Research Square

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Preliminary Qualitative HPTLC Fingerprinting of Triterpenoid Saponins in Gymnema sylvestre and Protoberberine Alkaloids in Berberis aristata

Shivaprasad Hudeda
JSS Ayurveda Medical College and Hospital
Spandana Malatesha
spandanam03@gmail.com

JSS Ayurveda Medical College and Hospital

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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 reference HPTLC identity data for their hydroalcoholic extracts remain limited. This study reports a preliminary qualitative HPTLC fingerprinting investigation of the hydroalcoholic extract of *Gymnema sylvestre* and the hydroalcoholic and aqueous extracts of *Berberis aristata*. For *Gymnema sylvestre* extract (leaf, 14% w/w hydroalcoholic yield), chromatography on silica gel 60 F_{254} with toluene: ethyl acetate: formic acid: methanol (3:5:0.5:2) and post-derivatization visualization (10% sulphuric acid in methanol) resolved three bands at Rf 0.52, 0.33 and 0.19, developing light blue, dark blue and light blue colouration respectively, consistent with an oleanane-type triterpenoid saponin class and tentatively corresponding by co-chromatography to gymnemagenin, gymnemic acid IV and deacyl gymnemic acid. For *Berberis aristata* extracts (root; hydroalcoholic yield 8% w/w, aqueous yield 6% w/w), the mobile phase butanol: ethyl acetate: formic acid: water (3:5:0.5:1) resolved two bands at Rf 0.58 and 0.47 in both extracts, showing yellowish-green fluorescence under UV light (254/366 nm) without derivatization, consistent with the protoberberine alkaloid chromophore and tentatively corresponding to berberine and palmatine; the jatrorrhizine standard resolved at a distinct Rf of 0.52 but was not detected in either extract. These preliminary, tentative fingerprints are reported as a qualitative reference profile only; quantitative estimation, inter-batch replication, and formal validation remain necessary before these methods can be regarded as standardization tools.

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].

HPTLC-based identification of the marker compounds in each species individually is not new. Gymnemagenin-referenced HPTLC standardization of *Gymnema sylvestre* was first reported by Puratchimani and Jha, who resolved gymnemagenin at Rf 0.43 on silica gel 60 F_{254} with chloroform: methanol (9:1) after acid hydrolysis of the gymnemic acid glycosides [7], and the method was subsequently refined by Raju et al. to correct an aglycone-conversion error in the original protocol [8]. For *Berberis aristata*, Basera et al. developed and validated a simultaneous HPTLC estimation method for five protoberberine alkaloids-berberine, berbamine, palmatine, magnoflorine and jatrorrhizine- in root

extracts, using densitometric quantification with full ICH-type validation [9]. What has not been reported, to our knowledge, is a direct, single-study qualitative comparison of the two chemically distinct marker classes- non-chromophoric, derivatization-dependent saponins versus inherently UV-fluorescent, derivatization-free alkaloids -across these two specific, commonly co-formulated herbs, alongside a comparison of extraction solvent (hydroalcoholic versus aqueous) for the alkaloid-bearing species. This gap, rather than the individual chromatographic systems themselves, is the basis for the present preliminary investigation.

It should be stated clearly at the outset that the present work is restricted to qualitative Rf- and colour/fluorescence-based fingerprinting; no densitometric quantification, calibration, or replicate/statistical analysis was performed, and no formal method validation (e.g., precision, robustness, or spectral confirmation of peak purity) was undertaken. The study is therefore presented as a preliminary qualitative reference profile for both extracts rather than as a standardization method, and any equivalence to marker compounds is tentative, based solely on co-chromatography (matching Rf and colour/fluorescence) with reference standards.

2. Experimental

2.1 Plant Material Procurement and Authentication

Leaves of *Gymnema sylvestre* R. Br. ex Sm. and roots of *Berberis aristata* DC. were procured from Himalaya Wellness Company, Bengaluru, India (batch numbers NPD/035/2025 and NPD/150/2024, respectively). Material was authenticated by Dr Prasanna Kumar C N, Associate Research Scientist, Department of Pharmacognosy, Himalaya Wellness Company, Bengaluru, based on pharmacognostical characters in the Ayurvedic Pharmacopoeia of India. Collection complied with the Biological Diversity Act, 2002 (Government of India) under the National Biodiversity Authority (NBA), and followed Good Field Collection Practices (GFCP) governed by the National Medicinal Plants Board (NMPB), India. No specific permits were required for commercially cultivated material. Voucher specimens (NPD/035/2025 and NPD/150/2024) were deposited at Himalaya Wellness Company, Bengaluru, India.

