



Received on 02 June 2026; received in revised form, 18 June 2026; accepted, 19 June 2026; published 01 October 2026

PHARMACOGNOSTIC STANDARDIZATION AND PHYTOCHEMICAL PROFILING OF ROOT BARK OF *PAJANELIA LONGIFOLIA* AND *RADERMACHERA XYLOCARPA*, TWO LESS-KNOWN ETHNOMEDICINAL TREES OF BIGNONIACEAE

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Keywords:

Ethnomedicinal, *Pajanelia longifolia*, *Radermachera xylocarpa*, Powder microscopy, ICP-AES, GC-MS analysis

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ABSTRACT: Pharmacognostic evaluation becomes a simple and reliable method for the authentication of crude herbal drugs, once the morphological characters are lost during processing. This study presents a comprehensive pharmacognostic, physicochemical, and elemental characterization of the root bark of two ethnomedicinally important but underexplored trees- *Pajanelia longifolia* and *Radermachera xylocarpa* of Bignoniaceae family. The roots of both species share some common histological characters like stone cells and calcium oxalate crystals, but differ in odour, cork thickness, and crystal size. Minor differences in ash values indicate variation in inorganic content. For the first time, this study reports the mineral and heavy metal elemental profiles of the root bark of *P. longifolia* and *R. xylocarpa*. Calcium was identified as the predominant mineral in *P. longifolia* (268.54 mg Kg⁻¹) and *R. xylocarpa* (328.43 mg Kg⁻¹), followed by magnesium, highlighting their potential nutritional and therapeutic significance. Major toxic heavy metals such as Al, Ni, Pb, Hg and Co were found to be below detectable levels in both species, while copper was detected only in *R. xylocarpa*. HPTLC analysis of root bark extracts showed that *P. longifolia* possessed 9 resolved peaks distributed mainly in the lower and mid R_f regions (0.02–0.65), on the other hand, *R. xylocarpa* displayed a broader phytochemical profile extending from R_f 0.02 to 0.80 with 12 resolved peaks. The dominant HPTLC peak at R_f 0.03 in both the root barks indicates the occurrence of a major common phytoconstituent. GC–MS analysis of *P. longifolia* root bark extracts revealed coumaran as the major phytoconstituent, represented by the highest peak at a retention time of 11.33 min, whereas the predominance of compounds such as quinic acid, guaiacol (2-methoxy phenol), isovanillic acid, β-sitosterol, and coumaran reflects the phytochemical complexity of *R. xylocarpa*. The generated diagnostic standards provide a scientific basis for the authentication, quality assurance, and safe medicinal use of these underexplored ethnomedicinal roots.

INTRODUCTION: Medicinal plants have been an integral component of traditional healthcare systems since ancient times, serving as valuable sources of therapeutic agents.

Their medicinal applications evolved through empirical observations and the accumulated knowledge of traditional healers.

Through centuries of practice and observation, ethnomedicinal knowledge has guided the identification of medicinal plants with therapeutic potential, many of which have subsequently evolved into evidence-based medicines through modern scientific validation. *Pajanelia longifolia* (Willd.) K. Schum. and *Radermachera xylocarpa* (Roxb.) K. Schum. are two ethnomedicinally

QUICK RESPONSE CODE 	DOI: 10.13040/IJPSR.0975-8232.17(10).3021-35 This article can be accessed online on www.ijpsr.com DOI link: https://doi.org/10.13040/IJPSR.0975-8232.17(10).3021-35
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important yet scientifically underexplored trees of the family Bignoniaceae. *P. longifolia*, commonly known as 'Tender wild jack', is an inhabitant of deciduous and semi-evergreen forests of India, Myanmar, Peninsular Malaysia and Sri Lanka ¹. It holds significance in Indian folklore medicine, with reported antioxidant, antimicrobial, and anthelmintic activities ^{2, 3}. Tribal populations inhabiting various parts of India use this plant to treat arthritis, jaundice, and skin diseases ^{4, 5}. HRLC-MS analysis revealed the presence of a diverse spectrum of bioactive phytoconstituents, several of which have been documented in the literature to possess a broad range of pharmacological activities, including anticancer, anti-arthritis, anti-inflammatory, and antiviral properties ⁶. Computational analysis of bioactive phytochemicals from the methanolic extract of *P. longifolia* revealed promising interactions with the cancer-associated targets epidermal growth factor receptor and Transforming growth factor beta, suggesting their potential anticancer activity ⁷. *R. xylocarpa* is a tree species endemic to India, confined to the dry deciduous landscapes of Central India and the Western Ghats, where it thrives at elevations of approximately 1,200 feet ⁸. The resinous stem exudate of this tree is traditionally used to treat cutaneous disorders ^{9, 10}. The bark paste is traditionally used to treat rheumatoid arthritis and joint pain ^{11, 12}. At the same time, its fruit paste is applied to snakebite wounds or administered orally for the expulsion of snake venom from the body ¹³⁻¹⁵. The classical Ayurvedic formulation *Dasamoola*, comprising ten medicinal roots, includes 'Patala' and 'Syonaka' as key ingredients. According to Ayurvedic literature, the root of *R. xylocarpa* is recognised as an alternative source for *Patala*, which is authentically obtained from *Stereospermum suaveolens* ¹⁶. Conversely, the root of *P. longifolia* is considered as an unauthorised substitute for *Syonaka*, which is traditionally and authentically sourced from *Oroxylum indicum*.

Despite the ethnomedicinal relevance of *P. longifolia* and *R. xylocarpa*, detailed pharmacognostic characterization, mineral analysis and heavy metal profiling of these species has not been reported. Pharmacognostic evaluation remains one of the most dependable and economical approaches for the authentication and quality

control of herbal drugs, even when the raw materials are reduced to powder form and lose their morphological identity ¹⁷. The present study addresses the lack of scientific documentation on these species by establishing diagnostic parameters for root identification in both raw and powdered forms, while also providing detailed phytochemical characterization to elucidate the scientific basis underlying the therapeutic potential of their root bark.

MATERIALS AND METHODS:

Collection of Plant Samples: Plant materials were collected from the Chittur region along the banks of the Bhavani River in Attappadi, Mannarkkad Taluk, Palakkad District, Kerala, during April 2023, after obtaining prior permission from the Divisional Forest Officer, Mannarkkad Forest Division. Collected materials were identified and authenticated by Dr. P.S. Udayan, Senior Consultant, National Medicinal Plants Board, Regional Cum-Facilitation Centre (Southern Region), Kerala Forest Research Institute, Thrissur District. The authenticated voucher specimens were deposited at the Calicut University Herbarium (CALI), with voucher numbers CALI359–366 for *P. longifolia* and CALI367–375 for *R. xylocarpa*. The pharmacognostic standardization studies were conducted in the laboratories of Government College for Women, Thiruvananthapuram. Inductively Coupled Plasma- Atomic Emission Spectroscopy (ICP-AES) and Gas Chromatography–Mass Spectrometry (GC–MS) profiling were carried out at the Centre for Analytical Instrumentation, Kerala Forest Research Institute (KFRI), Peechi, Thrissur, Kerala.

