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Research Article

EVALUATION OF PHYSIOCHEMICAL , PHARMACOGNOSTICAL AND PHYTOCHEMICAL PARAMETERS OF *PREMNA HERBACEA* .

D. THIRUMALAI[1], M. PARIDHAVI[2] AND M. GOWTHAM[2*]

[1] VIT University, Vellore, Tamilnadu, India-632014 ,[2]Rajiv Gandhi Institute of Pharmacy, Trikaripur, Kasaragod, Kerala, India-671310.
Email: gowthamm1984@gmail.com

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ABSTRACT

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. *Premna Herbacea* belonging to genus Premna and family Verbenaceae was one among them. Morphological and microscopical characters of the plant were studied. Preliminary photochemical analysis of ethanol, chloroform, petroleum ether and aqueous extracts of these plants were done. The results showed strong presence of triterpenoids and alkaloids with trace amounts of carbohydrates and flavonoids. The physiochemical parameters of the plant were within the limits. Root powder was treated with different reagents and observed for fluorescence under visible light and under UV light of short and long wavelength. They exhibited fluorescence.TLC and HPTLC of various extracts of the plant also yielded satisfactory results. Further evaluation needs to be carried out on *Premna Herbacea* 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;Antiinflammatory; Antidiabetic; 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 plant medical traditions in the world[1]. 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 Plant *Premna Herbacea* was a small bushy tree which was used as herbal drugs in Ayurveda. The plant has got antiinflammatory, analgesic and antiulcer activity[5,6].

MATERIALS AND METHODS

Collection and Authentication

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

Morphological evaluation

Roots of *Premna Herbacea* 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, water soluble ash, extractable matter, loss on drying, foaming index and swelling index etc [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 photochemical 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 a 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 (1cm 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. When 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 for 15 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 Herbacea* was found as Table1.

Table 1:Morphological evaluation of roots of Premna Herbacea.

Sl no	Character	When fresh	After drying	Powder
1	**Colour**	Light brownish yellow	Dark brown	Brownish yellow
2	**Odour**	Characteristic	Characteristic	Characteristic
3	**Taste**	Bitter	Bitter	Bitter
4	**Shape**	Globular	Globular	-
5	**Texture**	Hard	Hard	-

Microscopic studies

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

a)T. S of the root showed-

The root is circular in outline but the margin is broken due to the secondary growth hence it appears to be undulated. A root was surrounded by thick layer of periderm. It was crushed with the periderm by the secondary Xylem. No thin walled cells were found in the root. The Periderm was followed by secondary xylem. The xylem mainly composed of trachieds and vessels. The trachieds which were three or four sided, possess thick wall and the lumen was completely filled with secondary cell depositions, hence it gives much strength and makes the root harder. The vessels were broad primary vessels and were found towards the inner sides of the xylem. The vessels were either round or elliptical in shape. The xylem was traversed by numerous ray parenchymatous cells and these cells were also thick due to the secondary cell wall depositions. The middle portion of the root was occupied by thick walled cells.

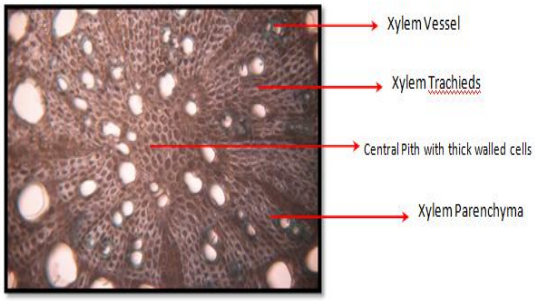

Fig.1:Central portion of the root in transverse section.

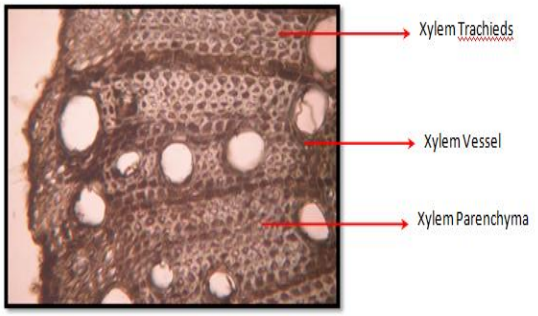

Fig. 2:Perpheral region of the root in transverse section.

b)Powder microscopy analysis of root showed that-

Fibres

The broken fibres of different length were found in the powder. The majority of the portion of the root consists of well developed xylem. Hence this was possible a xylem fibre and the fibres were found to be single and groups.