2.2 Extraction Methodology

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 (1000 g) 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, yielding 140 g of dried extract (14% w/w).

Hydroalcoholic extraction of *Berberis aristata* root powder (1000 g) 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, yielding 80 g of dried extract (8% w/w).

Aqueous extraction of *Berberis aristata* root powder (1000 g) was carried out by a dual-stage hot maceration/reflux method: a first wash with 6 L of water (1:6 drug-to-solvent ratio) followed by a second wash of the marc with 4 L of water (1:4 drug-to-solvent ratio), each stage maintained at 60–80°C for 3 h. The combined aqueous filtrate was concentrated and tray-dried, yielding 60 g of dried extract (6% w/w). Both the hydroalcoholic and aqueous extracts were stored under refrigeration in sealed containers until further analysis.

2.3 HPTLC Instrumentation and Materials

Sample and standard solutions were applied as 10 mm bands (10 µL per track) using a CAMAG Linomat 5 semi-automatic sample applicator. HPTLC fingerprinting was performed on precoated silica gel 60 F_{254} plates (10 × 10 cm, E-Merck) as the stationary phase. 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. Developed plates were documented using a CAMAG TLC Visualizer under white light and UV light (254 nm and 366 nm). Analytical (AR) grade solvents and reagents were used throughout. Reference standards-berberine hydrochloride (≥ 98%), palmatine chloride (≥ 97%), jatrorrhizine chloride (≥ 95%), gymnemagenin (≥ 96%), gymnemic acid IV (≥ 95%) and deacyl gymnemic acid (≥ 95%) were procured from TCI (Tokyo Chemical Industry) and used as received without further purification. No densitometric scanning, peak integration, or quantitative evaluation was performed; the instrumentation described above was used solely for band application and qualitative visual/photographic documentation.

2.4 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 under UV light (254 nm and 366 nm) prior to derivatization and in white light after derivatization; Rf values and band colours were recorded and compared against the reference standard profile for tentative identification only.

2.5 HPTLC Fingerprinting of *Berberis aristata* Hydroalcoholic and Aqueous Extracts

The mobile phase employed was butanol: ethyl acetate: formic acid: water (3:5:0.5:1 v/v/v/v). Samples were prepared by dissolving 5 mg each of the hydroalcoholic extract and the aqueous extract separately in methanol in 25 mL volumetric flasks, 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 both extract fingerprints were compared against the reference standard profile for tentative identification only.

3. Results and Discussion

3.1 Extraction Yield

Extraction yields obtained for both plant materials are summarized in Table 1. The hydroalcoholic extraction of *Gymnema sylvestre* leaf gave a substantially higher yield (14% w/w) than either extraction of *Berberis aristata* root (hydroalcoholic 8% w/w; aqueous 6% w/w), consistent with the differing tissue composition (leaf versus root) and extraction solvent polarity.

Table 1
Extraction yields of Gymnema sylvestre and Berberis aristata extracts

Plant material / extract	Starting quantity	Dried extract obtained	% Yield (w/w)
Gymnema sylvestre leaf (hydroalcoholic)	1000 g	140 g	14%
Berberis aristata root (hydroalcoholic)	1000 g	80 g	8%
Berberis aristata root (aqueous)	1000 g	60 g	6%

3.2 HPTLC Fingerprint of *Gymnema sylvestre* Hydroalcoholic Extract

Under the mobile phase system used, 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 2). 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 oleanane-type saponins, which lack a chromophore capable of direct UV detection and therefore require chemical derivatization for visualization. The light-blue-to-dark-blue colour reaction on treatment with sulphuric acid-methanol reagent is characteristic of oleanane-type triterpenoid saponins, and the

three resolved bands co-chromatographed tentatively with gymnemagenin, gymnemic acid IV and deacyl gymnemic acid respectively; this correspondence is based solely on matching Rf and colour and has not been confirmed by co-spotting overlays, spectral (UV-Vis) scanning, or any other orthogonal method, and should therefore be regarded as tentative rather than confirmed identification.

