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Quantification of Procyanidin B_2 in Bark and Leaves of *Saraca Asoca* (Roxb.) Willd: Pharmacognostic Profiling, TLC And HPLC Approaches

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KEYWORDS

Saraca asoca, Sita-ashok, Procyanidin B_2, TLC, HPLC, pharmacognosy, plant-part substitution, conservation

ABSTRACT:

Saraca asoca (Roxb. Willd.; Sita-ashok) is a medicinal tree of high ethnopharmacological importance. Overharvesting of its bark for therapeutic use has contributed to IUCN listing as a threatened taxon; therefore, replacement of destructive harvest (bark) with sustainable plant parts (leaves) is an urgent conservation priority. Procyanidin B_2 (a B-type proanthocyanidin) is a pharmaceutically valuable flavonoid dimer reported from S. asoca and implicated in anti-inflammatory and neuroprotective activity. In the present study, a pharmacognostic and chromatographic instrumental TLC and HPLC evaluation was conducted for the qualitative and quantitative profiling of Procyanidin B_2 in methanolic extracts of Saraca asoca bark and leaves. Macroscopic and microscopic features, physicochemical constants, Instrumental TLC fingerprinting, and an HPLC method (C18 column; gradient elution; detection 290 nm; RT ≈ 2.8 min) were developed and applied. Yields of methanolic maceration (1 kg starting material each) were 54.0 g (bark) and 48.8 g (leaves). Using the in-house developed HPLC method, Procyanidin B_2 was detected in both bark and leaf extracts; assay values were 444 ppm for bark and 509 ppm for leaves, corresponding to the reported assay percentages of 22.2% w/w (bark) and 25.5% w/w (leaves). These preliminary data support the feasibility of leaf substitution as a conservation-oriented strategy while retaining the target marker Procyanidin B_2.

1. Introduction

Saraca asoca (Roxb. Willd.), commonly known as Sita-ashok, is a tree of cultural and medicinal importance in the Indian subcontinent. The bark is used extensively in traditional systems for gynecological disorders, bleeding, inflammation and other indications [1, 2]. Intensive harvesting of bark has contributed to the species' threatened status under IUCN criteria and has prompted conservation and substitution initiatives promoted by agencies such as the National Medicinal Plants Board (NMPB). Sustainable use strategies that replace destructive harvest of bark with renewable plant parts such as leaves can reduce pressure on wild populations while maintaining supply of bioactive constituents [3].

Saraca asoca (Roxb. Willd.) is recognised as a conservation-sensitive species and has been assessed as Vulnerable under IUCN criteria (B1+2c), largely owing to intensive bark extraction for medicinal use. The National Medicinal Plants Board (Ministry of AYUSH) therefore promotes R&D into sustainable supply strategies, under scheme conservation and sustainable development of medicinal plants including the use of renewable plant parts such as leaves. Given the high commercial demand for bark-derived phytochemicals, systematic phytochemical profiling and standardisation

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of leaves are essential to evaluate their suitability as a conservation-oriented substitute [4]. This study compares bark and leaf matrices with Procyanidin B_2 as a marker to inform substitution and quality-control frameworks.

Procyanidin B_2 is a dimeric proanthocyanidin (catechin–catechin/B-type linkage) with reported antioxidant, anti-inflammatory and neuroprotective properties; its commercial value and therapeutic relevance make it a suitable chemical marker for *S. asoca* quality control [5, 6]. Analytical fingerprinting (TLC and HPLC) supports authentication and quantification of marker compounds in plant extracts.

The present research demonstrates a standard pharmacognostic and chromatographic study design: organoleptic and microscopic evaluation, physicochemical constants, TLC fingerprinting, and HPLC quantification of Procyanidin B_2 in bark and leaves with the explicit aim of assessing whether leaves provide a chemically comparable source for this high-value phytomarker, thereby supporting plant-part substitution as a conservation measure.