Trachieds

The trachieds were found in group. They were joined with each other in lateral position. The lateral wall of the trachieds was provided with bordered pits. The trachieds were much broader than the fibres and shorten in length. In some places the bundle of fibres and trachieds were found to be joined with each other along with parenchyma cells. In the root powder of *Premna Herbacea* the trachieds with spiral thickening were also found.

Parenchyma cells

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

Sclerids

The Sclerids are a kind of sclerenchyma cell, which provide mechanical support to the plant. In the powder of *Premna Herbacea* a few number of macrosclerids are found here and there.

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.

Epidermis

The powder consisted of broken piece of tissues of various sizes. The cell found in the epidermal tissues were found to be dry and shrink

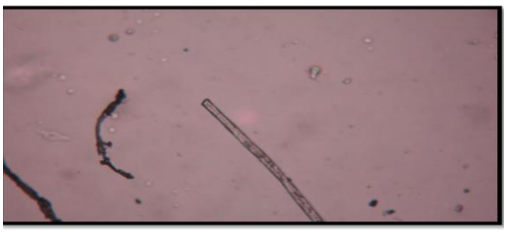

Fig.3: Fibres

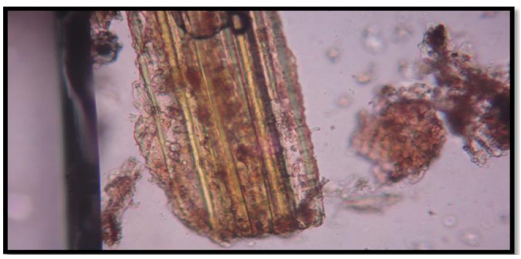

Fig.4: Bunddle of Tracheids

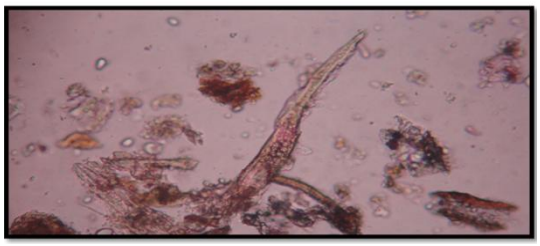

Fig. 5: Parenchyma cells.

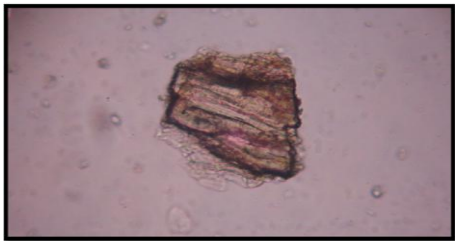

Fig.6:Sclerids

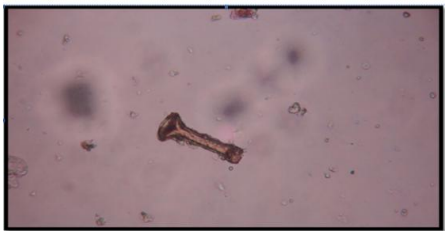

Fig.7:A broken Trichome.

