

Pharmacognostic, Physicochemical and Phytochemical Characterization with Chromatographic Fingerprinting of *Premna Serratifolia* Root

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Abstract: *Premna serratifolia* is a medicinal plant of the genus *Premna* with a documented history of traditional use and a chemically diverse phytochemical profile. The present study focused specifically on pharmacognostic standardization, physicochemical characterization, preliminary phytochemical screening and chromatographic fingerprinting of authenticated *P. serratifolia* root. Fresh, dried and powdered roots were evaluated for organoleptic characteristics, while anatomical examination, fluorescence analysis and physicochemical parameters were used to establish a quality profile. Petroleum ether, chloroform, ethanolic and aqueous extracts were screened qualitatively for major phytochemical classes. Thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) were employed to compare solvent-dependent chemical profiles. The roots showed characteristic light- to dark-brown coloration, bitter taste, distinctive odour and hard texture. Loss on drying ranged from 4.2–4.7%, while total ash, water-soluble ash and acid-insoluble ash were within the limits reported in the study. Alcohol-soluble and water-soluble extractive values were 7.59–8.35% and 6.10–6.89%, respectively. Phytochemical screening demonstrated marked solvent-dependent distribution of alkaloids, flavonoids, steroids, triterpenoids, glycosides, saponins and starch. The ethanolic extract showed an intense alkaloid reaction and contained flavonoids and triterpenoids. HPTLC produced the most complex fingerprint for the alcoholic extract, with 11 detectable spots at 366 nm and 8 at 554 nm. The findings provide baseline pharmacognostic and chromatographic parameters that can support authentication, quality control and future standardization of *P. serratifolia* root preparations.

Keywords: *Premna serratifolia*; pharmacognosy; physicochemical parameters; phytochemical screening; TLC; HPTLC; standardization; medicinal plant.

I. INTRODUCTION

Medicinal plants remain important sources of traditional remedies and lead molecules for pharmaceutical investigation. Their scientific evaluation requires more than demonstration of biological activity because botanical identity, crude-drug quality, extraction conditions and chemical composition can substantially influence reproducibility. Pharmacognostic standardization therefore provides the foundation for subsequent phytochemical and pharmacological work.

Premna serratifolia belongs to the genus *Premna* and has attracted ethnobotanical, phytochemical and pharmacological interest. The thesis literature review describes traditional applications involving headache, neuralgia, fever, cough,



gastrointestinal disorders and selected hepatic and cardiovascular complaints. Previous investigations have reported diverse metabolite classes, including diterpenoids, iridoid glycosides and flavonoids. However, variation in plant part, solvent, geography and nomenclature can complicate comparison among studies.

The root is particularly relevant because it represents a medicinally used underground plant part and may contain chemically diverse constituents. A systematic profile combining organoleptic examination, microscopy, physicochemical evaluation, qualitative phytochemistry and chromatographic fingerprinting can provide a practical identity and quality-control framework.

The objective of the present article was therefore to characterize authenticated *P. serratifolia* root using complementary pharmacognostic and analytical approaches. The study was intentionally focused on standardization and chemical profiling; detailed pharmacological outcomes from the broader thesis were not included.

II. LITERATURE REVIEW

The genus *Premna* has been reviewed for its ethnomedicinal uses, phytochemistry and pharmacological properties, with substantial chemical diversity reported across species and plant parts. Dianita and Jantan highlighted the importance of the genus as a source of diterpenoids, iridoid glycosides, flavonoids and other secondary metabolites. Such diversity provides a rationale for systematic species-level standardization before biological interpretation.

Pharmacognostic evaluation traditionally includes macroscopic and microscopic characterization, physicochemical constants and fluorescence behaviour. These parameters are particularly useful for crude drugs because they provide relatively simple means of confirming identity and detecting gross variation or adulteration. Moisture content, ash values and extractive values provide additional information regarding purity, inorganic residue and solvent-soluble matter.

Chromatographic fingerprinting complements these conventional tests. TLC offers a rapid comparative method for separating extract constituents, while HPTLC improves reproducibility through controlled sample application, development and densitometric assessment. For complex botanical preparations, a fingerprint can capture several constituents simultaneously and can be used for authentication and batch comparison.

