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Research Article

PHYSIOCHEMICAL, PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF *PREMNA LATIFOLIA*

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ABSTRACT

Objective: Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. *Premna latifolia* belonging to genus Premna and family Verbenaceae was one among them. The objective of the present study is to investigate the pharmacognostical and phytochemical evaluation of premna latifolia.

Methods: Morphological and microscopical characters of the plant were studied and preliminary photochemical analysis of ethanol, chloroform, petroleum ether and aqueous extracts of these plants were done. Root powder was treated with different reagents and observed for fluorescence under visible light and UV light of short and long wavelength. TLC and HPTLC studies of various extracts of the plant also were done.

Results: The powder microscopy showed presence of fibres, parenchyma cells and tracheids. The phytochemical results showed strong presence of triterpenoids and alkaloids with trace amounts of carbohydrates and flavonoids. The physiochemical parameters of root powder were within the limits. The root powder also showed fluorescence. TLC and HPTLC of various extracts of the plant also yielded satisfactory results.

Conclusion: The comparative and multidisciplinary approach of the study of *Premna latifolia* helps in understanding its identification, taxonomical determination and medicinal importance in depth. Further evaluation needs to be carried out on *Premna latifolia* in order to investigate the obscured areas and their practical clinical applications, which can be used for the welfare of the mankind.

Keywords: Premna; Genus;Physiochemical ; Phytochemical; Verbanaceae.

INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. Since time immemorial man has been using herbs/plant products as medicine for developing immunity or resistance against diseases. India has one of the richest medical plant traditions in the world [1]. It has a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. The development of these traditional systems of medicines with the perspectives of safety, efficacy, and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in healthcare. There were estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India [2, 3]. Premna genus belonging to the family Verbenaceae was one among them [4].

The detailed physiochemical, pharmacognostical and phytochemical evaluation of *Premna latifolia* give valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs. The Plant *Premna latifolia* grows as a small bushy tree which was used as herbal drug in Ayurveda. The plant is believed to have got antiinflammatory, analgesic and antiulcer activity [5, 6]. Here in this work a in depth physiochemical, pharmacognostical and phytochemical evaluation of *Premna latifolia* . The findings of this study will be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

MATERIALS AND METHODS

Collection and Authentication

Roots of *Premna latifolia were* collected from three distinct regions like Kerala, Mumbai and Delhi, in a quantity sufficient for all the experiments in a single batch and was authentified by Dr.H.B.Singh, NISCAIR, New Delhi (Ref.No- NISCAIR/RHMD/Consult-/2011-12/1922/222). The plant material was authenticated by NISCAR, Delhi. The roots of *Premna latifolia* was washed and shade dried (30°C, 50 ± 5% relative humidity) for 15days [7,8]. Then they were powdered and stored in an air tight container for further use.

Morphological evaluation

Roots of *Premna latifolia* were subjected to morphological evaluation for parameters like colour, odour, taste, shape and texture. [9, 10]

Microscopic studies

The microscopic analysis of T.S of root and root powder were done using standard procedures [11-15].

Physiochemical studies

Coarse powder of the plant root was used to perform quality control parameters such as total ash, acid insoluble ash and water soluble ash, and extractable matter, loss on drying, foaming index and swelling index etc as per WHO guidelines [16, 17]. Three determinations were carried out for each parameter.

Fluorescence analysis of drug powder

The root powder as such and after treatment with various solvents was subjected to fluorescence analysis. Observations were made under visible light and under UV light of short wave length and long wave length separately [18].

Preliminary phytochemical studies of various root extracts

The air-dried powdered material was extracted with chloroform, petroleum ether,ethanol and water in a Soxhlet extractor. Each extract was concentrated, evaporated to dryness, until semi-solid masses were obtained [19, 20]. Then preliminary phytochemical screening was performed to establish a chemical profile of crude drug [21].

Thin layer chromatography

Slurry of silica gel G was prepared in distilled water and poured over a glass plates to form a thin layer. The prepared plates were air dried for setting and then kept in an oven at 100-120°C (30min) for activation. The extracts were dissolved in respective solvents and spotted over an activated plate (2cm above from the bottom). The spotted plates were kept in a previously saturated developing chamber containing mobile phase and allowed to run 3/4th of the height of the prepared plate. The developed TLC plates were observed under UV light, iodine chamber and after derivitisation in 15% Ethanolic sulphuric acid followed by heating at 105^{0}c for15 min [22, 23].

HPTLC analysis

Application of bands

Sample were applied in duplicate on pre-coated silica gel 60GF254 aluminium sheets [(3x10) cm] with the help of Linomat 5 applicator attached to CAMAG HPTLC system, which was programmed through WIN CATS software [24].

Development of chromotogram

After the application of spots, the chromatogram was developed in twin trough glass chamber [(20 x 10) cm saturated with respective solvents for specified time.

Detection of spots

The air-dried plates were viewed in ultra violet radiation of 366nm and 544nm after derivitisation in iodine chamber. The chromatograms were scanned and photo documentation was done. The Rf values and fingerprint data were recorded by WINCATS software.

Peak development of different extracts

Two separate concentrations of 2.5µ L and 5µL of each extract were performed separately, and separate track was maintained for each concentration with separate peak development for each extract with two concentrations separately [25].

