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PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF LEAF OF PREMNA INTEGRIFOLIA LINN

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ABSTRACT

Premna integrifolia Linn. is a large shrub or small tree belonging to the family Verbenaceae. The present study deals with the pharmacognostical and phytochemical study of leaf including chromatographic evaluation. *Premna integrifolia* Linn. leaf is obovate or elliptic, acuminate at apex, entire or finely serrate at margin, cuneate or rounded at base and coriaceous, glabrous in texture. Leaf of the plant can be identified microscopically by the presence of hook shaped and sessile glandular trichomes, starch grains, oil globules, diacytic type of stomata and prismatic crystal. Purity test shows loss on drying (4.508% w/w), total ash (2.215% w/w), alcohol soluble extractive (5.362% w/w) and water-soluble extractive values(14.557% w/w), PH 6.5. Preliminary analysis revealed the presence of carbohydrates, steroid, alkaloids, tannin and phenol. HPTLC study of alkaloid showed the presence of five spots in short and four spots in long UV rays. The information generated by this study provides relevant Pharmacognostical and physico-chemical data needed for proper identification and authentication of leaf of *Premna integrifolia* Linn.

KEYWORDS: Premna Integrifolia, Trichomes, Starch Grains, Oil Globules.

INTRODUCTION

Premna integrifolia Linn.(*Premna serratifolia* Linn.) is a large shrub or small tree reaching 9m high. Bark yellowish, lentcellate. Young parts glabrous or slightly pubescent. Trunk and large branches are sometime spinous. Leaves 5-9 by 3.2-6.3cm, broadly elliptic, obtuse, very shortly acuminate, glabrous, entire or the upper part dentate, base rounded or subacute. Petioles 1-1.6cm long. Flowers small, greenish yellow, with a disagreeable odour, in terminal pubescent panickle corymbose cymes. Bracts minute, lanceolate. Calyx 2.5cm long, thick, glabrous. Corolla glabrous outside. Fruit 4mm long, pear-shaped, 4-seeded.[1] The study was conducted to identify and standardize the botanical identification of plant specie.

MATERIALS AND METHODS

Collection and Authentification: The plant growing in Sasoi botanical garden, Jamnagar, Gujarat, was authenticated by expert taxonomist as on the basis of characters given in Indian Medicinal plants[2] and various floras. The fresh plant sample was collected from its natural habitat, Gujarat, in the month of May 2015 and voucher specimen had been preserved in the pharmacognosy laboratory of IPGT and RA, vide no 6188/15. The collected plant sample was shaken to remove adherent soil and dirt. The leaves were separated from the stem and then washed with running fresh water and few pieces stored in solution of AAF (Alcohol: Acetic acid: Formalin) in the ratio of (90:5:5)[3] to utilize them for microscopic studies. The remaining leaves were shade dried and then powdered with mechanical grinder and passed through mesh no.80# and preserved in an air-tight glass container.

Morphological characters were studied by observing the leaf as such and also with the help of the dissecting microscope. For detailed microscopical observation, free hand thin transverse section passing through the midrib of leaf was taken and cleared with chloral hydrate and observed as such for the presence of any crystals, then were stained with Phloroglucinol and Hydrochloric acid to notice the lignified elements like fibers, vessels etc. of the meristele and other parts.[4] Photographs of the section were taken with the help of Canon digital camera attached to Zeiss microscope. Powder characters were observed and histochemical tests carried out, according to the standard guidelines of practical pharmacognosy.[5] Physicochemical parameters and Phytochemical screening were carried out as per the guidelines of Ayurvedic Pharmacopoeia of India.[6] Physicochemical parameters and Phytochemical screening were also carried out as per the guidelines of Ayurvedic Pharmacopoeia of India. HPTLC[7] was carried out for the analysis.

OBSERVATIONS AND RESULTS

Table No. 1: External morphology of leaf of *Premna integrifolia* Linn.