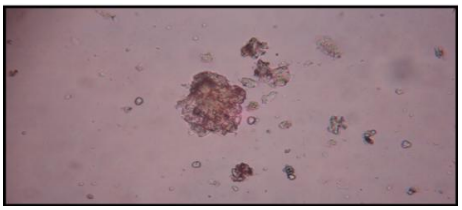

Fig. 8: Epidermis

Physiochemical studies

Physiochemical studies revealed that -

Table 2:Physiochemical studies of *Premna Herbacea* Root

Parameters	Kerala	Delhi	Mumbai	Limit
LOD	3.9%	4.2%	4.5%	NMT 4.5%
Total Ash	0.95%	0.88%	0.89%	NMT 0.95%
Water Soluble Ash	0.14%	0.08%	0.16%	NMT 0.16%
Acid Insoluble Ash	0.72%	0.71%	0.75%	NMT 0.75%
Sulphated Ash	0.81%	0.85%	0.79%	NMT 0.85%
Alcohol Soluble Matter	7.02%	6.09%	7.34%	NLT 6.09%
Water Soluble Matter	5.16%	5.75%	5.66%	NLT 5.16%
FOM	1.62%	1.71%	1.82%	NMT 1.82%
pH- 1% Aq.sol	6.5	6.4	6.4	6.4-6.5
pH- 10% Aq.sol	6.2	6.3	6.3	6.2-6.3

Fluorescence analysis

Many phytochemicals showed fluoresce 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 substituents.

Root powder was treated with different reagents and observed showed following results-

Table 3:Fluorescence analysis of root powder of *Premna Herbacea*.

Sl 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**	Yellowish brown	Yellowish brown	Yellowish brown
03	**Powder + 1N HCl**	Yellowish brown	Greenish brown	Golden yellow
04	**Powder + 50% HNO3**	Light brown	Yellowish black	Golden yellow
05	**Powder + 5% KOH**	Yellowish red	Greenish black	Dark black
06	**Powder +MeOH**	Light brown	Greenish brown	Yellowish black
07	**Powder + 1N NaOH**	Golden yellow	Greenish black	Dark black
08	**Powder +Dist.Water**	Black	Greenish black	Sulphur yellow
09	**Powder +Picric acid**	Sulphur yellow	Water green	Dark black
10	**Powder +5% Iodine sol.**	Light brown	Black	Sulphur yellow
11	**Powder +5% Fecl3**	Black	Dark brown	Dark black
12	**Powder +5% Acetic acid**	Light brown	Yellowish brown	Dark brown
13	**Powder + Ammonia**	Reddish brown	Greenish black	Sulphur yellow

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.

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

Sl no	Chemical Constituents	Pet. Ether ext.	Chloroform ext.	Ethanolic ext.	Aqueous ext.
1	**Carbohydrates**	-	-	-	+
2	**Triterpenoid**	+	+	+	+
3	**Alkaloids**	-	+	+	+
4	**Saponins**	-	-	-	+
5	**Tannins**	-	-	-	-
6	**Resins**	-	-	-	-
7	**Proteins**	-	-	-	-
8	**Fixed oil**	-	-	-	-
9	**Volatile oil**	-	-	-	-
10	**Saponins**	-	-	-	+
11	**Glycosides**	+	+	-	-
12	**Starch**	-	-	+	+
14	**Flavanoids**	+	-	+	+

Thin layer chromatography

TLC profile of different root extracts of two plants were found as per Table 5 when 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 for 15 min.