Phytochemical screening is a preliminary qualitative tool. Positive reactions indicate the presence or non-detection of broad chemical classes under particular test conditions; they do not establish the concentration or identity of individual molecules. Accordingly, qualitative screening should be regarded as an initial guide for subsequent chromatographic and spectroscopic characterization rather than definitive chemical identification.

III. OBJECTIVES

The study aimed to establish a pharmacognostic profile of *P. serratifolia* root; determine selected physicochemical quality parameters; characterize the solvent-dependent distribution of major phytochemical classes; and develop TLC and HPTLC fingerprints suitable for preliminary quality-control and standardization studies.

IV. RESEARCH METHODOLOGY

4.1 Plant material and authentication

Authenticated *P. serratifolia* roots were used as the plant material. The thesis records taxonomic authentication of the submitted specimen and documents collection of root material from Trikaripur, Kasaragod district, Kerala. The roots were washed, shade-dried, coarsely powdered and sieved before analysis.

4.2 Pharmacognostic evaluation

Fresh, dried and powdered roots were examined for colour, taste, odour, texture and shape. Anatomical sections were prepared after fixation, dehydration and paraffin processing; sections of approximately 12 μ m were stained with safranin and methylene blue and documented by photomicrography. Fluorescence observations were used as an additional diagnostic parameter.



4.3 Physicochemical evaluation

The crude root was evaluated for loss on drying, total ash, water-soluble ash, acid-insoluble ash, sulphated ash, alcohol-soluble extractive, water-soluble extractive, foreign organic matter and pH of aqueous solutions. The study compared samples designated as Kerala, Delhi and Mumbai samples.

4.4 Qualitative phytochemical screening

Petroleum ether, chloroform, ethanolic and aqueous extracts were subjected to qualitative tests for carbohydrates, proteins, alkaloids, saponins, tannins, flavonoids, steroids, fixed oil, volatile oil, triterpenoids, glycosides, starch and resins. Results were recorded as absent, present or intensely present.

4.5 TLC and HPTLC

TLC was performed on silica gel G plates activated at 110 °C. Developed plates were examined under ultraviolet light at 254 and 366 nm, followed by iodine visualization and derivatization. HPTLC was performed on silica gel 60 WR F254 plates using extract-specific mobile phases. The thesis reports 5 µL sample application, controlled humidity and temperature, a 60-mm migration distance and densitometric scanning with a CAMAG Scanner II at 254 nm.

4.6 Data interpretation

The article presents descriptive findings from the thesis. No new statistical analysis was applied to qualitative pharmacognostic or chromatographic observations. Rf values and spot counts are reported as observed under the stated conditions.

V. RESULTS AND DISCUSSION

5.1 Organoleptic characteristics

The fresh root was light brownish, whereas dried and powdered material was dark brownish. The root was bitter, had a distinctive odour and hard texture; the fresh and dried material was described as globular in shape. These simple characters provide a useful first-line identity profile for the crude drug.

Table 1. Organoleptic characteristics of *P. serratifolia* root

Feature	Fresh	Dried	Powdered
Colour	Light-brownish	Dark-brownish	Dark-brownish
Taste	Bitter	Bitter	Bitter
Smell	Distinctive	Distinctive	Distinctive
Texture	Hard	Hard	Hard
Shape	Globular	Globular	—

5.2 Physicochemical parameters

The three geographical samples showed relatively close values. Loss on drying ranged from 4.2 to 4.7%, total ash from 1.12 to 1.30%, water-soluble ash from 0.89 to 0.98%, and acid-insoluble ash from 0.68 to 0.73%. Alcohol-soluble extractive values ranged from 7.59 to 8.35%, while water-soluble extractive values ranged from 6.10 to 6.89%. The values indicate comparatively consistent crude-drug characteristics across the sampled materials.