Parameters	*Premna integrifolia* Linn.
Colour	Bright green
Size	9×3.4cm
Apex	Acuminate
Shape	Obovate or elliptic
Odour	Characteristic
Taste	Pungent and bitter
Margin	Entire or finely serrate
Base	Cuneate or rounded
Texture	Coriaceous, glabrous
Venation	Reticulate, 4-5pairs

Microscopic characters of leaf of *Premna integrifolia* Linn.

Transverse section of petiole

Diagrammatic T.S. of petiole showed presence of epidermis, cortex, pericyclic fibres, phloem, xylem and central pith.

Epidermis was single layered, cuticularised, with compactly arranged oval to square shaped cells. Some of the epidermal cells were seen interrupted by multicellular warty, hooked and glandular sessile trichomes, which also consist of oil globules.

Ground tissue was differentiated into two layers of hypodermis, which was formed by thick collenchyma cells followed by parenchyma cells which were filled with acicular crystals and prismatic crystals of calcium oxalate. Parenchymatous layer was followed by layers of pericyclic fibres. Endodermis was observed to be single layered.

Vascular bundles were located nearly at the centre, having boat shaped appearance, open and collaterally arranged. Xylem was seen made up of xylem parenchyma and phloem was made up of sieve elements of phloem fibres.

Transverse section through mid-rib

T.S. of leaf through midrib showed presence of upper and lower epidermis and centrally located vascular bundle.

Epidermis: Upper epidermis was observed single layered made up of rectangular shaped cells and covered with thick cuticle. Some of the epidermal cells were interrupted by presence of multicellular warty, hook shaped and sessile glandular trichomes. Lower

epidermis was found to be oval to rectangular in shape and was interrupted by multicellular warty and sessile glandular trichomes and also showed interruption by some stomatal openings.

Mesophyll was differentiated into upper layer of palisade parenchyma cells which were found to be arranged compactly without any intracellular space and was highly loaded with chlorophyll pigments. Lower parenchyma showed 3-4 layers of spongy parenchyma, whose cells were round to irregular in shape and having air spaces between them and also loaded by chlorophyll pigments. Both the spongy and palisade parenchyma layers showed presence of oil globules. Between palisade and spongy parenchyma cells strands of vascular strands were present. Single layer of collenchyma cells was present on both upper and lower epidermis. Discontinuous bands of pericyclic fibers covered the vascular bundle.

Vascular bundle

Showed phloem towards lower epidermis and xylem towards upper epidermis. Phloem was made up of phloem fibers and some sieve plates while xylem consists of xylem parenchyma and its fibers.

Surface study

Lower epidermis

Diacytic type of stomata was found to be present, which measured 0.8×0.4mm, 0.8×0.5mm, 0.7×0.4mm at different angles with their mean size to be around 0.8 × 0.4mm. Glandular sessile trichomes were found all over periphery, which had a radius of 5mm, 6mm and 5mm, having mean radius of 5.3mm. Multicellular warty trichomes were also present with 8 celled structures. Stomatal Index i.e. ratio of number of stomata present to the number of epidermal cells surrounding each stomata was observed to be 28.45. Two glandular sessile trichomes were found to be present in per square mm area.

Upper epidermis

Greenish chlorophyll pigments were found more in upper epidermis section as compared to lower epidermis. Diacytic type of stomata were present which measured 0.7×0.4mm, 0.8×0.3mm, 0.9×0.4mm at different angles with mean size of 0.8 × 0.4mm. Glandular sessile trichomes were also present with radius of 5mm, 4mm, 5mmat various angles with average radius of 4.6mm. Stomatal Index observed was 25.19. Only one glandular trichome was present in per square mm area.

Leaf powder microscopy: Leaf powder was dark green in colour, somewhat coarse in touch with characteristic irritant smell and bitter-pungent taste. Microscopic examination showed presence of multicellular warty trichomes, spongy parenchyma cells, simple fibres, oil globules, spiral vessels, prismatic crystals, brown contents, epidermal cells, glandular trichome fragments.

Table No. 2: Micrometric evaluation of *P. integrifolia* Linn. leaf powder.