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

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	Chloroform: Ethylacetate(9.5:0.5)	3 (0.04,0.15, 0.27)	4 (0.07,0.12,0.36, 0.56)	8 (0.03,0.08,0.12, 0.15, 0.26,0.37,0.55, 0.97)	2 (0.26,0.97)
	Toluene: Ethylacetate(9.5:0.5)	3 (0.04,0.16, 0.25)	4 (0.07,0.09,0.15, 0.56)	6 (0.03,0.09,0.12, 0.16,0.56,0.95)	2 (0.26,0.97)
	Toluene: Chloroform(8:2)	2 (0.04,0.15)	3 (0.07,0.16,0.56)	5 (0.09,0.12,0.26, 0.55,0.97)	2 (0.26,0.97)
	Chloroform: Methanol(9:1)	2 (0.15,0.26)	3 (0.15,0.26,0.44)	5 (0.05,0.12,0.15, 0.56,0.83)	2 (0.16,0.56)
	Chloroform:Toluene:Ethylacetate (3:6:1)	2 (0.05,0.18)	3 (0.08,0.12,0.67)	4 (0.05,0.25,0.45, 0.83)	3 (0.03,0.26, 0.95)
Chloroform extract	Chloroform: Ethylacetate(9.5:0.5)	3 (0.04,0.20, 0.81)	5 (0.04,0.20,0.45, 0.82,0.97)	7 (0.05,0.11,0.17, 0.30 0.46,0.71,0.97)	2 (0.11,0.56)
	Chloroform: Methanol(9:1)	2 (0.04,0.81)	3 (0.20,0.48,0.89)	6 (0.06,0.10,0.30. 0.39,0.57,0.85	2 (0.12,0.95)
	Methanol:Toluene:Ethylacetate(0.3:9:0.7)	2 (0.07,0.57)	4 (0.17,0.25,0.48, 0.58)	5 (0.06,0.17,0.22, 0.77,0.89)	3 (0.17,0.32,0.83)
	Toluene: Chloroform(8:2)	2 (0.17,0.57)	3 (0.07,0.25,0.59)	6 (0.05,0.12,0.21, 0.46,0.70,0.95)	3 (0.21,0.37)
	Toluene: Chloroform: Ethylacetate(6:3:1)	3 (0.17,0.30, 0.88)	3 (0.05,0.30,0.88)	6 (0.05,0.12,0.21, 0.46,0.70.0.97)	2 (0.05,0.88)
Petroleum ether extract	Toluene:Chloroform(8:2)	2 (0.05,0.64)	4 (0.06,0.28, 0.54, 0.64,0.70,0.85)	7 (0.05,0.12,0.28, 0.55, 0.64,0.70,0.85)	2 (0.05,0.34)
	Toluene: Methanol8:2)	2 (0.06,0.12)	2 (0.05,0.85)	5 (0.05,0.12,0.28, 0.55,0.85)	1 (0.55)
	Chloroform:Ethylacetate(9:1)	1 (0.28)	5 (0.05,0.28, 0.55, 0.64,0.85)	6 (0.05,0.12,0.28, 0.55, 0.64,0.70)	2 (0.05,0.28)
	100% Chloroform	2 (0.54,0.64)	3 (0.05,0.12, 0.28)	5 (0.05,0.12,0.28, 0.64,0.85)	1 (0.64)
	Toluene:Ethylacetate:Diethylamine(7:2:1)	2 (0.05, 0.54)	4 (0.06,0.28, 0.64,0.70)	6 (0.05,0.12,0.28, 0.55, 0.64,0.70)	2 (0.05,0.48)
Aqueous extract	100% 1-Propanol	1 (0.94)	3 (0.34,0.56,0.94)	4 (0.34,0.56,0.66,0.94)	1 (0.67)
	1-Propanol: Water (8:2)	1 (0.56)	2 (0.34,0.94)	3 (0.34,0.56,0.94)	2 (0.34,0.66, 0.94)
	1-Propanol:Methanol: Water (5:2:3)	1 (0.45)	2 (0.45,0.96)	3 (0.48,0.56,0.95)	2 (0.45,0.96)
	Ethanol: Water (6:4)	1 (0.44)	2 (0.45,0.96)	2 (0.45,0.96)	1 (0.88)
	1-Propanol:Butanol: Water (5:1:4)	2 (0.45,0.96)	2 (0.46,0.96)	3 (0.34,0.56,0.94)	1 (0.97)