2. Experimental

2.1 Chemicals and materials

HPLC-grade acetonitrile (ACN), methanol (MeOH), formic acid (GAA or formic acid reagent grade), and trifluoroacetic acid (TFA) were used as supplied by standard vendors. Procyanidin B_2 standard (certified reference material) was procured from an established supplier. TLC plates: silica gel 60 F254 (20 × 20 cm). Milli-Q water (18.2 MΩ·cm) was used for aqueous phases. All solvents and reagents employed were of analytical/HPLC grade and were used at the highest available purity.

2.2 Plant material collection and authentication

Shade-dried bark and leaves of Saraca asoca were collected in October 2023, under project R&D/MH-01/2021-22 from Vikri Vibhag Maharaj Bagh Ropvatika, Nagpur, Maharashtra. Voucher specimen (No. 10739) was deposited in the Department of Botany, R.T.M.N.U., Nagpur. Samples were authenticated by the departmental taxonomist prior to extraction.

2.3 Preparation of extracts and yield determination

Coarse powdered shade-dried bark and leaves (1 kg each) were macerated in methanol for 3 days with periodic agitation, filtered, and concentrated under reduced pressure (rotary evaporator). Reported yields from maceration were: bark 54.0 g; leaves 48.8 g (methanolic extracts).

2.4 Pharmacognostical evaluation

2.4.1 Macroscopic evaluation

The macroscopic characteristics of the powdered bark and leaves of *Saraca asoca* were systematically evaluated by visual inspection in accordance with standard pharmacognostic and taxonomic protocols, and the observations were cross-verified using authoritative floras, botanical monographs, and established pharmacognosy references specific to this species [7].

2.4.2 Organoleptic characters

The colour, odour, taste, and texture of *Saraca asoca* bark and leaves were systematically documented through careful visual and sensory evaluation in accordance with standard pharmacognostic procedures. Transverse sections of *S. asoca* bark and leaf were examined on a Leica compound microscope (Leica DM series) equipped with a Leica digital camera; micrographs were captured using 10× and 40× objectives (total magnifications ca. 100× and 400×) under Köhler illumination [8].

2.4.3 Microscopic evaluation

Transverse sections highlight periderm/cork and secondary phloem in bark and dorsiventral mesophyll with lignified parenchyma in leaves, and representative photomicrographs were recorded for the pharmacognostic profile. Environmental conditions during microscopy were maintained at 30 ± 1 °C and 55 ± 5% relative humidity to ensure consistency in imaging and sample stability [9].

2.4.5 Analytical study of *Saraca indica*

Physicochemical parameters including loss on drying, total ash, acid-insoluble ash, and solvent extractive values were determined for the bark and leaves of *Saraca asoca* following the standard procedures [5]. Methanolic extracts of both plant parts were prepared and subjected

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to preliminary phytochemical screening to detect major classes of bioactive constituents.

2.4.6 TLC fingerprinting

Instrumental TLC was employed to detect the presence of Procyanidin B_2 in the methanolic extracts of leaves and bark of *Saraca asoca*. The analysis was perfomed inhouse developed HPTLC method for Procyanidin B_2 quantification, following the CAMAG HPTLC protocol (CAMAG, Muttenz, Switzerland). For TLC analysis, a Procyanidin B_2 standard (1.0 mg·mL^{-1}) and sample extracts (10.0 mg·mL^{-1}) were prepared in HPLC-grade methanol. Each solution was vortexed, sonicated for 10 min and centrifuged at 2,500 rpm for 3 min prior to application. Aliquots were delivered as 6 mm bands onto silica gel 60 F254 plates (20 × 10 cm) using an automated applicator. Chromatographic development was performed in a twin-trough glass chamber pre-saturated for 20 min with toluene–ethyl acetate–glacial acetic acid (5.5:4.5:0.3, v/v). The developed plates were air-dried and visualized under UV light at 254 and 366 nm. Densitometric scanning was performed at 306 nm using a TLC scanner controlled by visionCATS software (version 4.0) [10, 11].