S.N	Character	*Premna integrifolia* Linn.	Measurements
1	**Crystal –** **Prismatic**	 Present	 0.6×0.3
2	**Trichomes –** **Warty** **Multicellular** **Hook shaped** **Glandular- oil filled**	 Present Present Present Present	 7.5×0.5 4.0×0.5 1.5×0.4 1.2×0.5
3	**Stomata – (fragments)** **Diacytic**	 Present	 0.8×0.4
4	**Fragments of fiber**	Present	8.7×0.3
5	**Fragments of spongy parenchyma**	Present	-
6	**Fragments of epidermal cells**	Present	-
7	**Fragments of annular vessels**	Present	-
8	**Oil globules**	Present	-
9	**Starch grains**	Present	-

Table No. 3: Phyto-constituents confirmation test.

S. No	Reagent	Observation	Characteristics
1.	Iodine solution–**Starch**	Macroscopic- it become blackish Microscopic- found round shape particles	+
2.	Ferric chloride–**Tannin**	Samples become blackish	+
3.	HCl–**Calcium**	It produced bubbles	+
4.	HCl–**Lignin**	Lignified elements become red in colour	+
5.	Sudan III solution–**Oil globules**	It becomes red in colour	+

Table No. 4: Physicochemical parameters of leaf powder of *P.integrifolia* Linn.

S. N	Parameters	*P.integrifolia* Linn. Leaf
1.	**Loss on Drying (%w/w)**	4.508
2.	**Total Ash Content (%w/w)**	2.215
3.	**Water Soluble Extractive Value (%w/w)**	14.557
4.	**Alcohol Soluble Extractive Value (%w/w)**	5.362
5.	**pH**	6.5

Table No. 5: Phytochemical evaluation of *P.integrifolia* leaf.

Sr No	Tests	*P. Integrifolia* leaf
1.	Proteins	
a.	Biuret test	–ve
b.	Ninhydrin test	–ve
c.	Xanthoproteic test	–ve
d.	Hopkins-cole test	–ve
e.	Sulphur test	–ve
2.	Carbohydrate test for starch	
a.	Molisch's test	–ve
b.	Iodine test	+ve
c.	Fehling's test	+ve
d.	Benedict's test	–ve
e.	Test for non reducing sugar such as sucrose	–ve
3.	Tannins	
a.	Gelatin test	+ve
4.	Anthrocyanins	
a.	Aqueous NaOH test	+ve
b.	Conc. H_2SO_4 test	–ve
5.	Glycosides	
a.	Molisch's test	+ve
b.	Conc. H_2SO_4 test	+ve
c.	Keller Kiliani test	–ve
J	Saponin	
a.	Foam test	–ve
7.	Flavonoids	
a.	Flavonoid test	–ve
b.	Shinoda test	–ve
d.	Aqueous NaOH test	–ve
e.	Conc. H_2SO_4 test	+ve
8.	Phenols	
a.	Phenol test	+ve
9.	Steroids	
a.	Salkowski's test	+ve
10	Alkaloids	
b.	Dragendroff's test	+ve

Table No: 6 HPTLC Analysis of *P.integrifolia* Linn. Leaf.

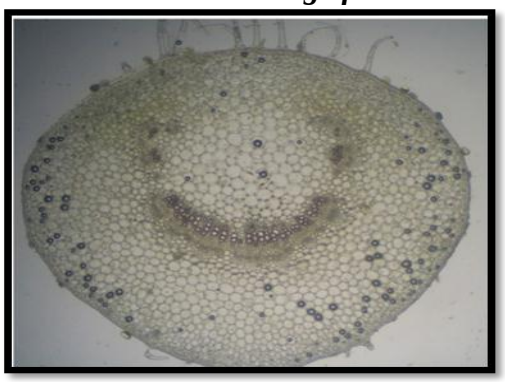
Sample	Solvent System (V/V)	Short UV 254 nm		Long UV 366 nm	
		No. of spots	Rf value	No. of spots	Rf value
PIL	Toluene: Ethyl acetate: Acetic acid 7: 2: 0.5	5	0.59, 0.65, 0.70, 0.71, 0.82, 0.84	4	0.03,0.59, 0.65, 0.70, 0.82, 0.84, 0.98

***Premna integrifolia* Linn. Plant.**

Inflorescence.