RESULTS AND DISCUSSION

Morphological evaluation

It is assumed that macroscopical evaluation of any plant drug is considered to be the primary step for establishing its quality control profile. Proper authentication of a drug depends almost entirely on macroscopical characters.

Morphological evaluation of roots of *Premna latifolia* was found as Table1.

Table1: Morphological evaluation of roots of *Premna latifolia.*

S. No.	Character	When fresh	After drying	Powder
1	Colour	Light brown	Dark brown	Dark brown
2	Odour	Characteristic	Characteristic	Characteristic
3	Taste	Bitter	Bitter	Bitter
4	Shape	Globular	Globular	-
5	Texture	Hard	Hard	-

Microscopic studies

Microscopic study of the plant drug either in entire or powdered form is one of the important aspect of its histological evaluation.

T. S of the root showed

The root was circular in outline but the margin was broken due to the secondary growth hence it appears to be undulated. The exposed portions of cortex were protected by periderm. But due to the continuous growth of xylem the periderm was also broken in many places. At the same time new periderm was also produced subsequently, hence a few numbers of alternate layers of outer cortex and periderm were observed in some portions of the cell. The outer cortex was made up of thin walled parenchyma cells and it was broken and ripped in many places. The inner cortex looked uniform and was made up of collenchymatous cells. The inner cortex was followed by secondary xylem and no phloem cells were found. The xylem mainly composed of tracheids and vessels. The tracheids possess thick wall and narrow lumen. The vessels were broad and were found on both inner and outer sides of the xylem. The xylem was transversed by numerous parenchymatous ray cells. The cell inclusions were found in all parts of the root.