2.4.7 HPLC method development and conditions

Procyanidin B_2 was identified and quantified in methanolic extracts of *Saraca asoca* bark and leaves by outsourced HPLC analysis (Pharmaregtech Pvt. Ltd., Butibori, Nagpur, Maharashtra, India). Procyanidin B_2 standard (2.0 mg) was accurately weighed into a 2.0-mL volumetric flask, dissolved in 1.0 mL methanol, sonicated for 5 min and diluted to volume with methanol. An aliquot of 0.5 mL of this stock was further diluted to 10.0 mL with methanol to prepare the working standard; serial dilutions were made to construct a multi-point calibration curve. For HPLC analysis, fresh test samples were prepared by refluxing 2.5 g of finely powdered, shade-dried *Saraca asoca* bark and leaf material in methanol for 30 min. The extract was allowed to cool to room temperature and subsequently filtered through Whatman No. 1. The filtrate was made to 10.0 mL with methanol, and 0.5 mL of this solution was diluted to 10.0 mL with methanol; the final solutions were passed through 0.22-µm PTFE syringe filters prior to injection.

Chromatographic separation was performed on an Agilent HPLC system equipped with a Phenomenex/Agilent C18 column (250 × 4.6 mm i.d., 5 µm). Mobile phases were: A — acetonitrile : glacial acetic acid (98 : 2, v/v) and B — methanol: water : glacial acetic acid (95 : 3 : 2, v/v). The gradient program used was: 0.01 min A 93% / B 7%; 1.50 min A 93% / B 7%; 1.51 min A 2% / B 98%; 10.00 min A 2% / B 98%; 10.01 min A 93% / B 7%; 20.00 min A 93% / B 7%. The flow rate was 1.0 mL·min^{-1}, injection volume 20 µL, and column temperature ambient (≈25 °C). Detection was performed at 290 nm. Under these conditions Procyanidin B_2 eluted at approximately 2.8 min and the total run time was 20 min. System suitability tests and a multi-point calibration curve were employed for quantification; data acquisition and integration were carried out by the contract laboratory using their validated instrumentation and software.

3. Results

3.1 Organoleptic characters

The organoleptic characteristics of shade-dried powdered bark and leaves of *Saraca asoca* were recorded by macroscopic examination. The bark powder was brown to reddish-brown, exhibited a distinct astringent odour and a mildly bitter taste, and on tactile assessment presented a coarse, fibrous texture. The leaf powder was greenish-brown to olive-brown, showed a characteristic herbaceous odour with a mild astringent–bitter taste, and had a finer, brittle texture. These organoleptic observations are consistent with published descriptions of *Sitaashok* and support the botanical identity of the material.

3.2 Microscopic characteristics

Transverse sections of *Saraca asoca* bark and leaf were examined as shown in (Figure 1), on a Leica compound microscope (10× and 40× objectives; ~100× and 400× total magnification). Bark anatomy showed a well-developed periderm of suberized cork layers overlying cortical parenchyma, a distinct secondary phloem with sieve-element zones, phloem fibres and prominent medullary rays. Leaf sections exhibited a typical dorsiventral organisation with compact adaxial palisade parenchyma, abaxial spongy mesophyll and collateral vascular bundles surrounded by lignified bundle-sheath/sclerenchymatous cells. These diagnostic microscopic features provide reliable markers for

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authentication and quality control of *S. asoca* bark and leaves.

3.2 Analytical study

3.2.1 Instrumental TLC screening of Procyanidin B_2

Co-TLC with Procyanidin B_2 standard demonstrated co-migration of bands in both bark and leaf extracts under the selected mobile phase (poster TLC images: white light and UV 254 nm). Spectral overlays from densitometry suggest identity matches for the Procyanidin B_2 band in both extracts (Figure 2).