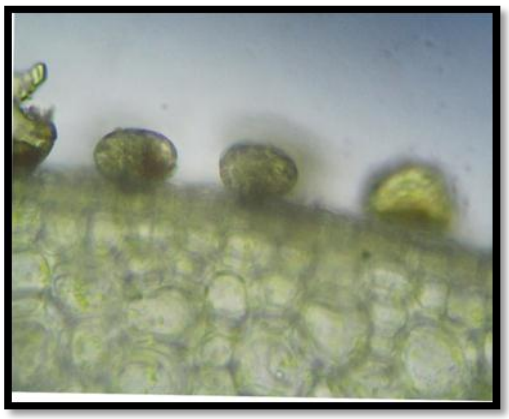
Fruit of *Premna integrifolia* Linn.

***Premna integrifolia* Linn.**

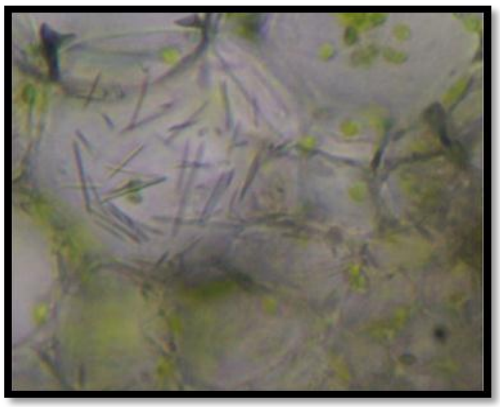


TS of Petiole.

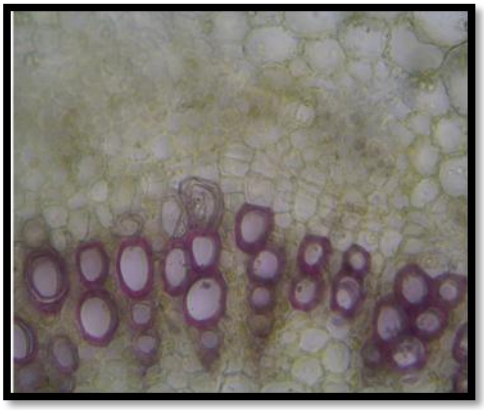
Hooked & sessile Trichome.



Sessile & glandular Trichome.

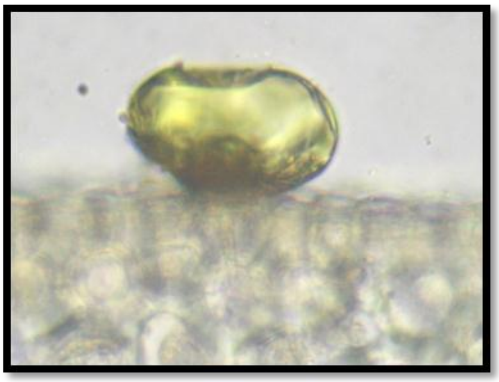


Oil globule.

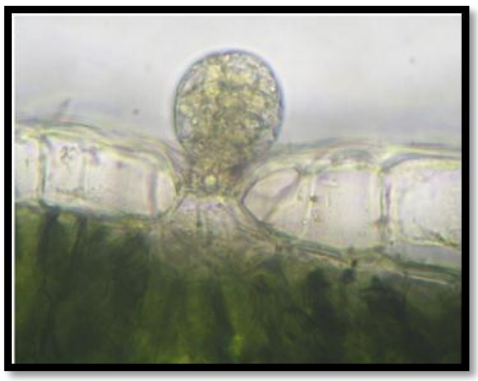


Vascular Bundle.