Preparation of Samples for Pharmacognostic Studies: The separated root barks were cut into small pieces, shade-dried and sieved through a 60 mesh size sieve to obtain a uniform powder, as per the methodology described in the Ayurvedic Pharmacopoeia of India ¹⁸. The powdered material was then preserved in airtight containers for further examination. Before drying, the organoleptic properties of both the fresh root bark and the powdered samples were noted.

Moisture Content of the Root Bark and Loss on Drying: The percentage of water content in the fresh root bark was calculated as [(Initial weight of

fresh bark – final weight of fresh bark) / Initial weight] $\times$ 100, according to the procedure mentioned in Indian Standards¹⁹. To determine the percentage loss on drying, which indicates the volatile matter in the powdered crude drug, 5 grams of crude drug powder was dried at 120 °C for 2 hours in a preheated oven and reweighed. The percentage of loss on drying is [(Initial weight of powder – Final weight of powder) / Initial weight] $\times$ 100.

Determination of Ash Value: Three grams of crude drug were incinerated in a tarred silica crucible at < 450 °C for 4 h to obtain total ash. The total ash in percentage was calculated as (Weight of total ash / Weight of crude drug) $\times$ 100. For finding water-soluble ash, the total ash was boiled in 25 mL of distilled water for 2 min, filtered, and the insoluble residue was oven-dried at 120 °C for 2 hours²⁰. Percentage of water-soluble ash = [(Total ash – Water-insoluble ash) / Weight of crude drug] $\times$ 100. For acid-soluble ash, the total ash was boiled with 25 mL of 2 M HCl for 5 min, filtered, washed, oven-dried at 120 °C, and weighed. The percentage of acid-insoluble ash = (Weight of acid-insoluble ash / Weight of crude drug) $\times$ 100.

Analysis of Mineral Content and Heavy Metals: One gram of powdered drug sample was boiled with 1% HNO₃, followed by the addition of perchloric acid and heating until fumes ceased. Aqua regia (HNO₃: HCl in 3:1 ratio) was then added and heated, ensuring complete decomposition of organic matter and release of elements. The solution was diluted to 100 mL with deionized water. Mineral and heavy metal content in the crude drug powder was determined using Inductively Coupled Plasma- Atomic Emission Spectrometry (ICP-AES) on a PerkinElmer Avio 200 instrument. Characteristic emission spectra of each element were subsequently recorded by the spectrophotometer.

Anatomical and Histochemical Studies: Anatomical observations were carried out using three to five root bark samples collected from authenticated plant materials, following the guidelines of WHO²¹. Fresh root barks were fixed in FAA (10% Formaldehyde, 50% Absolute alcohol, 5% acetic acid and 35% distilled water). Thin free-hand transverse sections were prepared

using a sharp stainless-steel razor blade. Representative sections were selected, stained with safranin, temporarily mounted in glycerine, and observed under a Leica DM1000 compound microscope at 100 x and 400 x magnification. For histochemical studies, sections were treated with respective reagents- phloroglucinol, iodine solution and ferric chloride and were observed under the microscope to detect the presence of lignin, starch and tannin, respectively.

Powder Microscopic Studies: A small amount of fine powder sample was soaked in distilled water for 1 hour, treated with chloral hydrate, and stained with phloroglucinol-HCl, safranine, and toluidine blue²². The material was mounted on grease-free slides and examined under a Leica DM 1000 microscope, and photomicrographs were captured using a DFC 295 camera.

HPTLC Analysis: HPTLC was performed on aluminium plates precoated with silica gel 60 F254 (0.25 mm thickness). The plates were prewashed with methanol and activated at 60 °C for 5 min before sample application. The plant extract was dissolved in 1 mL of chromatographic grade methanol. 5 μ L of each sample was applied as bands using a 25 μ L Camag automatic TLC sampler. The chromatographic development was carried out in a Camag automatic developing chamber (ADC, 20 $\times$ 10 cm, with two tracks maintained at a start position of 15 mm from the bottom edge and 15 mm inter-track distance. After optimization, the chromatographic chamber was saturated for 20 min with 100 mL of the mobile phase consisting of toluene:ethyl acetate:methanol (7:3:1) prior to development. Development was performed using 12 mL of mobile phase until appropriate separation was achieved. Developed plates were air-dried for 4 min, followed by heating for 1 min and densitometric scanning was performed using a Camag TLC scanner at 295 nm with a slit dimension of 6.00 $\times$ 0.30 mm and a scanning speed of 20 mm/s. The developed chromatograms were visualized under white light, 254 nm, and 360 nm using a Camag visualizer, and images were documented using a digital imaging system.

GC-MS Analysis: GC-MS analysis of the methanolic root bark extracts was performed using

a Shimadzu QP 2010 S instrument equipped with a 30 m × 0.25 mm × 0.25 µm column under electron impact mode (70 eV) with helium as carrier gas. The oven temperature was programmed from 80 °C to 260 °C at 10 °C min⁻¹ and held for 8 min. A 1 µl sample was injected in split mode and analyzed for 50 min. Compounds were identified by comparing retention times and mass spectra with NIST 05 and Wiley 8 libraries.

Statistical Analysis: All physicochemical analyses were performed in triplicate, and the results are expressed as mean ± standard deviation (SD). Statistical analysis was carried out using Microsoft Excel

RESULTS: The physicochemical parameters, elemental analysis and histochemical characteristics of the samples are presented in **Table 1, 2** and **3** respectively. **Fig. 1** illustrates the plants along with their medicinally important parts. The powder microscopic characteristics of the root barks of both species are depicted in **Fig. 2 & 3** and the results are explained in **Table 4**. Histochemical features of the root barks are presented in **Fig. 4** to **7**.

Fig. 8 and **9** represent the preliminary HPTLC plate of the methanolic root extracts of the two species under different wavelengths of light and their corresponding chromatograms, respectively. Root bark of *P. longifolia* revealed 10 resolved peaks with R_f values ranging from 0.03 to 0.65, indicating the presence of multiple phytoconstituents mainly distributed in the lower and middle R_f regions.

The major peaks were observed at R_f values of 0.03 (19.86% area), 0.07 (17.17%), and 0.09 (15.39%), suggesting the predominance of

relatively polar compounds **Table 5**. In comparison, the root bark extract of *R. xylocarpa* exhibited a relatively broader phytochemical profile with 12 resolved peaks spanning an R_f range of 0.03 to 0.79, indicating greater chemical diversity. The highest peak area was recorded at R_f 0.03 (29.67%), followed by notable peaks at R_f 0.22 (11.60%) and 0.79 (10.07%), reflecting the presence of both polar and less polar constituents **Table 6**.

In the absence of previously reported marker compounds for these species, the present preliminary HPTLC profiles may serve as baseline chromatographic fingerprints for their identification and future phytochemical investigations.

GC-MS chromatograms of the methanolic root bark extracts of two species are presented in **Fig. 10**. *P. longifolia* revealed 9 tentative phytochemicals with coumaran as the predominant phytoconstituent, accounting for 61.07% of the total peak area, followed by guanosine and 5-hydroxymethylfurfural.

On the other hand, *R. xylocarpa* revealed 12 tentatively identified compounds with retention times ranging from 7.616 to 42.270 min, indicating a chemically diverse phytoconstituent profile.