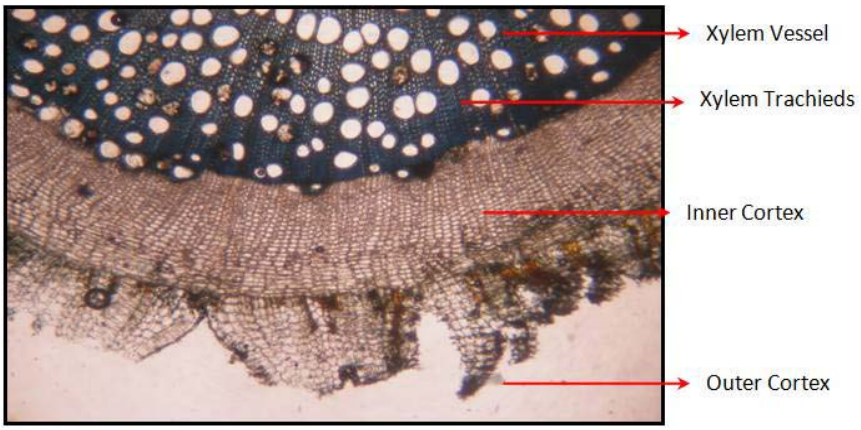


Fig. 1: T.S. of root

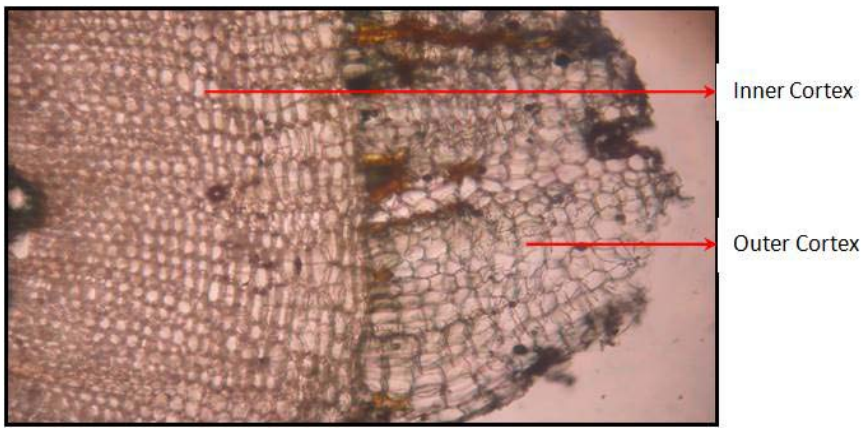


Fig. 2: Outer portion of the root

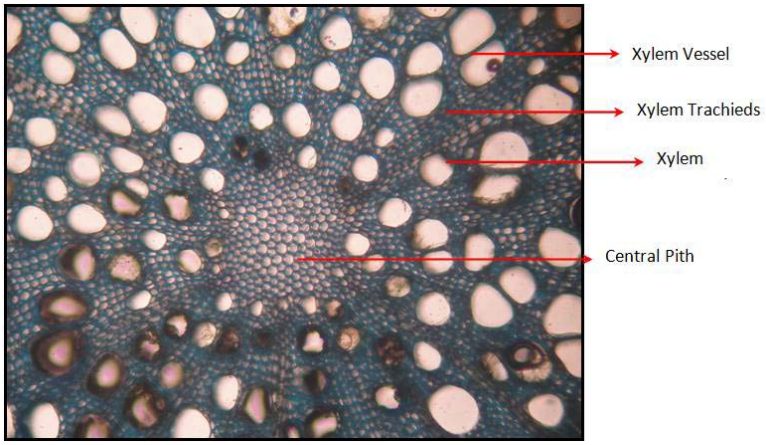


Fig. 3: Central portion of the root

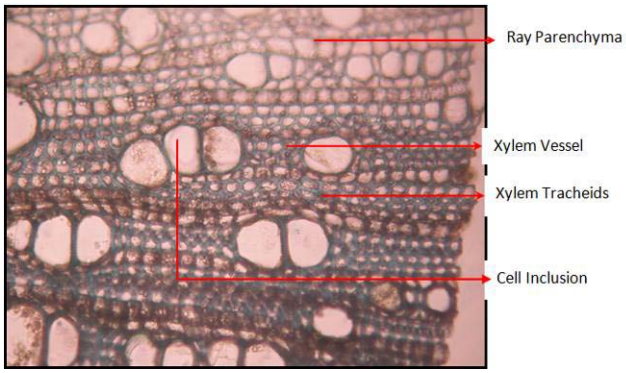


Fig. 4: A portion of xylem enlarged

Powder microscopy analysis of root showed that-

1. Fibres: The broken fibres of different length were found in the powder. The majority of the portion of this root consists of well developed xylem. Hence it may be xylem fibre which were found to be single and in groups.

2. Tracheids: The tracheids were found in group. They were joined with each other in lateral position. The lateral wall of the tracheids was provided with bordered pits. The tracheids were much broader than the fibres and shorten in length. In some places the bundle of fibres and tracheids were found to be joined with each other along with parenchyma cells.

3. Oil globules: The thin oil globules were found in the powder which was in the form of small spherical structures.

4. Parenchyma cells: Group of Parenchyma cell were found throughout the powder. The parenchyma cell was thin walled. In some places the parenchyma cell were provided with some inclusions.

5. Sclereids: In the root powder, group of thick walled dark red coloured sclereids were found.

6. Trichomes: The powder also consists of a very few number of thick celled trichomes. The trichomes were multicellular and non – glandular. The basal cell of the trichome was broad at the place where it was attached to the epidermis.

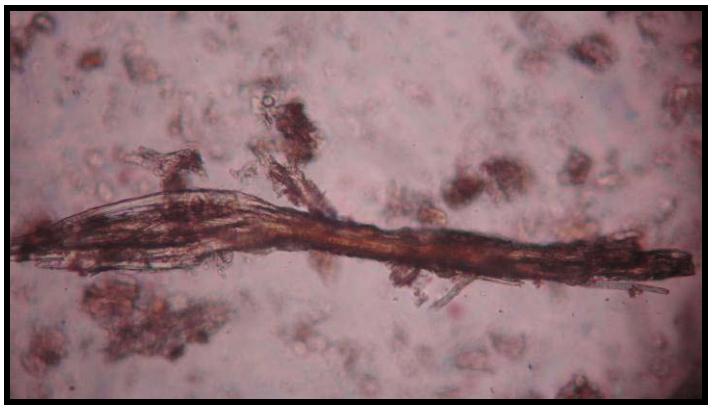


Fig. 5: Bundles of fibres.

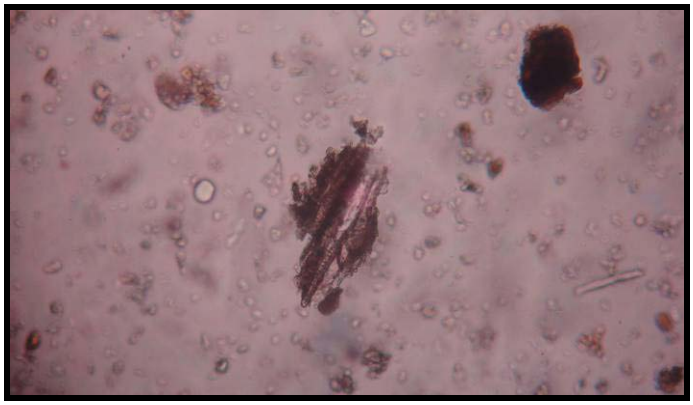


Fig. 6: Tracheids with spiral thickening.

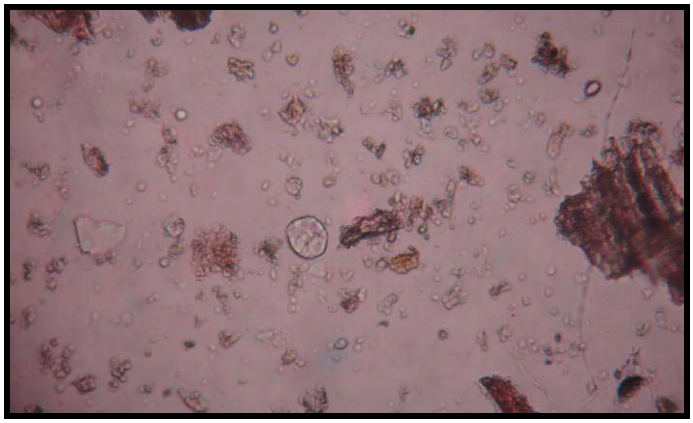


Fig. 7: Parenchyma cells with oil inclusions.