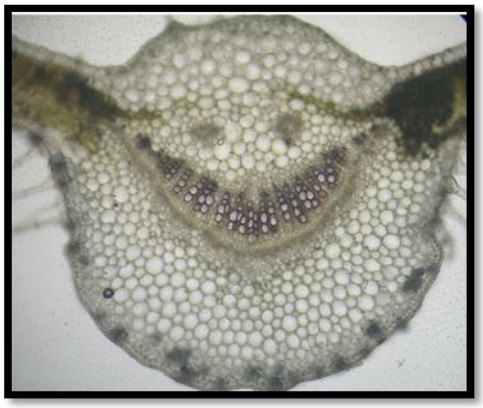
Multicellular warty Trichome.



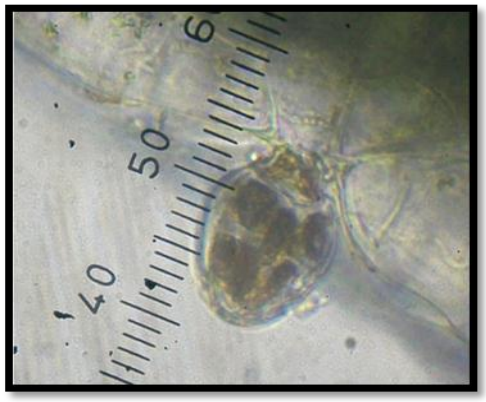
Oil filled sessile Trichome.



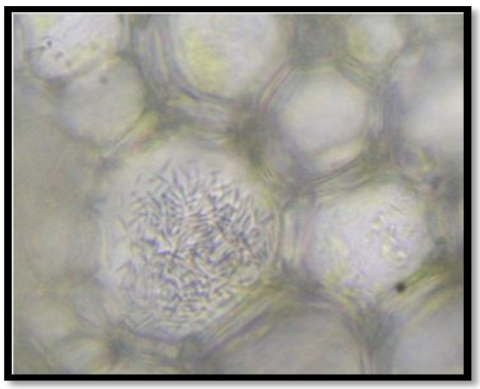
Oil filled glandular Trichome.



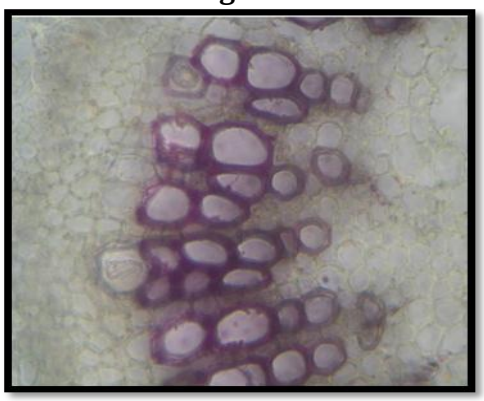
Section through Mid rib.



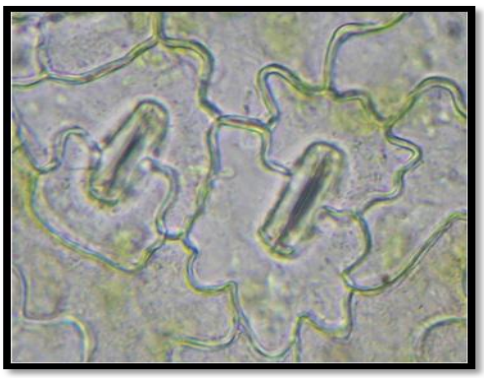
Measurement of glandular Trichome.



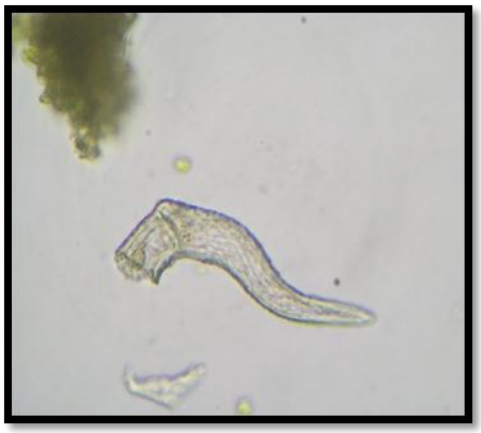
Starch grain with oil globule.