Among them, guanosine (24.79% area) and quinic acid (22.02% area) were the major constituents, followed by isovanillic acid (10.26%) and β-sitosterol (9.12%), suggesting their potential contribution to the medicinal properties of the plant. The results indicate that *P. longifolia* is dominated mainly by heterocyclic aromatic compounds, whereas *R. xylocarpa* exhibited comparatively greater phytochemical diversity with phenolics, nucleosides, and sterol derivatives.

TABLE 1: ORGANOLEPTIC AND PHYSICO-CHEMICAL CHARACTERISTICS OF THE ROOT BARKS OF *P. LONGIFOLIA* AND *R. XYLOCARPA*

Character	<i>P. longifolia</i>	<i>R. xylocarpa</i>
Colour of fresh root bark	Reddish-brown	Reddish brown
Odour of fresh root bark	Pungent	Aromatic
Taste of fresh root bark	Bitter	Bitter
Moisture content of bark (%)	69.6±1.83	63.8±0.83
Moisture content of crude powder (%)	8.20±0.21	8.60±0.32
Total ash (%)	13.6±0.24	12.8±0.31
Water soluble ash (%)	3.20±0.23	3.10±0.52
Acid insoluble ash (%)	2.20±0.42	1.80±0.33

*values represent mean ±SD

TABLE 2: MINERAL AND HEAVY METAL COMPOSITION OF ROOT BARK POWDER OF *P. LONGIFOLIA* & *R. XYLOCARPA*

Mineral (mg Kg ⁻¹)							
	Ca	Mg	Mn	Fe	Zn	Na	Cr
<i>P. longifolia</i>	268.54	120.68	0.950	65.399	BDL	0.421	3.428
<i>R.xylocarpa</i>	328.431	149.60	1.033	70.177	5.031	2.357	3.877
Heavy metals (mg Kg ⁻¹)							
	Al	Cd	Cu	Ni	Pb	Hg	Co
<i>P. longifolia</i>	BDL	0.902	BDL	BDL	BDL	BDL	BDL
<i>R. xylocarpa</i>	BDL	0.583	21.09	BDL	BDL	BDL	BDL

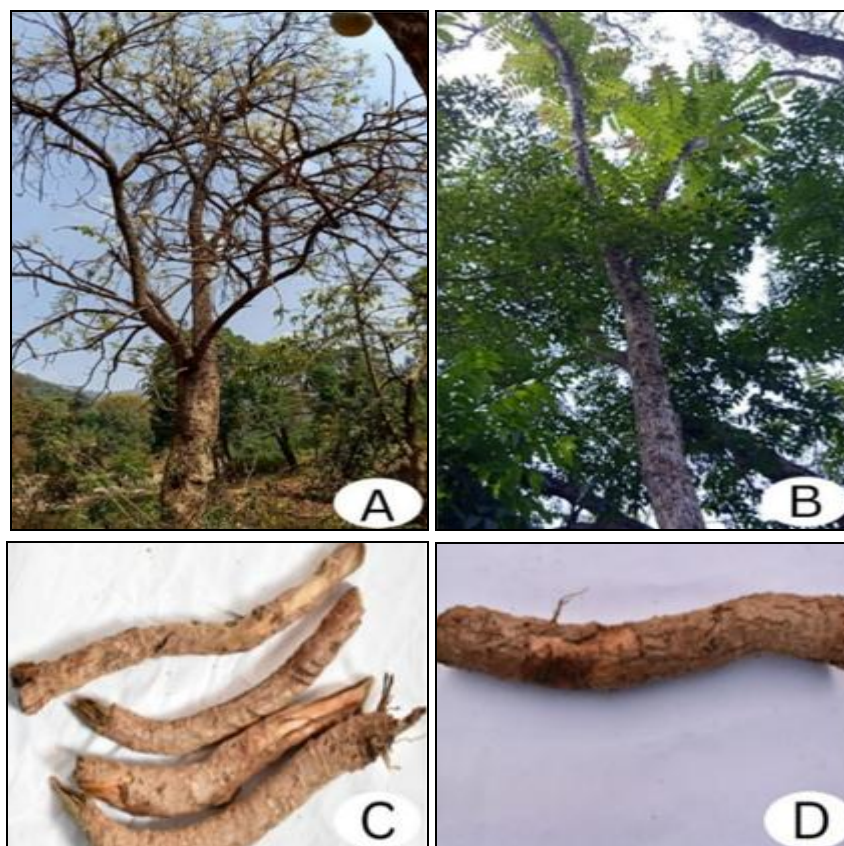
*BDL- below detectable live

TABLE 3: HISTOLOGICAL CHARACTERS OF ROOT BARK OF *P. LONGIFOLIA* & *R. XYLOCARPA*

Characters	<i>P. longifolia</i>	<i>R. xylocarpa</i>
Thickness of cork layer	500-800 µm	180-220 µm
No. of layers in the cork	20 to 40	15-25
No of layers of cork cambium	3-5 layers	7-9 layers
Nature of parenchyma cells in primary cortex	Round crushed	Round intact
Measurement of primary cortical cells	40-70 µm	30-50 µm
Medullary rays	Multiseriate	Triseriate
Cork cells	40-70 µm	30-40 µm)

TABLE 4: POWDER MICROSCOPIC ANALYSIS OF ROOT BARK POWDERS OF *R. XYLOCARPA* & *P. LONGIFOLIA*

Microscopic characters	<i>P. longifolia</i>	<i>R. xylocarpa</i>
Sclerified stone cells in secondary cortex	Present (70-80 µm)	Present (60-70µm)
Measurement of the stone cell group	180 -220 µm	100 -180 µm
Acicular crystals of calcium oxalate	Present (15-25 µm)	Present (25-35µm)
Phloem fibres with tapering end & wide lumen	Present	Present
Type of thickening in xylem vessels	Reticulate	Reticulate

**FIG. 1: THE TREES IN THEIR NATURAL HABITAT IN ATTAPPADI FOREST A. *R. XYLOCARPA* B. *P. LONGIFOLIA*, C. ROOT OF *R. XYLOCARPA*, D. ROOTS OF *P. LONGIFOLIA***