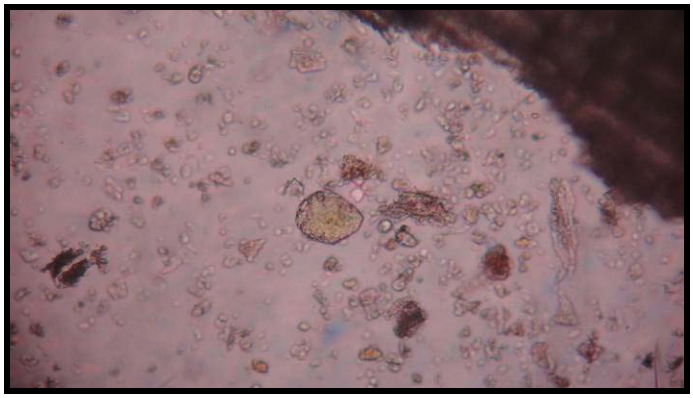


Fig. 8: Parenchyma cells

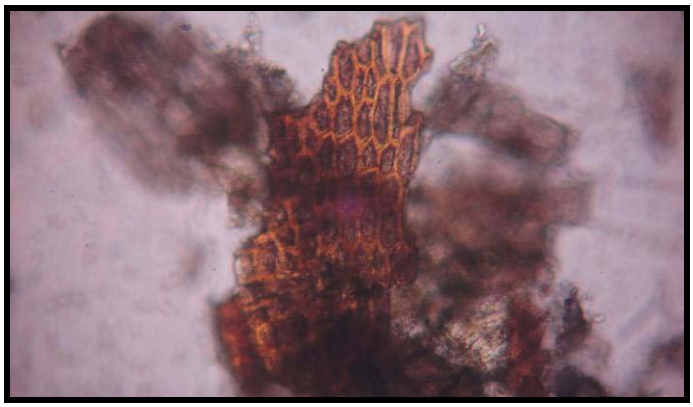


Fig. 9: Sclereids

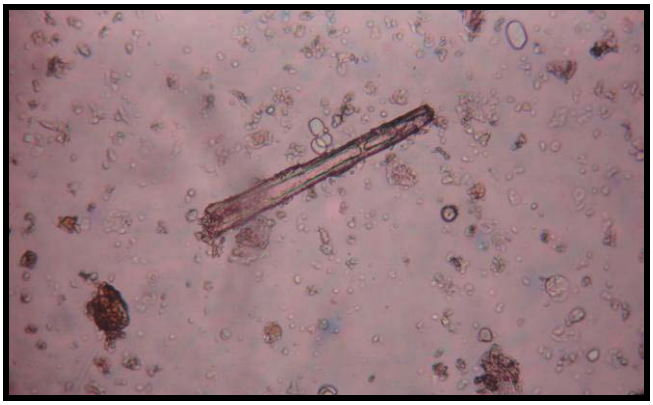


Fig. 10: Trichomes.

Physiochemical studies

Extractive values are primarily useful for the determination of adulterated drugs. Acid insoluble ash is used to know the percentage of dirt and sand while total ash values of a drug give information about inorganic compounds such as carbonated, phosphates, silica and silicates, which are naturally occurring in drug or deliberately added to it as a form of adulterant.

Table 2: Physiochemical studies of Premna *latifolia* Root

Parameters	Kerala	Delhi	Mumbai	Limit
LOD	4.6%	4.9%	4.8%	NMT 4.9%
Total Ash	1.35%	1.12%	1.16%	NMT 1.35%
Water Soluble Ash	0.98%	0.89%	0.94%	NMT 0.98%
Acid Insoluble Ash	0.68%	0.72%	0.75%	NMT 0.75%
Sulphated Ash	0.79%	0.82%	0.80%	NMT 0.82%
Alcohol Soluble Matter	7.65%	8.12%	8.35%	NLT 7.65%
Water Soluble Matter	6.21%	6.52%	6.89%	NLT 6.21%
FOM	1.52%	1.54%	1.60%	NMT 1.60%
pH- 1% Aq.sol	6.4	6.3	6.2	6.2-6.4
pH- 10% Aq.sol	6.2	6.2	6.3	6.2-6.3

Table 3: Fluorescence analysis of root powder of *Premna latifolia*

S. No.	Particulars of treatment	Under ordinary light	Under UV light	
			Short Wavelength (254nm)	Long Wavelength (366nm)
01	Root Powder as such	Brown	No florescence	No florescence
02	Powder + 50% H2SO4	Light brown	Greenish black	Dark brown
03	Powder + 1N HCl	Light brown	Greenish black	Dark brown
04	Powder + 50% HNO3	Golden yellow	Water green	Black
05	Powder + 5% KOH	Milky coffee	Greenish black	Light brown
06	Powder +MeOH	Light brown	Light brown	Black
07	Powder + 1N NaOH	Dark brown	Greenish brown	Dark black
08	Powder +Dist.Water	Brown	Greenish brown	Dark brown
09	Powder +Picric acid	Sulphur yellow	Dark brown	Dark black
10	Powder +5% Iodine sol.	Light brown	Dark brown	Dark black
11	Powder +5% Fecl3	Dark brown	Dark brown	Dark black
12	Powder +5% Acetic acid	Light brown	Dark brown	Dark brown
13	Powder + Ammonia	Sulphur yellow	Cascade green	Light green