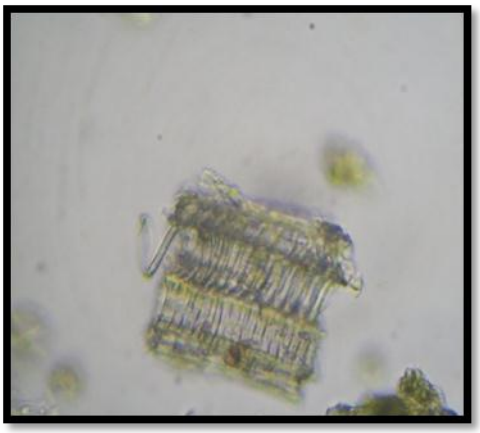
Vascular bundle with Xylem & Phloem.



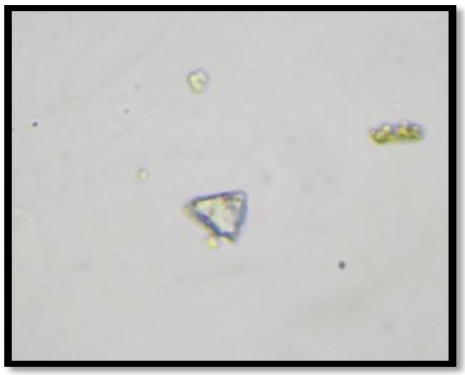
Diacytic Stomata



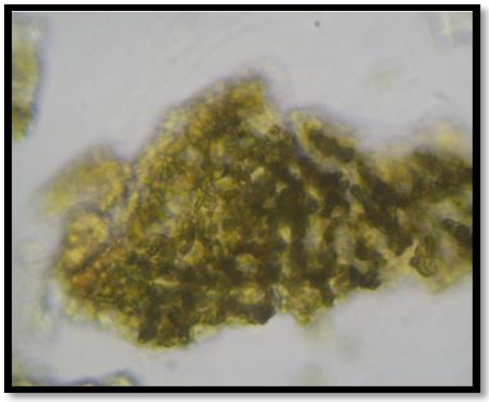
Multicellular warty Trichome.



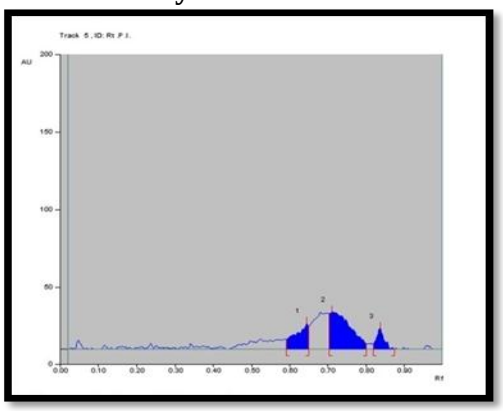
Spiral vessels Fragment.



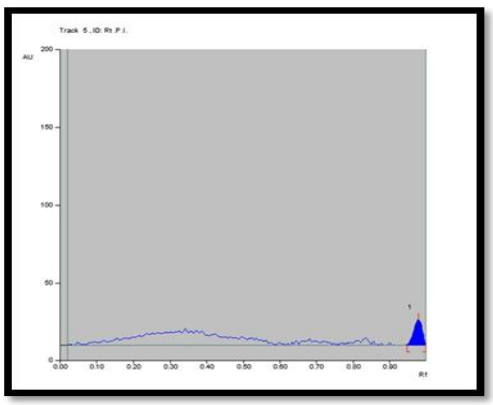
Prismatic Crystal of Calcium oxalate.



Brown content.



Densitogram at 254nm.



Densitogram at 366nm.

CONCLUSION

Premna integrifolia Linn. Leaf can be identified microscopically by the presence of hook shaped and sessile glandular trichomes, starch grains, oil globules, diacytic type of stomata and prismatic crystal. Obtained physico chemical and phytochemical parameters can be considered as the standards for genuinity of the plant. The observed pharmacognostical characters, phytochemical parameters and HPTLC findings may be useful to establish the botanical standards for identification and standardization of Leaf of *Premna integrifolia* Linn.

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