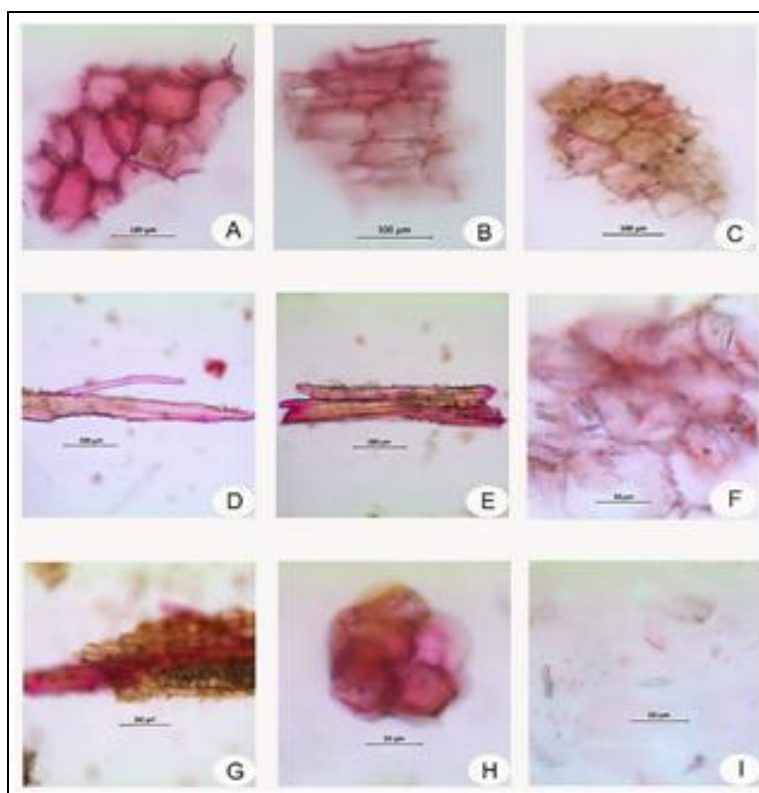


FIG. 2: POWDER MICROSCOPIC CHARACTERS OF ROOT BARK OF *P. LONGIFOLIA*. A. Surface view of suberized cork cells, B. Sectional view of cork cells, C. Cortical cells, D. Fragments of fibres, E. Xylem vessels, F. Cortical parenchyma embedded with acicular crystals of calcium oxalate, G. Phloem fibres with parenchyma cells. H. Group of stone cells. I. Acicular crystals of calcium oxalate.

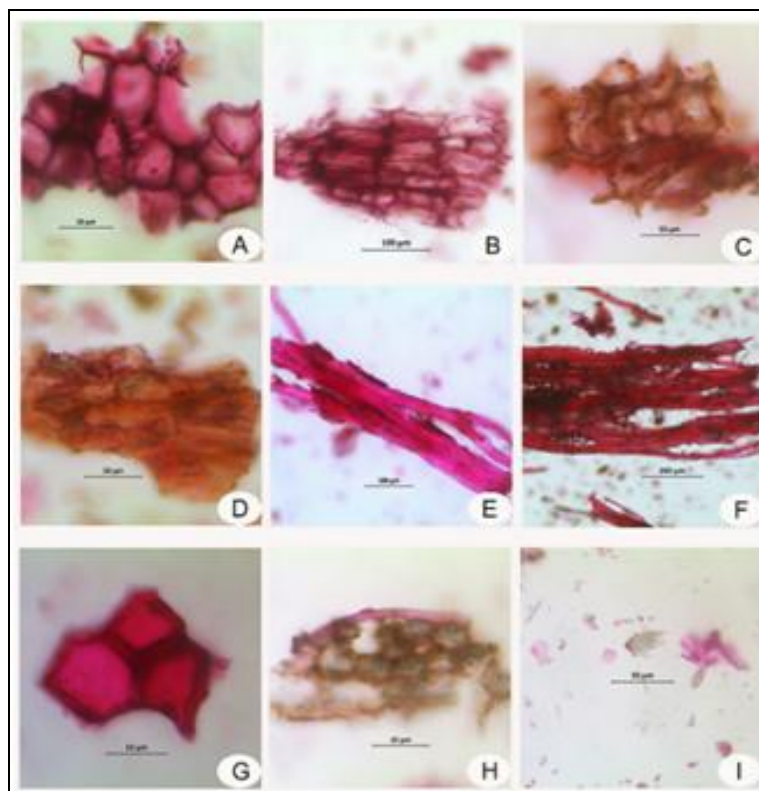


FIG. 3: POWDER MICROSCOPIC CHARACTERS OF ROOT BARK OF *R. XYLOCARPA*: A. Cork cells surface view, B. Cork cell sectional view, C. Cortical cells with calcium oxalate crystals, D. Sectional view of cortical cells, E. Fragments of fibres, F. Group of fibres associated with parenchyma cells. G. Hexagonal stone cells, H. Parenchyma cells embedded with calcium oxalate crystals, I. Acicular crystals of calcium oxalate.

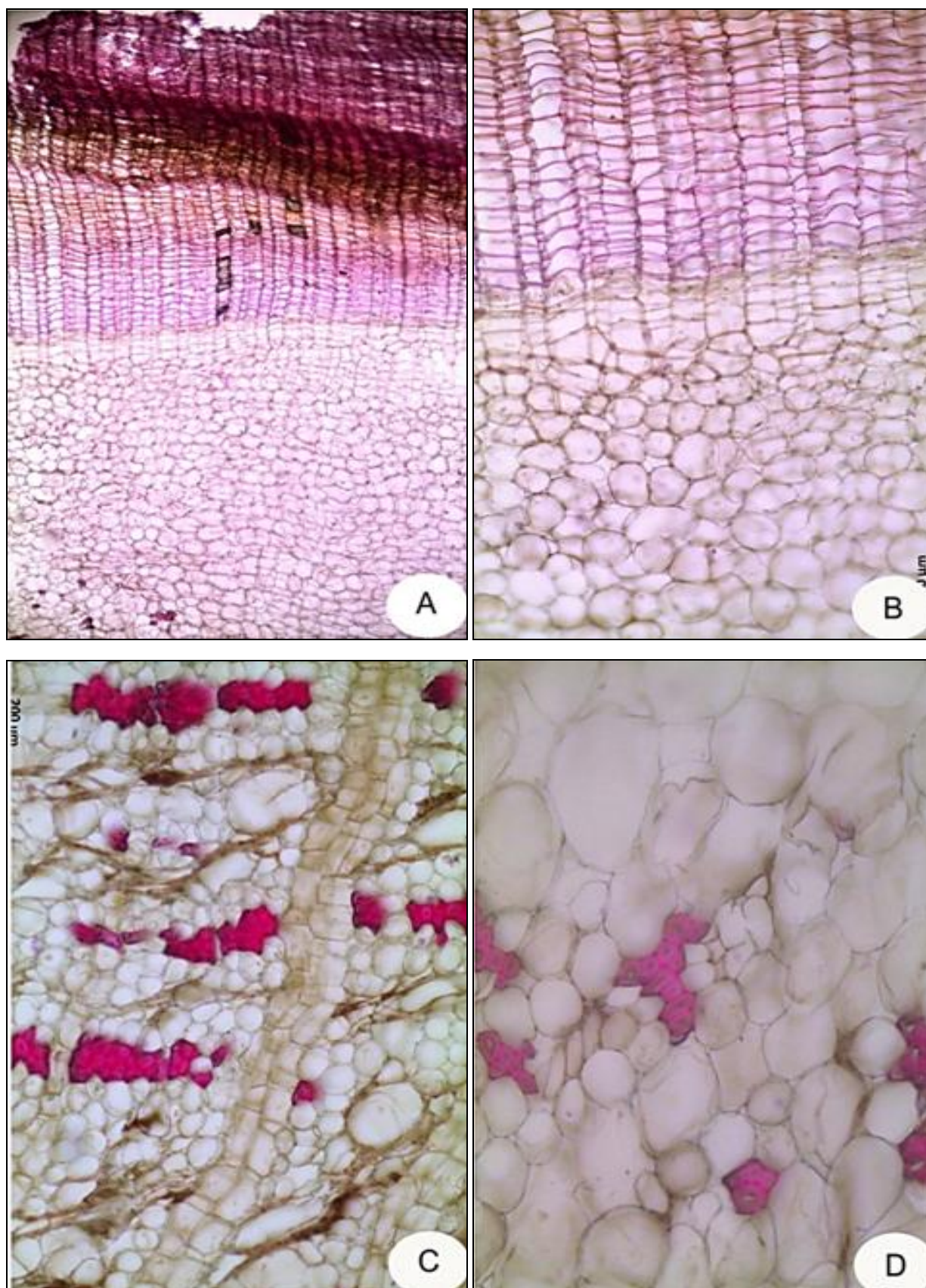


FIG. 4: ANATOMICAL FEATURES OF ROOT BARK OF *P. LONGIFOLIA*. **A:** T.S. of root bark showing cork, cork cambium and secondary cortex, **B:** Barrel-shaped suberized cork cells and round thin-walled parenchymatous secondary cortex, with thin-walled cambium in-between, **C:** Primary cortex interrupted with radial rows of medullary rays, and traversed by groups of lignified stone cells. **D:** Unequally sized parenchymatous cells in the primary cortex and lignified stone cells.