Table 2
Tentative Rf-based identification for Gymnema sylvestre hydroalcoholic extract (chromatographic conditions per Section 2.3*)*

Rf value	Colour under UV 254/366 nm	Colour after derivatization	Tentative compound class / marker
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)

3.3 HPTLC Fingerprint of *Berberis aristata* Hydroalcoholic and Aqueous Extracts

Both the hydroalcoholic and the aqueous extract of *Berberis aristata* resolved into the same two bands, at Rf 0.58 and 0.47, each exhibiting yellowish-green fluorescence under UV light at 254 nm and 366 nm without any requirement for chemical derivatization (Fig. 2, Table 3). This yellowish-green fluorescence is a well-recognized physicochemical property of quaternary isoquinoline (protoberberine) alkaloids, arising from extended aromatic conjugation and the quaternary nitrogen of the isoquinoline ring system. The band at Rf 0.58 co-chromatographed tentatively with berberine and the band at Rf 0.47 with palmatine, in both extracts. The jatrorrhizine standard resolved as a distinct, separate band at Rf 0.52- clearly differentiated from both the berberine (0.58) and palmatine (0.47) bands under this mobile phase- but no corresponding band was visible at Rf 0.52 in either the hydroalcoholic or the aqueous extract lane, indicating that jatrorrhizine was either absent or present below the visual detection limit in both extracts, rather than co-migrating with or being obscured by the berberine band.

The qualitatively similar band pattern (matching Rf values and comparable relative band intensities) observed for the hydroalcoholic and aqueous extracts suggests that both extraction media are capable of extracting the two major protoberberine alkaloid markers from *Berberis aristata* root; however, since no densitometric quantification was performed, this observation reflects only qualitative comparability and does not establish equivalence in extraction efficiency between the two solvents. As with the *Gymnema sylvestre* fingerprint above, all compound correspondences reported here are tentative, based on matching Rf and fluorescence colour with reference standards under a single mobile phase system, and have not been confirmed spectrally.

Table 3
Tentative Rf-based identification for Berberis aristata hydroalcoholic and aqueous extracts (chromatographic conditions per Section 2.4*)*

Rf value	Colour under UV 254/366 nm	Detected in	Tentative compound class / marker
0.58	Yellowish-green fluorescence	Hydroalcoholic and aqueous extract	Protoberberine alkaloid (berberine)
0.47	Yellowish-green fluorescence	Hydroalcoholic and aqueous extract	Protoberberine alkaloid (palmatine)
0.52	Yellowish-green fluorescence (standard only)	Not detected in either extract	Protoberberine alkaloid (jatrorrhizine- reference standard only)

3.4 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. This mechanistic contrast — a derivatization-dependent system for a non-chromophoric saponin class versus a derivatization-free, directly UV-fluorescent system for an alkaloid class — provides a rationale for HPTLC detection-mode selection when working with these two co-formulated botanicals.

The inclusion of formic acid as an acidic modifier suppresses ionization and tailing of the free carboxylic acid and hydroxyl groups on the triterpenoid aglycone, sharpening band resolution, while the higher proportion of polar modifiers (ethyl acetate and methanol) relative to the non-polar toluene component increases overall migration relative to a purely chloroform-methanol system. Similarly, the Rf values obtained for berberine and palmatine (0.58 and 0.47, butanol:ethyl acetate:formic acid:water) reflect a considerably more polar, aqueous-modified mobile phase, the acidic aqueous component in the present system is well suited to resolving the protonated, water-soluble quaternary ammonium alkaloids without recourse to ion-pairing reagents. These comparisons indicate that the mobile phase systems used here, while drawing on established chemistry for each marker class, were independently optimized for the present extracts rather than directly reproduced from prior reports.