Table 4: Preliminary phytochemical analysis of various root extracts of *Premna latifolia*

S. No.	Chemical Constituents	Pet.ether ext.	Chloroform ext.	Ethanolic ext.	Aqueous ext.
1	Carbohydrates	-	-	-	+
2	Proteins	-	-	-	-
3	Alkaloids	-	+	+	+
4	Saponins	-	-	-	+
5	Tannins	-	-	-	-
6	Flavonoids	+	-	+	+
7	Steroids	+	+	-	-
8	Fixed oil	-	-	-	-
9	Volatile oil	-	-	-	-
10	Triterpenoids	+	+	+	+
11	Glycosides	+	+	-	-
12	Starch	-	-	-	+
14	Resins	-	-	-	-

Table 5: TLC profile of different root extracts of Premna Latifolia

Extracts	Solvent system	Detecting reagents			
		UV(254nm) No. of Bands	UV(366nm) No. of Bands	Iodine chamber No.of Bands	After Derivitisation using Ethanolic sulfuric acid. No.of Bands
Ethanolic extract	Toluene:Ethylacetate (9.3:0.7)	3 (0.15,0.46,0.82)	4 (0.08,0.44,0.70,0.85)	9 (0.08,0.15,0.25, 0.45,0.56,0.65,0.70,0.82,0.85)	5 (0.08,0.15, 0.65,0.70,0.82)
	Chloroform: Ethylacetate (9.5:0.5)	2 (0.46,0.82)	3 (0.15,0.67,0.73)	6 (0.15,0.46,0.67,0.73,0.82,0.86)	3 (0.15,0.67,0.86)
	100% Chloroform	1 (0.15)	3 (0.15,0.45,0.84)	5 (0.08,0.15,0.56,0.72,0.80)	2 (0.15,0.72)
	Toluene: Chloroform: Methanol (2:6:2)	2 (0.08,0.26)	4 (0.08,0.16,0.66,0.80)	5 (0.08,0.16,0.52, 0.66,0.80)	3 (0.08,0.52,0.66)
	Toluene:Ethylacetate:Aceti c acid(8.7:1:0.3)	2 (0.17,0.29)	3 (0.10,0.17,0.87)	4 (0.10,0.17,0.29,0.87)	3 (0.10,0.29,0.87)
Chlorofor m extract	Toluene:Ethylacetate (9.3:0.7)	2 0.09,0.51)	4 (0.10,0.38,0.47,0.86)	7 (0.10,0.19,0.38,0.46,0.51,0.70, 0.85)	4 (0.10,0.19,0.38, 0.74)
	Chloroform: Methanol (9.5:0.5)	2 (0.11,0.87)	3 (0.19,0.47,0.87)	6 (0.11,0.19,0.35,0.47,0.55,0.87)	3 (0.19,0.47,0.87)
	Methanol:Toluene: Ethylacetate (0.3:9:0.7)	2 (0.20,0.85)	4 (0.12,0.20,0.32,0.85)	5 (0.12,0.20,0.32,0.44,0.85)	2 (0.20,0.85)
	Toluene:Ethylacetate:Aceti c acid (8.7:1:0.3)	2 (0.09,0.70)	4 (0.22,0.37,0.47,0.59)	6 (0.09,0.22,0.25,0.37,0.47,0.70)	2 (0.09,0.59)
	Chloroform: Ethylacetate (9.5:0.5)	1 (0.39)	3 (0.21,0.47,0.68)	5 (0.16,0.21,0.39,0.47,0.68)	3 (0.21,0.47,0.68)
Petroleu m ether extract	Chloroform: Ethylacetate (9.5:0.5)	4 (0.06,0.24,0.44,0. 53)	5 (0.06,0.24,0.44, 0.88,0.91)	8 (0.06,0.24,0.44,0.53,0.58,0.66,0. 84,0.88)	2 (0.53,0.91)
	Toluene: Chloroform: Methanol (2:6:2)	1 (0.45)	3 (0.45, 0.61,0.88,0.90)	5 (0.12,0.45,0.61,0.88,0.90)	2
	Toluene:Ethylacetate (9.3:0.7)	2 (0.15,0.50)	6 (0.10,0.15,0.35,0.50,0.57, 0.76)	7 (0.10,0.15,0.26,0.33,0.49,0.57,0. 76)	3 (0.35,0.50,0.57)
	100% Chloroform	2 (0.26,0.33)	4 (0.10,0.15,0.26,0.33)	6 (0.10,0.15,0.26,0.33,0.42,0.59)	1 (0.59)
	Toluene: Ethylacetate: Acetic acid (8.7:1:0.3)	2 (0.12,0.24)	4 (0.12,0.24,0.39,0.47)	6 (0.12,0.24,0.39,0.47,0.53,0.67)	2 (0.24,0.39)
Aqueous extract	100% 1-Propanol	1 (0.13)	2 (0.13,0.16)	2 (0.13,0.16)	2 (0.13,0.16)
	1-Propanol: Water (6:4)	1 (0.15)	1 (0.17)	2 (0.15,0.17)	2 (0.15,0.17)
	1-Propanol:Methanol: Water (5:1:4)	1 (0.09)	1 (0.09)	2 (0.09,0.12)	2 (0.09,0.12)
	Methanol: Water (6:4)	1 (0.08)	2 (0.08,0.11)	1 (0.11)	1 (0.11)
	1-Propanol:Butanol: Water (5:1:4)	1 (0.16)	1 (0.16)	2 (0.16,0.18)	1 (0.16)

Fluorescence analysis

Many phytochemicals show fluorescence when suitably illuminated. The fluorescence colour is specific for each compound. A non-fluorescent compound may fluoresce if mixed with impurities that are fluorescent. Hence, it is useful in detecting the adulterants and substituent. Root powder was treated with different reagents and observed for fluorescence under visible light and under UV light (short and long wavelength) and fluorescence was observed with many reagents.