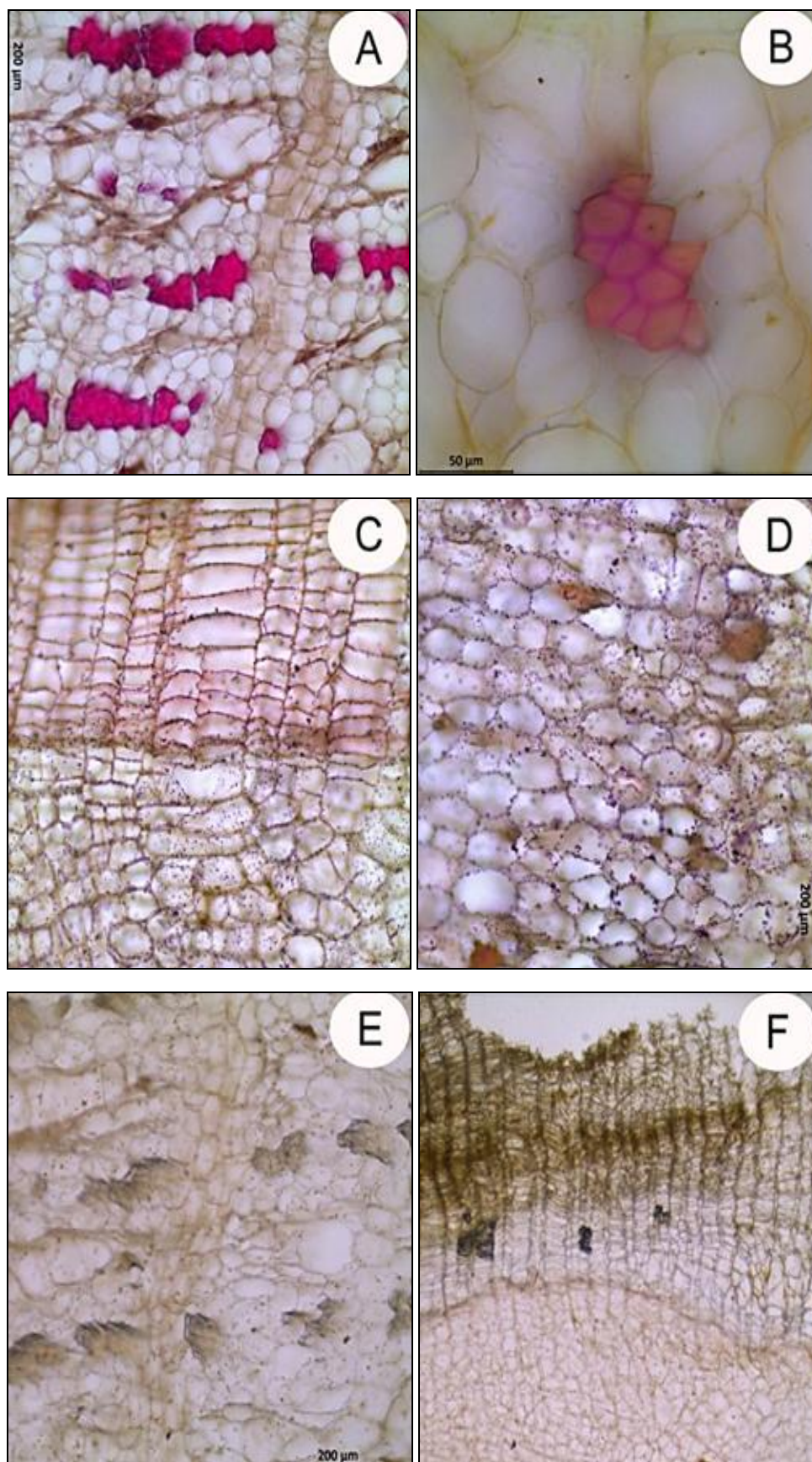


FIG. 5: HISTOCHEMICAL PECULIARITIES OF ROOT BARK OF *P. LONGIFOLIA*. A: Lignified stone cells in the cortical region, B: Group of stone cells, C & D: Oil droplets in the cambium and secondary cortex, E & F: Tannin deposition in the cortical cells and medullary rays.