3.3 HPLC identification and quantification of Procyanidin B_2

HPLC screening for Procyanidin B_2 was carried out using the gradient C18 method described above (detection 290 nm, run time 20 min). The Procyanidin B_2 reference standard produced a sharp, well-resolved peak at a retention time of ~2.8 min (AUC = 4,611). Both *S. asoca* extracts showed corresponding peaks that co-eluted with the standard (SABM, bark macerate RT ≈ 2.9 min, AUC = 6,145; SALM, leaf macerate RT ≈ 3.0 min, AUC = 7,043), confirming the identity of the analyte by retention time and spectral match. Quantification by external calibration (multi-point calibration curve) yielded assay values of 444 (SABM) and 509 (SALM), corresponding to the reported contents of 22.2% w/w and 25.5% w/w, respectively (Table 1, Figure 3). These data indicate a higher Procyanidin B_2 load in leaves than in bark for the studied samples

4. Discussion

The present study provides an integrated pharmacognostic and chromatographic comparison of *Saraca asoca* bark and leaves with Procyanidin B_2 as the target phylomarker. Macroscopic and microscopic analyses confirmed anatomical features for both plant parts, while instrumental TLC co-chromatography furnished rapid qualitative confirmation of Procyanidin B_2. HPLC profiling using a gradient C18 method produced sharp, well-resolved peaks that co-eluted with the authentic standard (RT ≈ 2.8 min) and permitted reliable peak-area quantification. Together, these complementary approaches establish both identity and measurable abundance of Procyanidin B_2 in the both extracts [12].

Comparative phytochemical analysis of *Saraca asoca* bark and leaves demonstrated the presence of Procyanidin B_2 in both plant parts: co-TLC (co-migration with authenticated standard) provided qualitative confirmation, while HPLC quantification yielded higher assay values in leaves (SALM; assay 509 ppm, 25.5% w/w) than in bark (SABM; assay 444 ppm, 22.2% w/w). The matched retention times and spectral overlays in the HPLC runs corroborated the co-TLC identity assignment [12]. These results indicate that leaves constitute a chemically comparable and richer source of Procyanidin B_2, supporting the feasibility of plant-part substitution as a conservation strategy [13, 14].

Adoption of leaves as an alternative raw material has broader supply-chain and policy implications. Promoting leaf use will require stakeholder engagement (collectors, manufacturers, regulatory authorities) and clear documentation (voucher specimens, chain-of-custody, sustainable-harvest SOPs). Drafting a monograph that specifies the analytical method, marker limits, and acceptable harvest/processing practices will facilitate uptake by industry and regulators and support conservation objectives.

5. Conclusion and future perspectives

This study evaluated the feasibility of plant-part substitution in *Saraca asoca* by comparing the pharmacognostic profile and Procyanidin B_2 content of bark and leaves. Qualitative co-TLC and quantitative HPLC analyses indicate that leaves are a chemically comparable in the present samples, and richer source of Procyanidin B_2. These findings support the National Medicinal Plants Board, Ministry of AYUSH objective to promote sustainable use of medicinal species through R&D on alternative plant parts and supply-side conservation measures [15].

For translation into practice, priority actions include comparative in-vitro/in-vivo bioactivity and safety testing to establish therapeutic equivalence, and systematic studies of seasonal, geographic and intra-population variability to define specification ranges. Adoption of leaves as an authorised raw material will also require harvest and processing SOPs, inclusion of validated leaf specifications in monographs, and engagement with collectors, farmers and industry.

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Beyond regulatory and analytical implications, this work provides a conservation-oriented research framework that (i) directs future investigations toward evidence-based, conservation-centric studies of medicinal plants, (ii) identifies alternative sources of valuable bioactives to reduce destructive bark harvesting, (iii) offers practical guidance to cultivators and collectors for sustainable cultivation and collection, and (iv) exemplifies application-driven research with direct societal and ecological benefit.

Collectively, these steps will enable evidence-based substitution that conserves wild *S. asoca* populations while maintaining pharmaceutical quality and supply security [16, 17].

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Competing Interests

The authors declare that there are no conflicts of interest.