Preliminary phytochemical studies

Preliminary phytochemical analysis of ethanol, chloroform, petroleum ether and aqueous extracts of the plant showed strong presence of triterpenoids and alkaloids with trace amounts of carbohydrates and flavonoids.

Thin layer chromatography

TLC profile of different root extracts of the plant was found as per Table 5 when developed TLC plates were observed under UV light, iodine chamber and after derivitisation with 15% Ethanolic sulphuric acid followed by heating at 105^0c for 15 min.

HPTLC Analysis

HPTLC analysis revealed the following data-

Ethanolic Extract-

At 366nm-

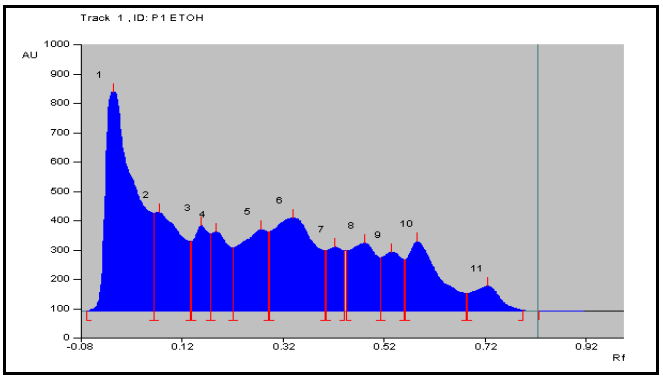


Fig. 11: HPTLC chromatogram of ethanol extract at 366nm

At 554nm-

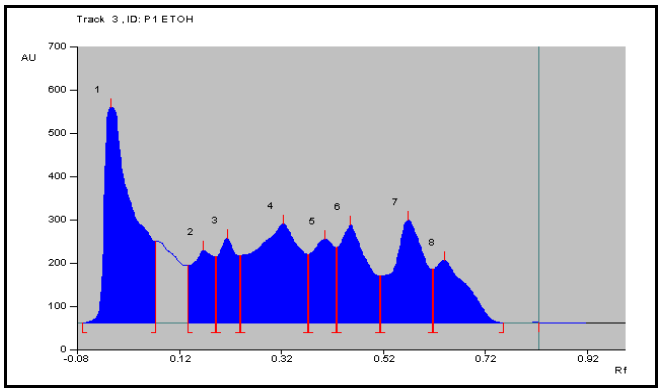


Fig. 12: HPTLC chromatogram of ethanol extract at 554 nm (after derivatization in iodine chamber)

Chloroform Extract

At 366nm-

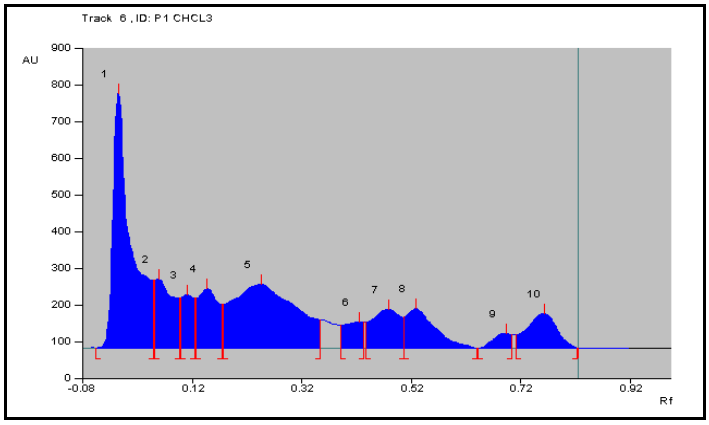
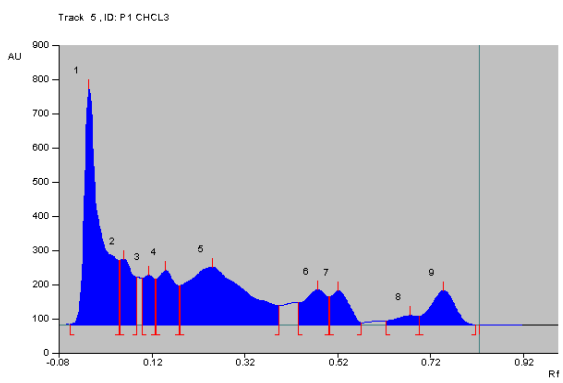
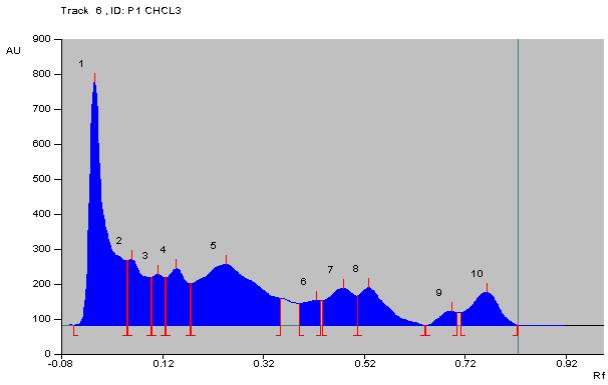