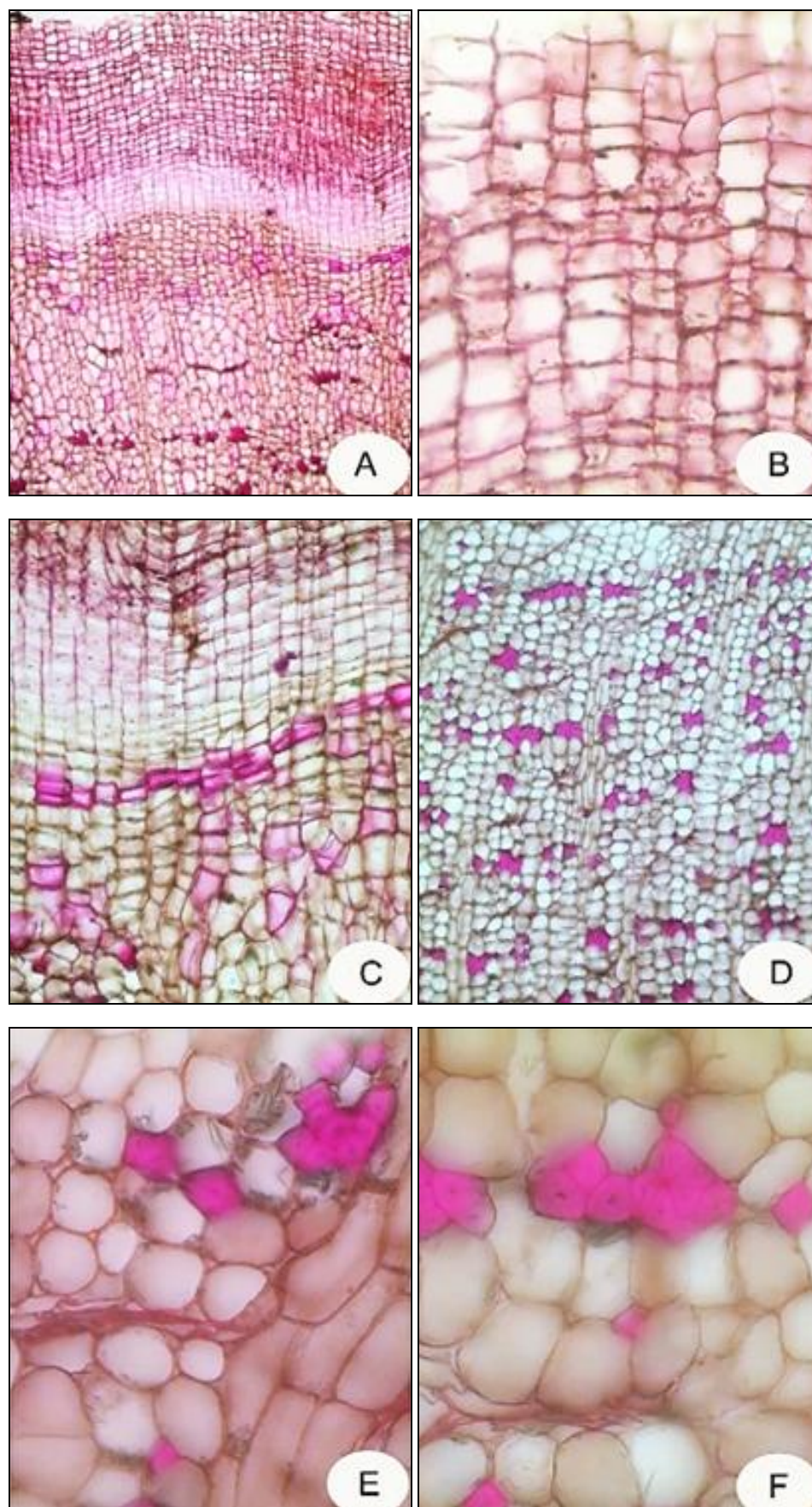


FIG. 6: ANATOMICAL FEATURES OF ROOT BARK OF *R. XYLOCARPA*. A. T.S of root cork, B. Suberised cork cells, C. Multi-layered cambium, D. Sclerenchymatous stone cells embedded in the isodiametric parenchyma cells in the cortex, E&F. Group of stone cells.

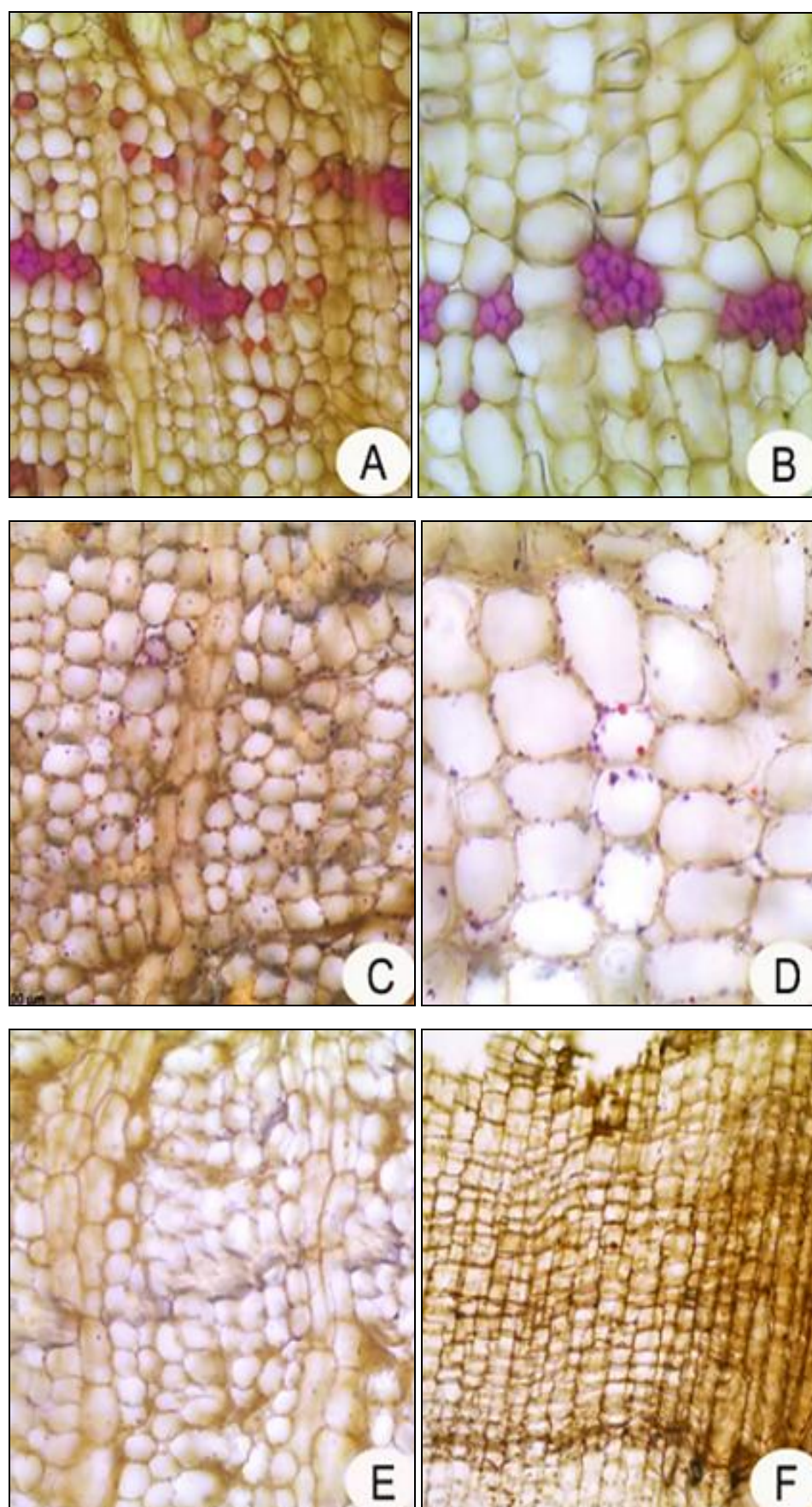


FIG. 7: HISTOCHEMICAL FEATURES OF ROOT BARK OF *R. XYLOCARPA*. A. Lignin detected in stone cells, B. Stone cells enlarged, C&D. Oil globules in cortical cells, E. Tannin in medullary rays, F. Tannin in cork cell.

