analytical foundation for subsequent quantitative chromatographic standardization.

1. Introduction

Diabetes mellitus remains one of the foremost global health emergencies, ranking among the leading causes of mortality worldwide [1, 2]. The escalating burden of Type 2 diabetes and the limitations associated with synthetic antihyperglycemic agents have intensified interest in standardized plant-derived therapeutics [3]. Among these, *Gymnema sylvestre* R.Br. (*Mesasringi*) and *Berberis aristata* DC. (*Daruharidra*) are well-documented Ayurvedic antidiabetic herbs, frequently co-formulated in polyherbal preparations. *Gymnema sylvestre* exerts its antihyperglycemic activity primarily through triterpenoid saponins collectively termed gymnemic acids, together with gymnemasaponins and the polypeptide gurmarin [4]. *Berberis aristata* owes its antihyperglycemic action chiefly to the protoberberine alkaloid berberine, which promotes glycolysis via increased glucokinase activity and suppresses hepatic gluconeogenesis and adipogenesis [5, 6].

The therapeutic reliability of both extracts is contingent upon consistent phytochemical quality, a factor that is often overlooked in herbal drug research. High-performance thin-layer chromatography (HPTLC) offers a rapid, reproducible and cost-effective preliminary fingerprinting tool for establishing the qualitative phytochemical identity of herbal extracts prior to more resource-intensive quantitative chromatographic methods. The present study was undertaken to develop and characterize HPTLC fingerprint profiles for the hydroalcoholic extracts of *Gymnema sylvestre* and *Berberis aristata* as a foundation for their qualitative standardization.

2. Experimental

2.1 Plant Material and Extraction

The leaves of *Gymnema sylvestre* R.Br. and the roots of *Berberis aristata* DC. were procured and authenticated on the basis of pharmacognostical characters described in the Ayurvedic Pharmacopoeia of India. Both raw materials were pulverized to a coarse powder to increase surface area for extraction.

Hydroalcoholic extraction of *Gymnema sylvestre* leaf powder was carried out by a two-stage hot reflux method using methanol:water (70:30 v/v) at a drug-to-solvent ratio of 1:10 (primary reflux, 80 ± 2°C, 3–5 h) followed by a secondary reflux of the marc for 3–4 h. The pooled filtrate was concentrated under reduced pressure (400–500 mmHg; bath temperature 60–70°C) and tray-dried at ≤ 50°C to constant weight.

Hydroalcoholic extraction of *Berberis aristata* root powder was carried out by a dual-stage hot reflux method using a 1:1 hydroalcoholic solvent system (primary reflux, 60–80°C, 3–4 h; secondary reflux, identical conditions, 3 h). The combined extract was vacuum-filtered, evaporated and tray-dried to obtain the solid extract, which was stored under refrigeration in a sealed container until further analysis.

2.2 HPTLC Instrumentation and Materials

HPTLC fingerprinting was performed on precoated silica gel 60 F_{254} plates (10 × 10 cm, E-Merck) as the stationary phase. Sample and standard solutions were applied as 10 mm bands (10 µL per track) using an automatic band applicator. Development was carried out in a Camag twin-trough glass chamber, pre-saturated with the respective mobile phase for 30 min, up to a solvent migration distance of 8 cm from the point of application, at ambient laboratory temperature. Analytical (AR) grade solvents and reagents were used throughout.

2.3 HPTLC Fingerprinting of *Gymnema sylvestre* Hydroalcoholic Extract

The mobile phase employed was toluene: ethyl acetate: formic acid: methanol (3:5:0.5:2 v/v/v/v). For sample preparation, 2.5 g of the hydroalcoholic extract (25 mg/mL) was refluxed with 40 mL of 50% v/v ethanol and 2 mL of 12% w/v potassium hydroxide for 1 h to saponify bound glycosides, followed by acid hydrolysis with 5.5 mL of 4N hydrochloric acid under reflux for a further 1 h. The hydrolysate was cooled, centrifuged, neutralized to pH 7.5–8.5 with 12% w/v potassium hydroxide, and concentrated to approximately 50 mL prior to spotting. Gymnemagenin, deacyl gymnemic acid and gymnemic acid IV (1 mg/mL each in methanol) served as reference standards. Sample and standard solutions (10 µL) were applied as 10 mm bands and developed as described in Section 2.2. After development, the plate was air-dried, derivatized by spraying with 10% sulphuric acid in methanol, and heated at 105°C for 5 min. The resolved bands were examined in white light after derivatization and under UV light (254 nm and 366

nm) prior to derivatization and Rf values and band colours were recorded; the sample fingerprint was compared against the reference standard profile for tentative identification.