Fig. 13: HPTLC chromatogram of chloroform extract at 366nm





At 554nm-

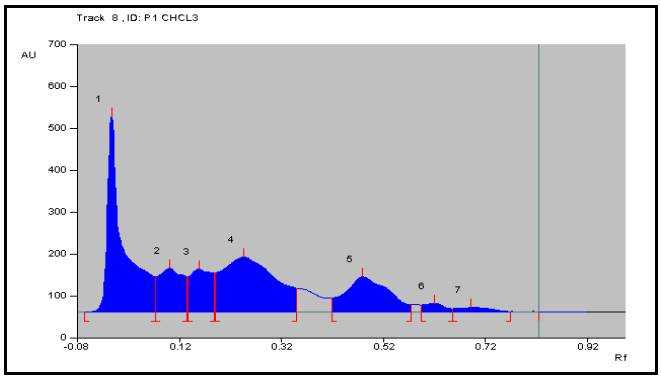


Fig. 14: HPTLC chromatogram of chloroform extract at 554 nm (after derivatization in iodine chamber)

Petroleum ether Extract

At 366nm-

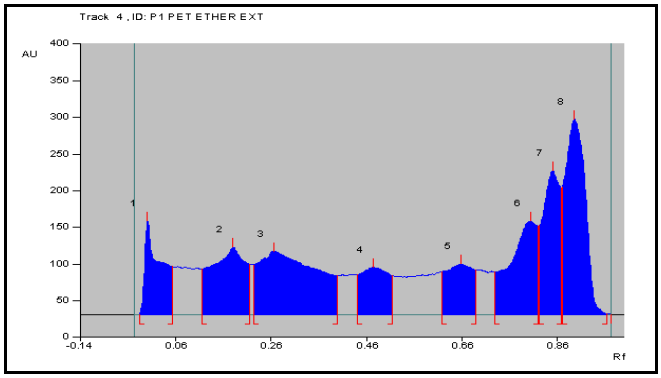


Fig. 15: HPTLC chromatogram of petroleum ether extract at 366nm

At 554nm-

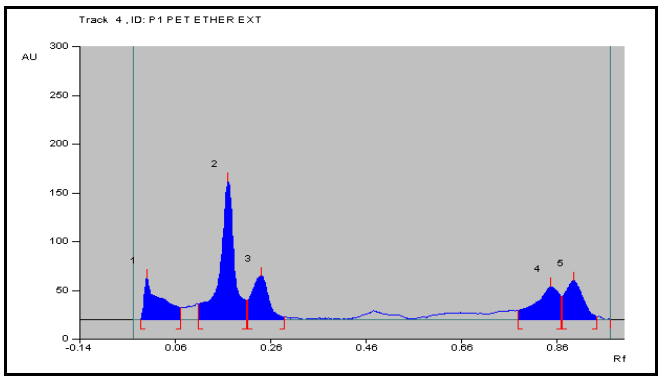


Fig. 16: HPTLC chromatogram of petroleum ether extract at 554 nm (after derivatization in iodine chamber)

Aqueous Extract

At 366nm-

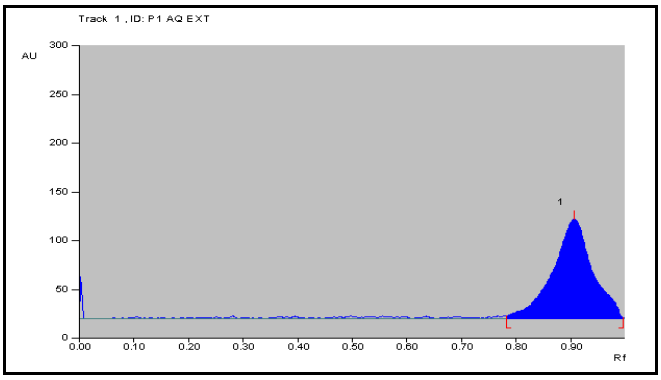


Fig. 17: HPTLC chromatogram of aqueous extract at 366nm

At 554nm-

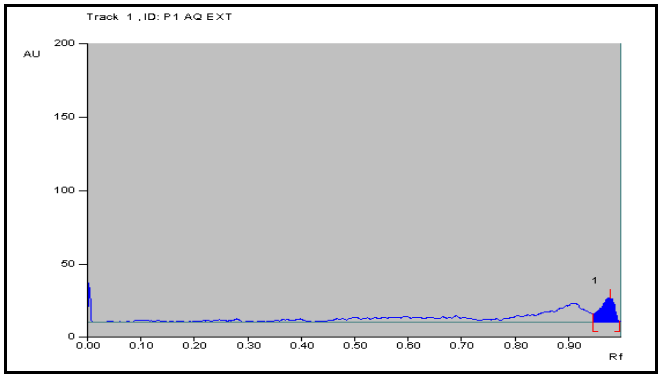


Fig. 18: HPTLC chromatogram of aqueous extract at 554 nm (after derivatization in iodine chamber)