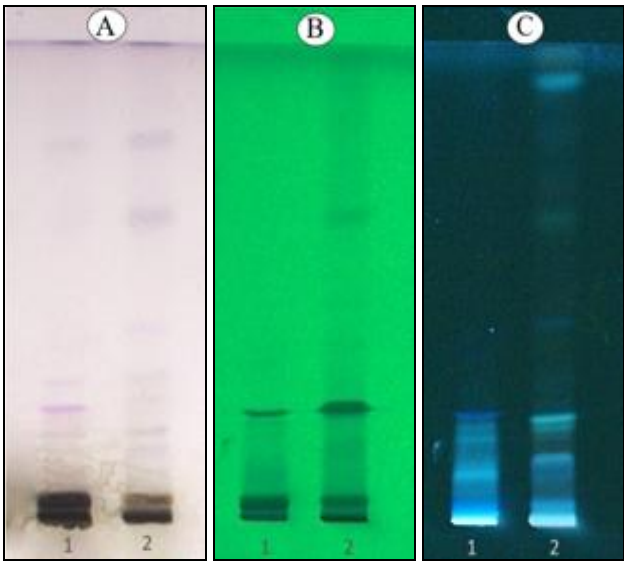


FIG. 8: HPTLC PLATE: TRACK 1: *P. LONGIFOLIA*, TRACK 2: *R. XYLOCARPA*. Viewed under A) visible light, B)254 nm, C) 366 respectively

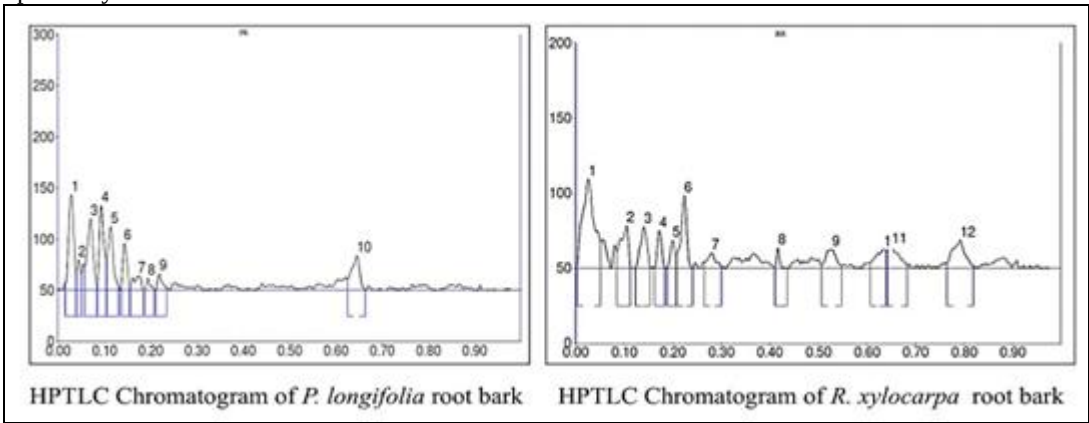


FIG. 9: HPTLC DENSITOMETRIC CHROMATOGRAM OF THE METHANOLIC ROOT BARK EXTRACTS OF *P. LONGIFOLIA* AND *R. XYLOCARPA* SHOWING DISTRIBUTION OF PHYTOCOMPOUNDS

TABLE 5: HPTLC FINGERPRINT PROFILE OF METHANOLIC EXTRACT OF *P. LONGIFOLIA* ROOT BARK

Peak	Start Rf	End Rf	Max Rf	Start height	End height	Max height	Percentage	Area	Area %
1	0.01	0.04	0.03	7.2	17.3	93.7	20.20	992.4	19.86
2	0.04	0.05	0.04	20.0	16.4	30.3	6.52	214.0	4.28
3	0.06	0.08	0.07	24.9	3.2	70.5	15.20	857.9	17.17
4	0.09	0.10	0.09	4.1	32.7	83.5	18.00	769.2	15.39
5	0.11	0.12	0.11	34.2	5.9	62.5	13.47	714.0	14.29
6	0.14	0.16	0.14	3.3	2.9	45.9	9.89	406.4	8.13
7	0.16	0.18	0.17	3.4	0.5	14.2	3.07	227.9	4.56
8	0.19	0.21	0.19	0.2	0.0	12.2	2.62	90.8	1.82
9	0.21	0.23	0.22	0.6	2.4	16.5	3.59	152.1	3.04
10	0.63	0.67	0.65	11.9	0.3	34.4	7.43	572.2	11.45

TABLE 6: HPTLC FINGERPRINT PROFILE OF METHANOLIC EXTRACT OF *R. XYLOCARPA* ROOT BARK

Peak	Start Rf	End Rf	Max Rf	Start height	End height	Max height	Percentage	Area	Area %
1	0.00	0.05	0.03	0.5	17.5	59.3	20.44	1340.7	29.67
2	0.09	0.11	0.11	11.1	2.0	28.5	9.81	425.6	9.42
3	0.12	0.15	0.14	0.8	1.4	27.7	9.54	338.8	7.50
4	0.16	0.18	0.17	0.4	0.2	25.1	8.64	211.0	4.67
5	0.19	0.21	0.20	1.0	8.3	18.6	6.40	164.8	3.65
6	0.21	0.24	0.22	9.2	0.2	48.5	16.73	524.2	11.60
7	0.26	0.30	0.28	3.8	1.1	10.4	3.59	170.6	3.77

8	0.42	0.44	0.42	0.8	0.2	13.5	4.65	76.9	1.70
9	0.51	0.55	0.53	2.0	2.0	12.7	4.39	249.3	5.52
10	0.61	0.64	0.64	3.6	11.6	12.7	4.36	245.9	5.44
11	0.64	0.68	0.65	11.6	2.1	14.6	5.03	315.5	6.96
12	0.77	0.82	0.79	4.0	1.9	18.6	6.42	455.2	10.07

TABLE 7: GC-MS COMPOUNDS TENTATIVELY IDENTIFIED FROM THE METHANOLIC ROOT BARK EXTRACT OF *P. LONGIFOLIA*

Peak no.	RT (min)	Area %	Height %	Compound Tentatively Identified	Base m/z
1	11.733	61.07	57.61	Coumaran	120.05
2	12.053	6.50	6.26	5-Hydroxymethylfurfural	97.05
3	12.173	7.04	5.77	Coumaran	120.05
4	17.716	4.68	7.68	Benzofuran-2-carboxaldehyde	145.00
5	19.858	4.41	0.77	3-Propionyloxytetradecane	57.00
6	20.122	6.51	5.58	Guanosine	57.00
7	31.006	5.15	7.15	Nonylphenol (mix of isomers)	107.05
8	39.340	2.48	5.27	1,2-Benzenedicarboxylic acid	149.00
9	39.674	2.14	3.91	Equilenin	223.00