The extraction yield data (Table 1) are also informative when read alongside the fingerprint results. The comparatively high yield from *Gymnema sylvestre* leaf (14% w/w) is consistent with the moderate polarity of the glycosylated gymnemic acid saponins, which partition efficiently into a 70% aqueous-methanol system. For *Berberis aristata* root, the modestly higher yield of the hydroalcoholic extract (8%

w/w) relative to the aqueous extract (6% w/w) is compatible with the co-extraction of both the water-soluble protoberberine alkaloid salts and additional less polar root constituents (e.g., resinous and lipophilic material) by the hydroalcoholic solvent, whereas the aqueous solvent selectively favours the alkaloid salts themselves. This is consistent with the observation that both extracts nonetheless produced qualitatively matching alkaloid band patterns (Rf 0.58 and 0.47) despite the yield difference, suggesting that water alone is sufficient to solubilize the principal marker alkaloids even though it recovers a smaller total mass of extractable material.

From an applied standpoint, planar chromatographic identity testing of this kind is consistent with the role assigned to TLC/HPTLC fingerprinting in international herbal quality control frameworks, where it is recommended as a rapid, low-cost, first-tier identity confirmation step ahead of more resource-intensive quantitative assays [10]. Framed in this way, the present fingerprints are best regarded as a starting reference point for two commonly co-formulated antihyperglycemic botanicals one for which prior single-species HPTLC precedents exist [7–9] but a direct, side-by-side qualitative comparison across both marker classes and both *Berberis aristata* extraction solvents has not previously been reported.

4. Limitations

The scope of this study was intentionally restricted to qualitative Rf- and colour/fluorescence-based fingerprinting; densitometric quantification, replicate/statistical analysis, and formal validation (e.g., robustness, inter-day precision, spectral confirmation) were not undertaken and are appropriately left to a dedicated follow-up study now that these preliminary marker profiles are established. Accordingly, all compound correspondences reported here are tentative, based on matching Rf and colour/fluorescence with reference standards under a single mobile phase system, and terms implying reproducibility or validated status have been avoided throughout. These next steps-calibration-based quantification, multi-batch replication, and spectral confirmation of the assigned bands represent the natural continuation of the present work.

5. Conclusion

This preliminary study reports qualitative HPTLC fingerprints for the hydroalcoholic extract of *Gymnema sylvestre* and the hydroalcoholic and aqueous extracts of *Berberis aristata*. Three tentative oleanane-type saponin bands (Rf 0.52, 0.33, 0.19) were resolved on derivatization for *Gymnema sylvestre*, while two tentative, directly UV-fluorescent protoberberine alkaloid bands (Rf 0.58, 0.47) were resolved in both the hydroalcoholic and aqueous extracts of *Berberis aristata*, with the jatrorrhizine standard resolving separately at Rf 0.52 but remaining undetected in either extract. These findings provide a preliminary qualitative reference profile only and do not constitute a validated standardization method; quantitative densitometric analysis, replicate/statistical evaluation, and formal validation are necessary next steps before these HPTLC systems can be applied for routine quality control of either extract.

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 or animals. Plant material was sourced from Himalaya Wellness Company, Bengaluru, India (batch numbers NPD/035/2025 and NPD/150/2024) and authenticated by Dr Prasanna Kumar C N, Associate Research Scientist, Department of Pharmacognosy. Collection complied with the Biological Diversity Act, 2002 (Government of India), under the National Biodiversity Authority (NBA), and with Good Field Collection Practices (GFCP) governed by the National Medicinal Plants Board (NMPB), India. Voucher specimens were deposited at Himalaya Wellness Company, Bengaluru, India.

Declarations of generative AI and AI-assisted technologies in the manuscript preparation process.

During the preparation of this work, the authors used Claude (Sonnet 5, Anthropic) to assist with drafting, merging, and formatting the manuscript text, and Quill Bot to assist with language paraphrasing and readability editing. After using these tools, the authors reviewed, edited, and verified all content for accuracy and take full responsibility for the content of the published article.