CONCLUSIONS

To ensure reproducible quality of herbal products, proper control of starting material is utmost essential. Thus in recent years there have been emphasis in Standardization of medicinal plants of therapeutic potential. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken.The macro and microscopical characters along with physicochemical and fluorescence characters of root powder *of Premna latifolia* was used to establish the pharmacognostical standards and qualitative parameters as per pharmacopoeia and WHO guide lines. The information obtained from the preliminary phytochemical screening will reveal the useful findings about the chemical nature of the drug and it showed that the roots of contains triterpenoids as the chief constituent which could be bioactive. The comparative and multidisciplinary approach of the study of *Premna latifolia* helps in understanding its identification, taxonomical determination and medicinal importance in depth.

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REFERENCES

1. Linnaeus C. Mantissa Plantarum. Laurentius Salvius, Stockholm, 1771, p.587.
2. Harley R M, Atkins S, Budantsev PD. The Families and Genera of Vascular Plants: Flowering Plant-Dicotyledons. In: K. Kubitzki, editor. Labiatae. Germany:Springer-Verlag, 2004, p.267-8.
3. Fletcher HR. The Siamese Verbenaceae.Bulletin of Miscellaneous Information. Royal Botanic Gardens, Kew, 1938, 10, p.401-45.
4. Moldenke HN. A sixth summary of the Verbenaceae, Avicenniaceae, Stilbaceae, Chloanthaceae, Symphoremaceae, Nyctanthaceae and Eriocaulaceae of the world as to valid taxa, geographic distribution and synonymy. Phytologia Memoirs; 1980, 2: 284-285.
5. Thai Plant Names Tem Smitinand. The Forest Herbarium. Royal Forest Department revised edition, 2001, p.810 .
6. Nadkami AK. Indian material medica. New Delhi: Popular prakashan,1985, p.1008.
7. The wealth of India. New Delhi: Raw Materials- Vol VIII.NISCAIR, CSIR; 2005.P.239.
8. Council of Scientific and Industrial Research, India. The wealth of India: a dictionary of Indian raw materials and industrial products. Raw materials. New Delhi.
9. Medicinal and aromatic plant abstracts. , 1995-2010, New Delhi: NISCAIR, CSIR.
10. Rajendran R, Suseela L, S undaran MR, Basha NS. Caridiac stimulant activity of bark and wood of P.serratifolia. Bangladesh J of Pharmacology; (2008), 3(2):107-113.
11. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African Journal of Biotechnology; (2008), 7 (12): 1797-1806.
12. Mukherjee K. P. Quality control of herbal drugs, published by business horizon pharmaceutical publishers, New Delhi, 2005.
13. Wagner H. and Bladt S., Plant Drug Analysis, 2 ed, Nirali prakashan –India, 2000.
14. Handa SS, Khanuja SPS, Longo G, Rakesh DD. Extraction Technologies for Medicinal and Aromatic Plants. International centre for science and high technology, Trieste, 2008:21-25.
15. Mojab, F., Kamalinejad, M., Ghaderi, N., Vanidipour, H.R.Phytochemicals screening of some species of Iranian plants.Iran. J. Pharm. Res., 2003, 3: 77-82.
16. Sanja SD, Sheth NR,Patel NK, Patel D and Patel B. Characterization and evaluation of antioxidant activity of Portulaca Oleracea .Int J Pharm Pharm Sci 2009 ; 1, suppl 1:74:84.
17. Khatri DK, Juvekar P, Juvekar AR. Phytochemical Investigation and In Vitro Antioxidant Activities Indigofera Cordifolia Seed Extracts. Int J Pharm Pharm Sci. 2013; 5, Suppl 2:71-75
18. Chase, C.R. and Pratt, R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, J. Am.Pharmacol. Assoc., (1949), 8: 324-331.
19. Harbone, J.B. Method of extraction and isolation, In: Phytochemical methods, Chapman & Hall, London, 1998, p.60-66.
20. Bhakuni DS, Dhar ML, Dhar MM. Screening of Indian plants for Biological activity. Part II. Indian J exp Biol; (1969), 7:p.250-262.
21. Pandya DJ, Desai TR, Nadpara NP, Mehta HA, Modi AM. Pharmacognostic study and establishment of quality parameters of leaves of Bombax insigne Linn. Int J Pharmacogn PhytochemRes, (2010); 2(3): 1-5.
22. Ali M. Text book of pharmacognosy. New Delhi: CBS Publishers &Distributers; 2008, p. 181-211.
23. Gokhale SB, Kokate CK. Practical pharmacognosy. 12th ed. Pune:Nirali Prakashan,2008;p. 24.
24. Khandelwal KR. Practical pharmacognosy. 18th ed. Pune: Nirali publication, 2007:p.10-14.
25. Gupta AK, Tandon N, Sharma M. Quality standards of Indian medicinal plants. New Delhi: Indian Council of Medical Research, 2008: p.246-255.