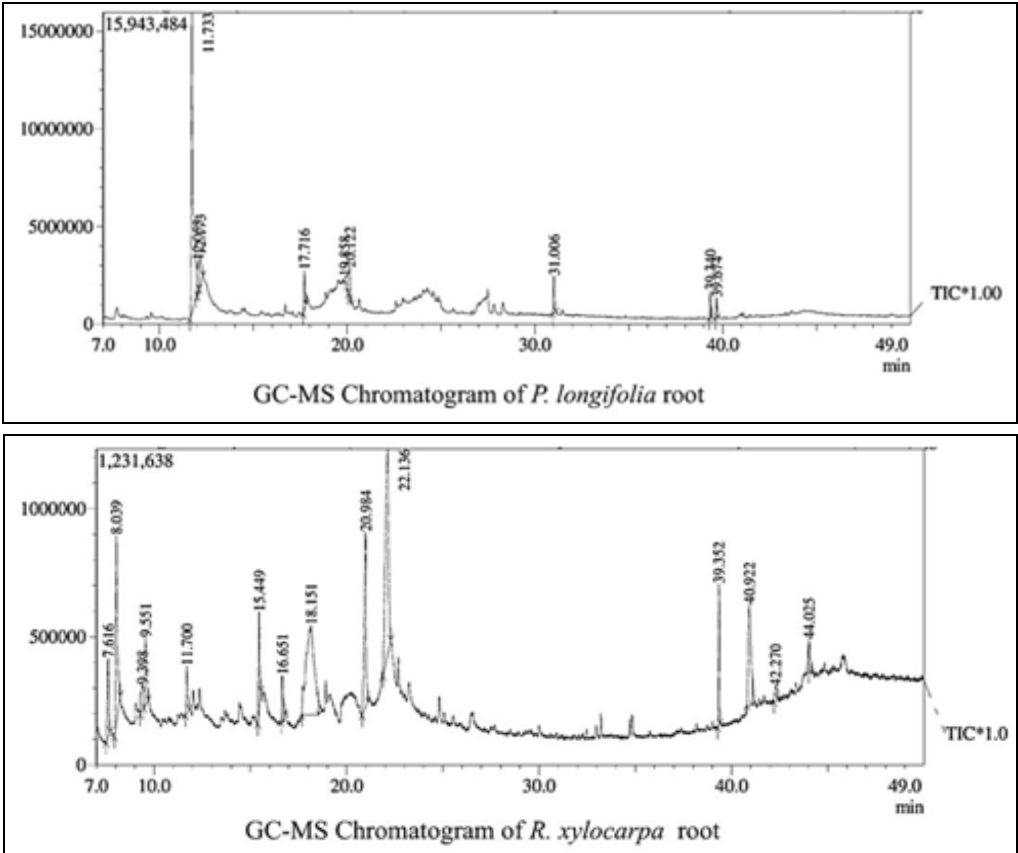


FIG. 10: GC-MS CHROMATOGRAM OF THE METHANOLIC ROOT BARK EXTRACT OF *P. LONGIFOLIA* AND *R. XYLOCARPA*

TABLE 8: GC-MS COMPOUNDS TENTATIVELY IDENTIFIED FROM THE METHANOLIC ROOT BARK EXTRACT OF *R. XYLOCARPA*

Peak no.	RT (min)	Area %	Height %	Compound tentatively identified	Base m/z
1	7.616	3.78	6.02	Thymine	126.00
2	8.039	10.33	14.35	Guaiacol	109.00
3	9.398	2.30	1.92	Erythritol (D)	61.00
4	9.551	3.66	5.18	Pyranone	144.00
5	11.700	2.11	3.62	Coumaran	120.05
6	15.449	3.43	7.49	Phenol, 2,6-dimethoxy-	154.00
7	16.651	2.07	3.57	3-[N'-(3H-Indol-3-ylmethylene)-hydrazino]-5-methyl-[1,2,4]triazol-4-ylamine	142.00

8	18.151	24.79	6.84	Guanosine	57.00
9	20.984	10.26	13.34	Isovanillic acid	168.00
10	22.136	22.02	15.59	Quinic acid	60.00
11	40.922	9.12	7.72	β -Sitosterol	57.05
12	42.270	1.12	1.14	Methyl commate C	218.10

DISCUSSION: Accurate identification of crude herbal drugs becomes increasingly challenging once diagnostic macromorphological characters are lost during processing and powdering. In the two species studied here, organoleptic differences, particularly the pungent odour of *P. longifolia* and the aromatic odour of *R. xylocarpa*, provide useful preliminary cues for crude drug identification. Although fresh bark showed high moisture content, the powdered samples (~8%) were within acceptable limits as mentioned in the Ayurvedic pharmacopoeia, indicating suitability for storage after proper drying. It was mentioned that a moisture content below 14% is good for herbal drug powder^{23, 24}. Slightly higher total, water-soluble, and acid-insoluble ash values in *P. longifolia* indicate marginally greater inorganic content, which correlates with the abundant calcium oxalate crystals observed microscopically in both species. The therapeutic efficacy of many Ayurvedic drugs is often attributed to their mineral constituents²⁵.

However, adequate screening of herbal medicines is essential to prevent heavy metal contamination and ensure their safety and quality²⁶. Elemental profiling carried out in *P. longifolia* and *R. xylocarpa* revealed appreciable levels of calcium, magnesium, and iron, supporting their traditional therapeutic relevance. Most heavy metals analyzed, including aluminium, nickel, lead, mercury, and cobalt, were below detectable limits; however, cadmium levels in both species slightly exceeded the WHO permissible limit of 0.33 mg/kg.

Anatomical and histochemical studies revealed several shared features like barrel-shaped cork cells, lignified stone cells, tannin deposits, and acicular calcium oxalate crystals, reflecting their taxonomic affinity. Distinguishing characters were also evident: *P. longifolia* possessed a thicker, easily detachable cork, while *R. xylocarpa* exhibited abundant pigmented cortical oil globules and longer calcium oxalate crystals. Powder microscopy further confirmed consistent markers like cork fragments, stone cell groups, phloem fibres, reticulate xylem vessels, and crystal-bearing

parenchyma, which are critical for identifying the drug in powdered form.

Since the phytochemistry of both species is poorly documented and no specific marker compounds have been established, the present HPTLC analysis was undertaken as a preliminary fingerprinting study to provide baseline chromatographic profiles for future investigations. Such species-specific chromatographic fingerprints are valuable for the authentication, differentiation, and quality control of medicinal plant materials and have been widely recognized as reliable tools for herbal drug standardization²⁷.

Among the GC-MS compounds tentatively identified from the root bark of *P. longifolia*, coumarans, 5-hydroxymethyl furfural, and 1,2-benzenedicarboxylic acid were reported to possess notable biological activities²⁸⁻³⁰. Guaiacol, a prominent phenolic constituent identified from the root bark of *R. xylocarpa*, has been widely reported to exhibit significant antioxidant and antimicrobial activities³¹. Likewise, β -sitosterol is well known for its apoptotic, antidiabetic, and anticancer properties³²⁻³⁴, while isovanillic acid, a phenolic acid derivative, has been associated with gastrointestinal stimulatory effects³⁵.

CONCLUSION: *P. longifolia* and *R. xylocarpa* exhibited distinct pharmacognostic and powder microscopic characteristics. GC-MS profile of both root bark extracts demonstrated the presence of several pharmacologically important bioactive compounds, highlighting their potential therapeutic significance and supporting their traditional medicinal applications. Their mineral composition further supports their significance in ethnomedicinal practices. The diagnostic standards developed in this study, together with the elemental parameters, provide a practical framework for the authentication, quality control, and safe medicinal use of these underexplored ethnomedicinal roots.

ACKNOWLEDGEMENTS: The author is thankful to the Centre for Analytical

Instrumentation, Kerala Forest Research Institute, Peechi, Thrissur, Kerala, for providing instrument facilities and to Dr. Udayan P.S., Senior Consultant, National Medicinal Plants Board, Regional Cum-Facilitation Centre, Kerala Forest Research Institute, Peechi, for giving the necessary information regarding the plants.

CONFLICT OF INTEREST: The authors declare that, there are no conflicts of interest.

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How to cite this article:

Smitha CK, Kavitha CH and Anupama SS: Pharmacognostic standardization and phytochemical profiling of root bark of *Pajanelia longifolia* and *Radermachera xylocarpa*, two less-known ethnomedicinal trees of bignoniaceae. *Int J Pharm Sci & Res* 2026; 17(10): 3021-35. doi: 10.13040/IJPSR.0975-8232.17(10).3021-35.

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