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Author Contribution

Hudeda and Malastesha designed the study. Malatesha 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. Malatesha 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 analyzed during the current study, including HPTLC plate images, are available from the corresponding author on reasonable request

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Figures

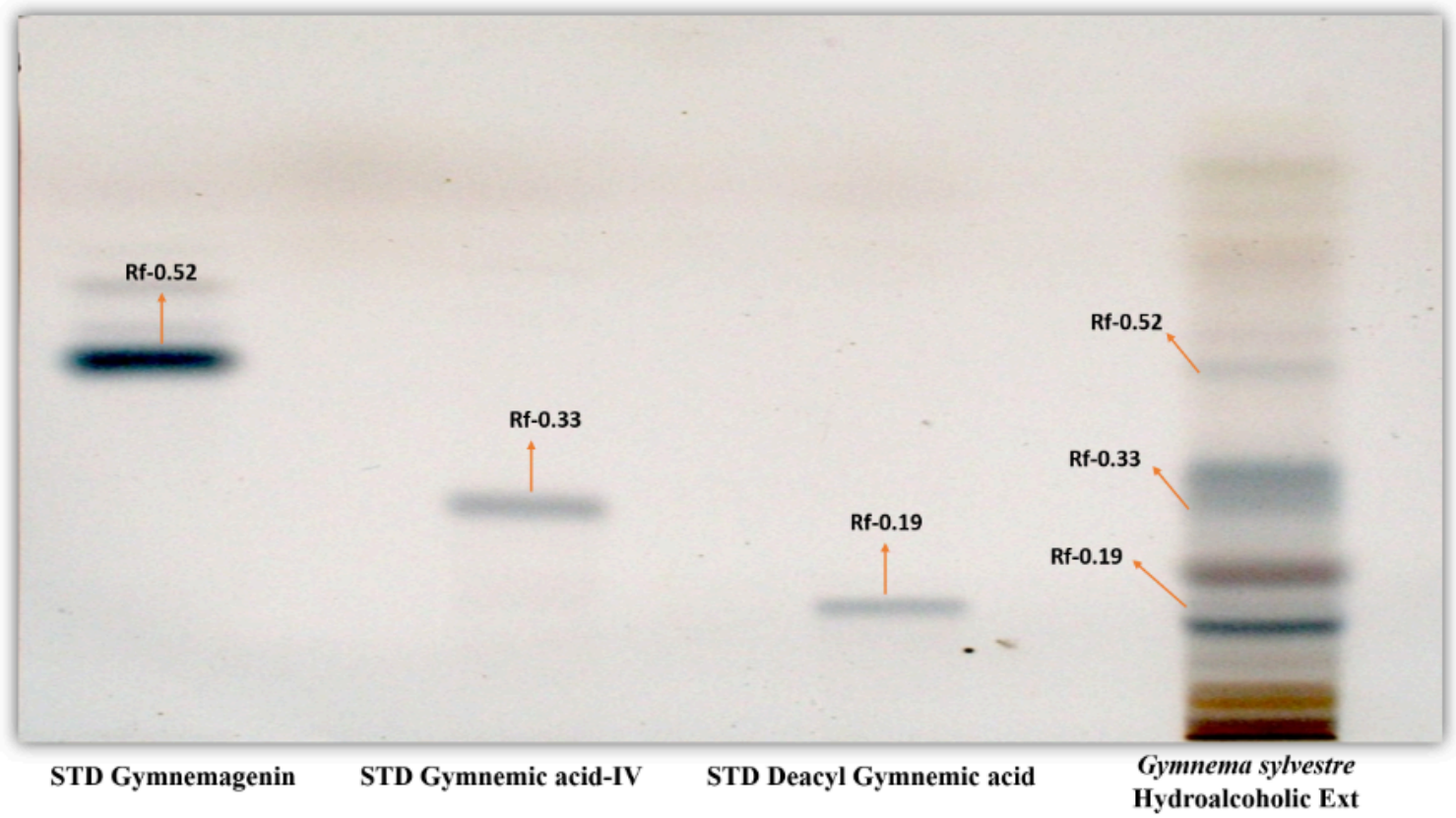


Figure 1

HPTLC 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); tentative, non-validated qualitative identification only