2.4 HPTLC Fingerprinting of *Berberis aristata* Hydroalcoholic Extract

The mobile phase employed was butanol: ethyl acetate: formic acid: water (3:5:0.5:1 v/v/v/v). The sample was prepared by dissolving 5 mg of the hydroalcoholic extract in methanol in a 25 mL volumetric flask with sonication for 10 min. Berberine, palmatine and jatrorrhizine (1 mg/5 mL each in methanol) served as reference standards. Sample and standard solutions (10 µL) were applied as 10 mm bands and developed as described in Section 2.2. Detection did not require chemical derivatization. The dried plate was directly visualized and photo-documented under UV light at 254 nm and 366 nm and the sample fingerprint was compared against the reference standard profile for tentative identification.

3. Results and Discussion

3.1 HPTLC Fingerprint of *Gymnema sylvestre* Hydroalcoholic Extract

Under the optimized mobile phase system, the hydroalcoholic extract of *Gymnema sylvestre* resolved into three distinct bands after derivatization at Rf 0.52, 0.33 and 0.19, developing light blue, dark blue and light blue colouration respectively (Fig. 1, Table 1). None of the bands showed absorption under UV light (254/366 nm) prior to derivatization, consistent with the non-aromatic, saturated triterpenoid ring system of gymnagenin-type saponins, which lack a chromophore capable of direct UV detection and therefore require chemical derivatization for visualization. The characteristic light-blue-to-dark-blue colour reaction on derivatization with sulphuric acid-methanol reagent is diagnostic of oleanane-type triterpenoid saponins, and the three resolved bands correspond tentatively to the three principal gymnemic-acid-related marker compounds of the extract-gymnemagenin, gymnemic acid IV and deacyl gymnemic acid.

Table 1
HPTLC conditions and Rf values for *Gymnema sylvestre* hydroalcoholic extract

Parameter	Detail
Stationary phase	Precoated silica gel 60 F_{254} (10 × 10 cm, E-Merck)
Mobile phase	Toluene: ethyl acetate: formic acid: methanol (3:5:0.5:2 v/v/v/v)
Chamber	Camag twin-trough chamber, pre-saturated 30 min
Development distance	8 cm from origin
Spotting volume	10 µL
Band width	10 mm
Derivatizing agent	10% sulphuric acid in methanol; heated at 105°C for 5 min
Visualization	White light after derivatization
Rf value	**Colour under UV (254/366 nm) → Colour after derivatization → Tentative compound class**
0.52	Not visualized → Light blue → Oleanane-type saponin (gymnemagenin)
0.33	Not visualized → Dark blue → Oleanane-type saponin (gymnemic acid IV)
0.19	Not visualized → Light blue → Oleanane-type saponin (deacyl gymnemic acid)

3.2 HPTLC Fingerprint of *Berberis aristata* Hydroalcoholic Extract

The hydroalcoholic extract of *Berberis aristata* resolved into two distinct bands at Rf 0.58 and 0.47, both exhibiting characteristic yellowish-green fluorescence under UV light at 254 nm and 366 nm without any requirement for chemical derivatization (Fig. 2, Table 2). This intense yellowish-green fluorescence is a well-recognized and diagnostic physicochemical property of quaternary isoquinoline (protoberberine) alkaloids-including berberine, palmatine, jatrorrhizine, columbamine and berberrubine- arising from extended aromatic conjugation and the quaternary nitrogen of the isoquinoline ring system. The two resolved bands correspond tentatively to berberine, the band of higher Rf value consistent with its greater lipophilicity relative to the mobile phase composition, and palmatine; the band corresponding to jatrorrhizine was faintly visible and closely overlapping with the berberine band owing to closely approximating Rf values. The inherent UV-active, fluorescent nature of the protoberberine chromophore permitted direct detectability of the alkaloids without chemical modification, in contrast to the derivatization required for the saponin-rich *Gymnema sylvestre* extract, illustrating the complementary nature of the two HPTLC systems developed for these phytochemically distinct herbal extracts.