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Appendix

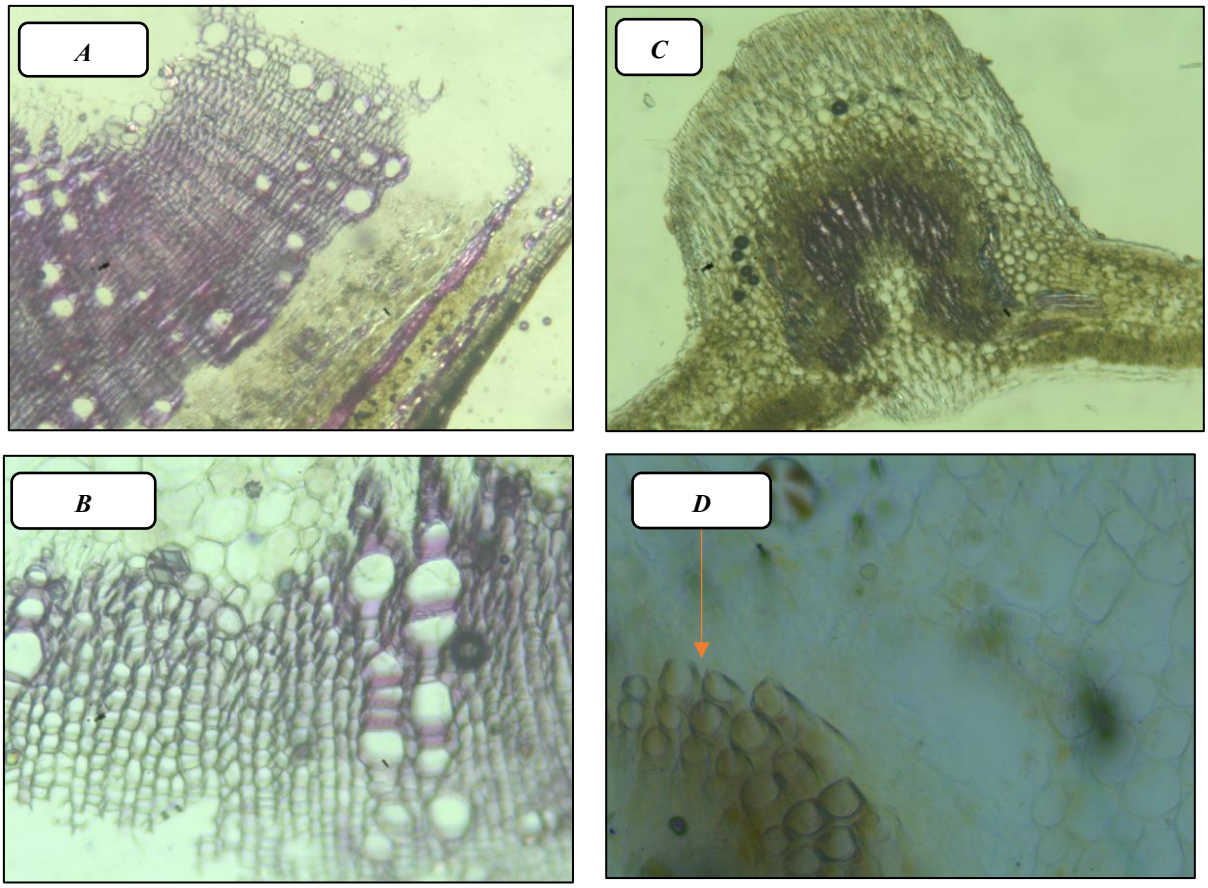

Figure 1. T. S. of Bark and Leaves of Sita ashok

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(T.S. of Bark A Periderm & Cork cells **B** Secondary Phloem **T.S. of Leaf C** Epidermis, Lignified parenchyma **D** Mesophyll cells)