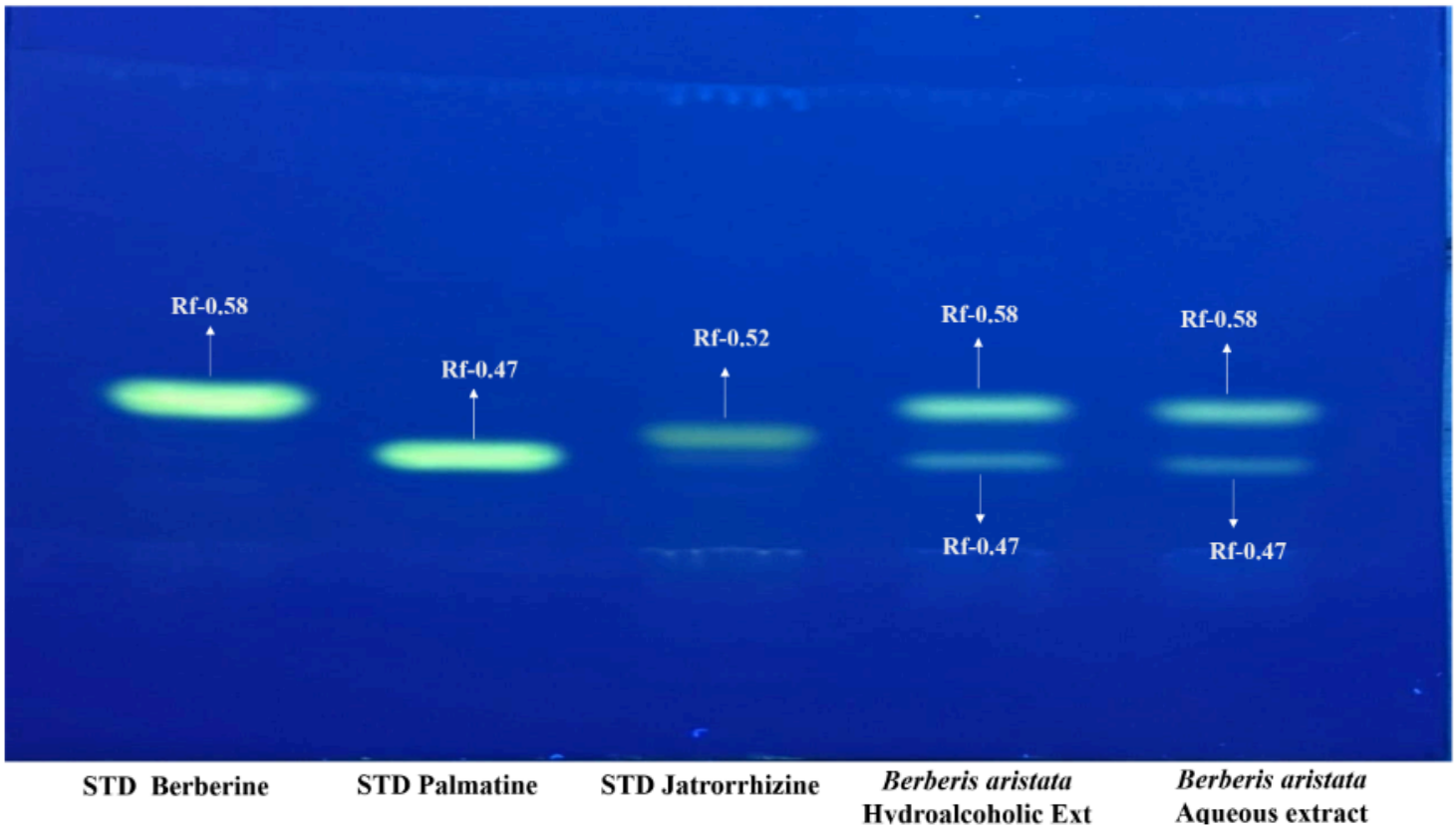


Figure 2

HPTLC plate of Berberis aristata hydroalcoholic and aqueous root extracts alongside reference standards berberine, palmatine and jatrorrhizine, visualized under UV light (254/366 nm) without derivatization (Rf 0.58 and 0.47 in both extracts; jatrorrhizine standard resolved separately at Rf 0.52, not detected in either extract); tentative, non-validated qualitative identification only

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