Table 2
HPTLC conditions and Rf values for *Berberis aristata* hydroalcoholic extract

Parameter	**Detail**
Stationary phase	Precoated silica gel 60 F_{254} (10 × 10 cm, E-Merck)
Mobile phase	Butanol: ethyl acetate: formic acid: water (3:5:0.5:1 v/v/v/v)
Chamber	Camag twin-trough chamber, pre-saturated 30 min
Development distance	8 cm from origin
Spotting volume	10 µL
Band width	10 mm
Derivatizing agent	Not derivatized
Visualization	UV 254 nm and 366 nm
Rf value	**Colour under UV (254/366 nm) → Tentative compound class**
0.58	Yellowish-green fluorescence → Protoberberine alkaloid (berberine)
0.47	Yellowish-green fluorescence → Protoberberine alkaloid (palmatine)

3.3 Comparative Discussion

The two HPTLC systems developed in this study were tailored to the dominant phytoconstituent class of each extract. The absence of UV absorbance in the *Gymnema sylvestre* extract prior to derivatization, together with the light-blue-to-dark-blue colour development on treatment with sulphuric acid-methanol reagent, is compatible with the saturated, non-aromatic triterpenoid ring system of oleanane-type saponins, which require chemical derivatization for visualization. Conversely, the direct, intense yellowish-green UV fluorescence of the *Berberis aristata* bands, requiring no derivatization, reflects the inherently chromophoric and fluorescent quaternary isoquinoline alkaloid skeleton common to the protoberberine class. These complementary qualitative fingerprints establish the identity of the principal phytoconstituents in both hydroalcoholic extracts and serve as validated preliminary tools guiding subsequent quantitative chromatographic standardization of these two Ayurvedic antihyperglycemic herbs.

4. Conclusion

HPTLC fingerprinting provided reproducible, phytoconstituent-class-appropriate qualitative profiles for the hydroalcoholic extracts of *Gymnema sylvestre* and *Berberis aristata*. Three oleanane-type saponin bands (Rf 0.52, 0.33, 0.19) were resolved and visualized on derivatization for *Gymnema sylvestre*, while two directly UV-fluorescent protoberberine alkaloid bands (Rf 0.58, 0.47) were resolved for *Berberis aristata*. These validated HPTLC fingerprints establish a preliminary qualitative standard for both extracts

and provide the analytical foundation for downstream quantitative chromatographic and bioanalytical standardization.

Declarations

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Ethics Approval

Not applicable. The study did not involve human participants, animals, or their biological material; only commercially and pharmacognostically authenticated plant material was used.

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Author Contribution

Hudeda and Malastesh designed the study. Malatesh performed plant extraction, HPTLC method development, sample and standard preparation, chromatographic analysis, and data acquisition. Hudeda supervised research and provided critical input on methodology and interpretation of results. Malatesh analyzed the data and drafted the main manuscript text, including all tables and figures. Hudeda critically revised the manuscript for important intellectual content. Both authors reviewed and approved the final manuscript.

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Data Availability

The datasets generated and/or analysed during the current study, including HPTLC densitograms, are available from the corresponding author on reasonable request.

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Figures



Gymnema sylvestre herb raw material dried leaves



Reflux extraction



Dry extract processed



Berberis aristata herb raw material- dried roots



Extraction and drying



Dry extract processed

Figure 1

Hydroalcoholic extraction method of *Gymnema sylvestre* and *Berberis aristata*



Figure 2

CAMAG Linomat 5: semi-automatic sample applicator designed for precise and reproducible application of samples onto TLC and HPTLC plates.