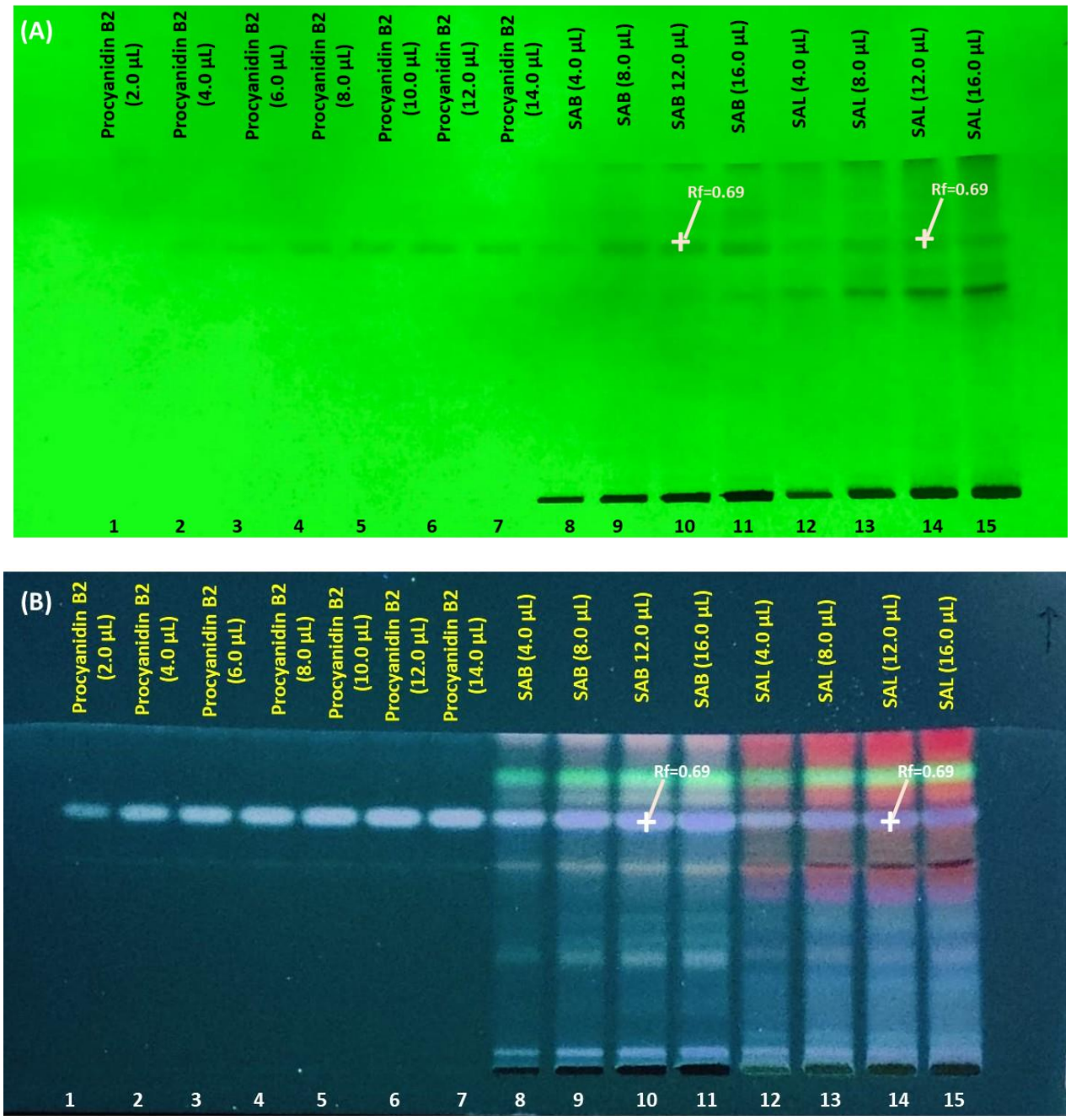

Figure 2: TLC chromatographic study of *Saraca asoca* bark (SAB) and leaves (SAL) extracts visualized under UV light at 254 nm **A** and 306 nm **B**, showing Procyanidin B2 at R_F = 0.69