Table 2. Selected physicochemical parameters of *P. serratifolia* root

Parameter	Kerala	Delhi	Mumbai
Loss on drying (%)	4.2	4.4	4.7
Total ash (%)	1.30	1.12	1.16
Water-soluble ash (%)	0.98	0.89	0.92
Acid-insoluble ash (%)	0.68	0.72	0.73
Sulphated ash (%)	0.79	0.89	0.80
Alcohol-soluble extractive (%)	7.59	8.12	8.35
Water-soluble extractive (%)	6.10	6.52	6.89



Foreign organic matter (%)	1.52	1.54	1.68
pH, 1% aqueous solution	6.2	6.4	6.3

5.3 Preliminary phytochemical profile

Marked solvent-dependent differences were observed. Triterpenoids were detected in all four extracts and were intensely present in petroleum ether. Alkaloids were detected in chloroform and aqueous extracts and were intensely present in ethanol. Flavonoids were detected in petroleum ether, ethanol and aqueous extracts. Steroids occurred in petroleum ether and chloroform, while fixed oil was detected in chloroform. Glycosides were detected in petroleum ether and chloroform. Saponins were detected only in the aqueous extract. Carbohydrates and starch were strongly associated with the aqueous fraction. Proteins, tannins, volatile oils and resins were not detected under the reported screening conditions.

Table 3. Qualitative phytochemical screening of *P. serratifolia* root extracts

Constituent class	Petroleum ether	Chloroform	Ethanol	Aqueous
Carbohydrates	Absent	Absent	Absent	Intensely present
Proteins	Absent	Absent	Absent	Absent
Alkaloids	Absent	Present	Intensely present	Present
Saponins	Absent	Absent	Absent	Present
Tannins	Absent	Absent	Absent	Absent
Flavonoids	Present	Absent	Present	Present
Steroids	Present	Present	Absent	Absent
Fixed oil	Absent	Present	Absent	Absent
Volatile oil	Absent	Absent	Absent	Absent
Triterpenoids	Intensely present	Present	Present	Present
Glycosides	Present	Present	Absent	Absent
Starch	Absent	Absent	Present	Intensely present
Resins	Absent	Absent	Absent	Absent

The solvent-dependent distribution illustrates why a single extract cannot represent the entire chemical composition of a medicinal plant. Ethanol showed an intense alkaloid reaction together with flavonoids and triterpenoids, whereas the aqueous extract displayed a strong polar profile. These observations are consistent with solvent polarity influencing extraction and partitioning. Importantly, qualitative detection of a class does not establish the identity, quantity or biological contribution of individual constituents.

5.4 TLC fingerprinting

The ethanolic extract generated a complex TLC pattern. Among the evaluated solvent systems, ethyl acetate:toluene (0.7:9.3) produced eight visible iodine bands, with recurrent zones around R_f 0.15, 0.65, 0.70, 0.82 and 0.85. The same system showed three bands at 254 nm and three at 366 nm under the reported conditions. Chloroform:ethyl acetate (9.5:0.5) also generated a comparatively rich profile. The different detection modes revealed different portions of the extract, emphasizing the importance of multimodal visualization.

Table 4. Selected TLC fingerprint observations reported in the thesis

Extract/system	254 nm bands	366 nm bands	Iodine bands	Derivatization bands
Ethanol; ethyl acetate:toluene (0.7:9.3)	3	3	8	4
Ethanol; chloroform:ethyl acetate (9.5:0.5)	2	3	6	3



Chloroform; toluene:ethyl acetate (9.3:0.7)	1	3	6	3
Petroleum ether; ethyl acetate:chloroform (0.5:9.5)	—	—	—	—

The TLC findings should be interpreted as fingerprints rather than definitive compound identification. Rf values are condition-dependent and can be affected by plate activation, chamber saturation, humidity and solvent composition. Nevertheless, recurrent zones can provide starting points for future marker selection and densitometric method development.

5.5 HPTLC fingerprinting

HPTLC provided a more detailed comparative fingerprint. The alcoholic extract displayed 11 spots at 366 nm and 8 spots at 554 nm. The chloroform extract showed 10 and 7 spots, respectively, while petroleum ether showed 8 and 5 spots. The aqueous extract showed only one detectable spot at each wavelength under the selected 100% 1-propanol system. The thesis interpretation emphasizes that the limited aqueous resolution should not be interpreted as proof of low chemical complexity because highly polar constituents may not resolve efficiently under a particular stationary-phase/mobile-phase combination.