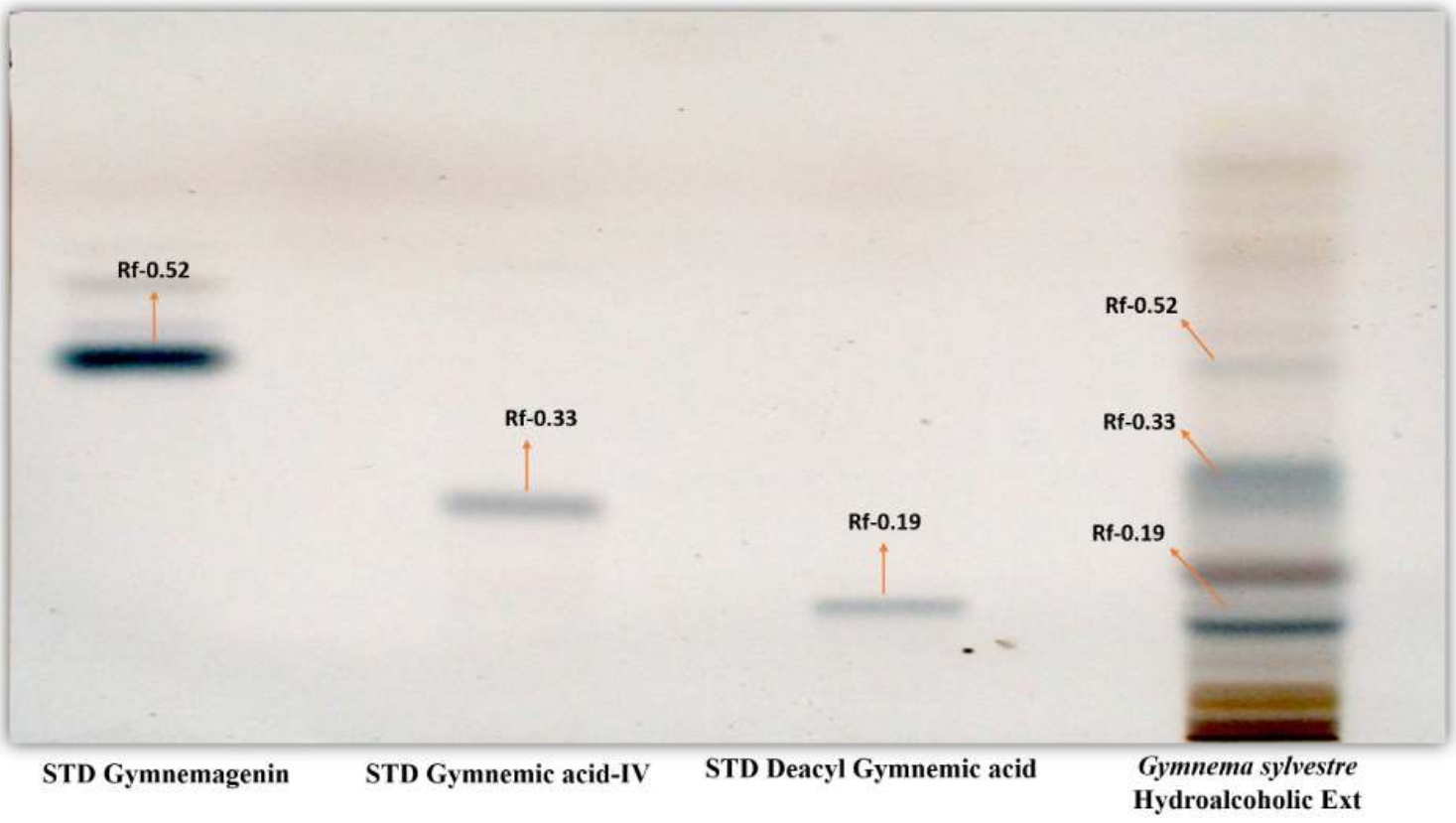


Figure 3

HPTLC densitogram plate of *Gymnema sylvestre* hydroalcoholic extract alongside reference standards gymnemagenin, gymnemic acid IV and deacyl gymnemic acid, visualized in white light after derivatization with 10% sulphuric acid in methanol; Rf 0.52, 0.33 and 0.19.