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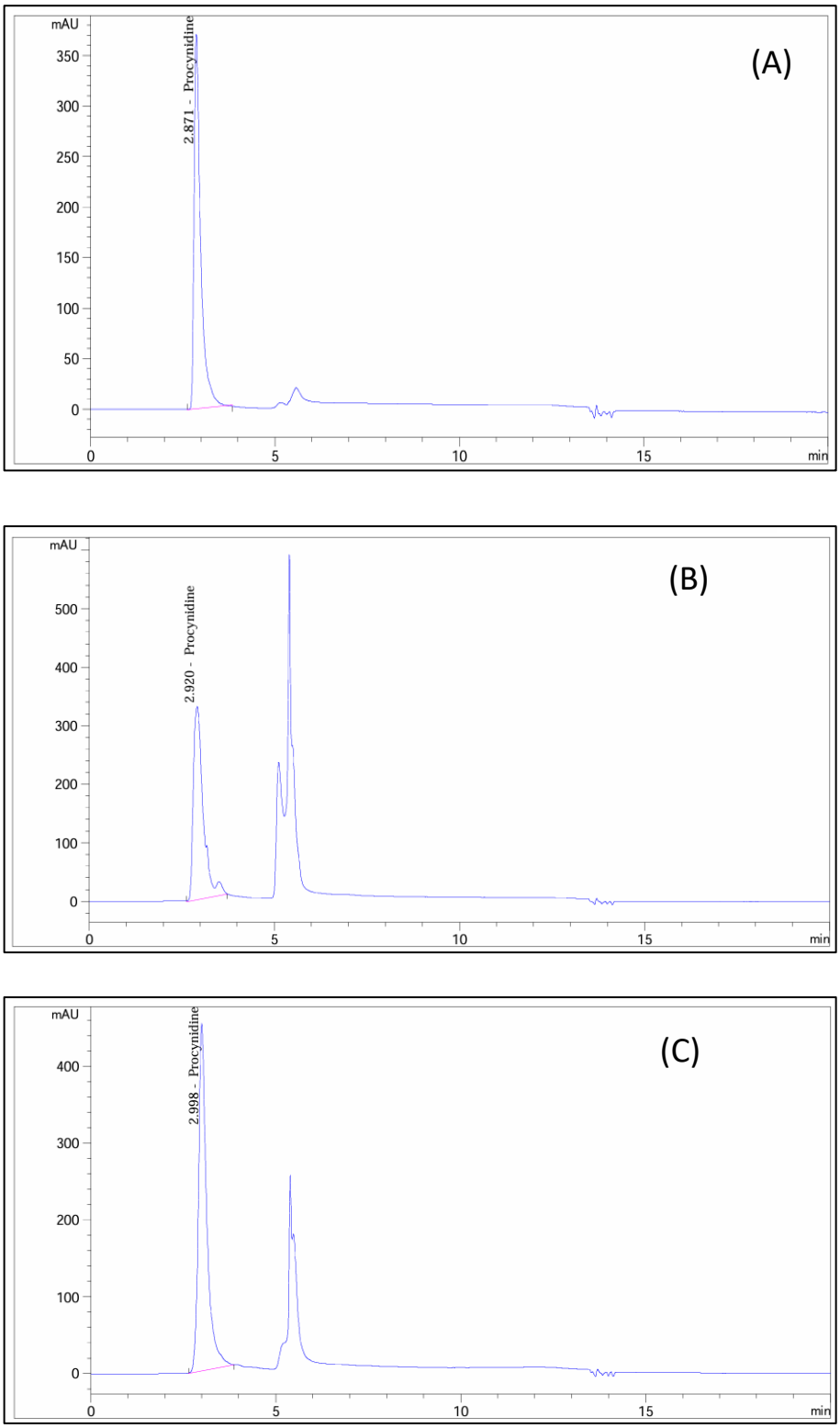

Figure 3: HPLC chromatograms showing **A** standard Procyanidin B2 and **B** Methanolic extract of *Saraca asoca* bark and **C** Methanolic extract of *Saraca asoca* leaves analyzed under identical chromatographic conditions

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Table 1: Quantification of Procyanidin B2 in *Saraca asoca* bark (SAB) and *Saraca asoca* leaves (SAL) extracts

Quantification - Sample Description		Results	
Code	Test Parameter	PPM	%
SAB	Assay	444	22.2
SAL	Assay	509	25.5