Table 5. HPTLC fingerprint summary

Extract	Mobile phase	366 nm: number of spots	554 nm: number of spots
Alcoholic	Toluene:ethyl acetate (9.3:0.7)	11	8
Chloroform	Ethyl acetate:toluene (0.7:9.3)	10	7
Petroleum ether	Ethyl acetate:chloroform (0.5:9.5)	8	5
Aqueous	100% 1-propanol	1	1

The alcoholic extract had the broadest HPTLC profile. Its 366-nm Rf values extended from 0.07 to 0.80, while eight spots were observed at 554 nm from 0.08 to 0.76. The chloroform profile similarly demonstrated substantial chemical diversity. These fingerprints complement the qualitative phytochemical data and provide a practical analytical layer between simple class tests and future quantitative chromatographic methods.

5.6 Integrated interpretation

The results demonstrate a coherent standardization framework. Organoleptic and microscopic observations provide identity-related information; physicochemical parameters provide measures of crude-drug quality; phytochemical screening identifies broad chemical classes; and TLC/HPTLC reveal extract-specific chromatographic patterns. The relative consistency of physicochemical values across geographical samples is useful for establishing preliminary specifications.

The strongest chemical diversity was observed in the ethanolic extract by HPTLC, which is compatible with the broad extraction capacity of ethanol. However, the qualitative phytochemical results also show that no single solvent recovered every detected class. This supports the use of solvent-specific profiling when developing a complete chemical fingerprint.

The present work also highlights the distinction between detection and identification. A positive alkaloid, flavonoid or triterpenoid reaction cannot establish which molecule is present or whether it is responsible for biological activity. Likewise, an HPTLC band cannot be assigned a compound identity solely from its Rf value. Marker assignment would require authentic standards and complementary spectroscopic or chromatographic evidence.



VI. RESULTS AND DISCUSSION—QUALITY-CONTROL IMPLICATIONS

For medicinal-plant standardization, reproducibility is as important as detection of individual constituents. The physicochemical values reported here can serve as preliminary specifications for future batches, particularly for loss on drying, ash values and extractive matter. The absence or non-detection of excessive foreign organic matter also supports the basic quality of the tested samples.

The chromatographic findings offer a complementary tool for authentication and batch comparison. The alcoholic extract fingerprint is particularly information-rich under the reported HPTLC conditions. Future work can evaluate whether selected recurrent bands remain stable across collection seasons, geographic sources, processing conditions and independent batches.

The study has limitations. The phytochemical tests are qualitative and do not quantify constituents. TLC/HPTLC fingerprints were developed under specific experimental conditions and were not presented as validated quantitative marker assays. The study therefore establishes baseline characteristics rather than a complete pharmacopoeial monograph. Further work using validated densitometry, HPLC or LC-MS/MS could determine marker compounds and their concentrations.

An additional consideration is that botanical material can vary with geography, harvest time, plant age and post-harvest processing. The close physicochemical values across the sampled materials are encouraging, but a larger sampling framework would be necessary before general specifications are proposed.

VII. CONCLUSION

Authenticated *P. serratifolia* root demonstrated characteristic organoleptic properties, acceptable physicochemical characteristics and a solvent-dependent distribution of phytochemical classes. TLC and HPTLC generated reproducible comparative fingerprints, with the ethanolic extract showing the most complex HPTLC profile under the reported conditions. Collectively, these findings provide a useful baseline for pharmacognostic identification and preliminary chemical standardization of *P. serratifolia* root. The findings should be viewed as a foundation for future marker-based standardization and detailed compound identification rather than as definitive evidence of the activity of any individual phytoconstituent.

VIII. ACKNOWLEDGEMENTS

The author acknowledges the guidance and institutional support provided by the supervisors and research facilities associated with Shri Jagdishprasad Jhabarmal Tibrewala University and Rajiv Gandhi Institute of Pharmaceutical Sciences and Research. The author also acknowledges the assistance of the staff involved in plant authentication, laboratory experimentation and analytical studies.

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