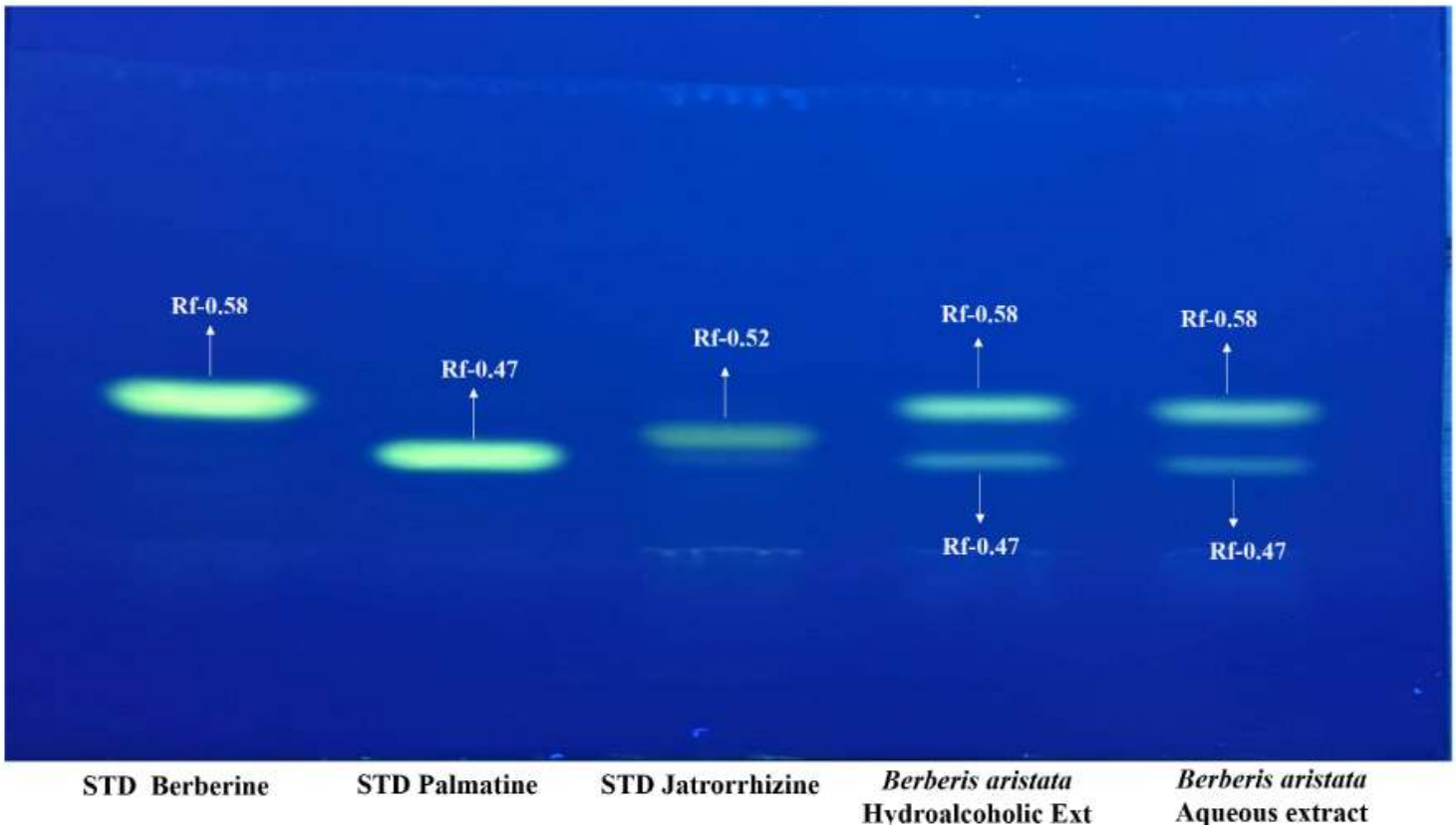


Figure 4

HPTLC plate of *Berberis aristata* hydroalcoholic extract alongside reference standards berberine, palmatine and jatrorrhizine, visualized under UV light (254/366 nm) without derivatization; Rf 0.58 and 0.47.