HPTLC Analysis

HPTLC analysis revealed the following datas-

1. Ethanol Extract-

a) At 366nm-

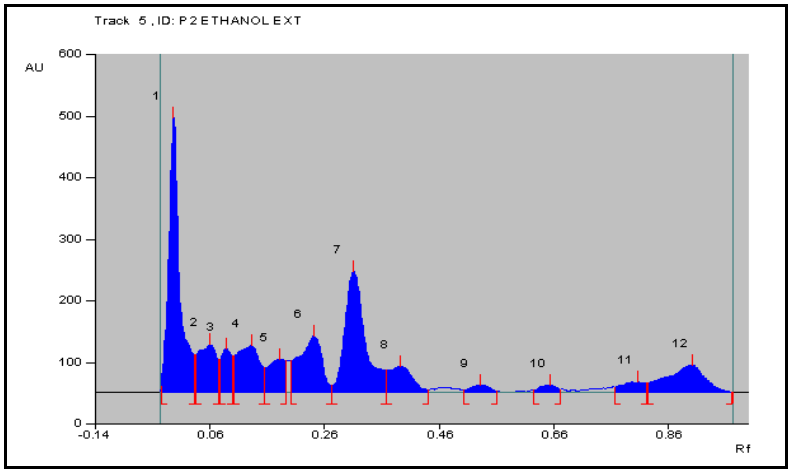

Fig. 9:HPTLC chromatogram of ethanol extract at 366nm

Table 6:Peak list and *Rf* value of the chromatogram of ethanol extract at 366nm

S.No	Peak	End Rf
1	1	0.03
2	2	0.07
3	3	0.1
4	4	0.15
5	5	0.19
6	6	0.27
7	7	0.37
8	8	0.44
9	9	0.56
10	10	0.67
11	11	0.82
12	12	0.97

b) At 554nm-

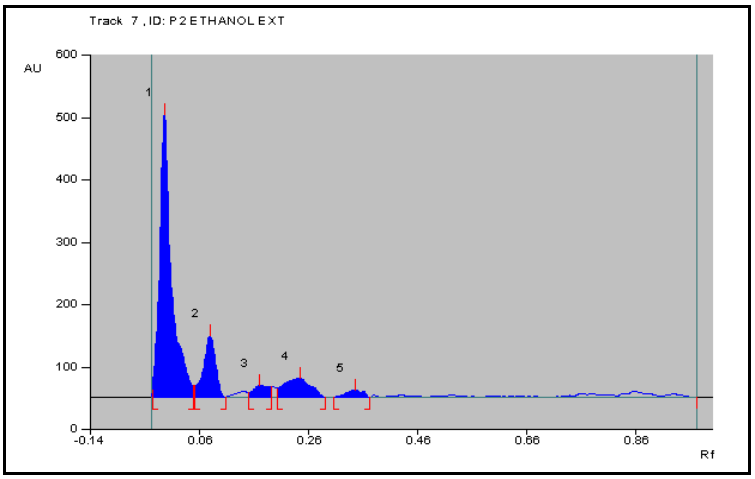

Fig.10:HPTLC chromatogram of ethanol extract at 554 nm (after derivatization in iodine chamber)

Table 7:Peak list and *Rf* value of the chromatogram of ethanol extract at 554 nm (after derivatization in iodine chamber)

S.No	Peak	End Rf
1	1	0.05
2	2	0.11
3	3	0.19
4	4	0.29
5	5	0.37

2. Chloroform Extract

c) At 366nm-

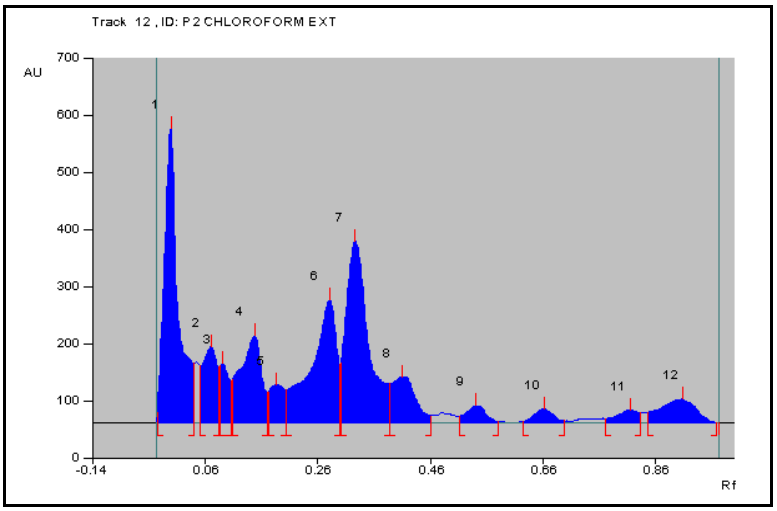

Fig.11:HPTLC chromatogram of chloroform extract at 366nm

Table 8-Peak list and *Rf* value of the chromatogram of chloroform extract at 366nm

S.No	Peak	End Rf
1	1	0.04
2	2	0.08
3	3	0.1
4	4	0.17
5	5	0.2
6	6	0.3
7	7	0.39
8	8	0.46
9	9	0.58
10	10	0.7
11	11	0.83
12	12	0.97

d) At 554nm-

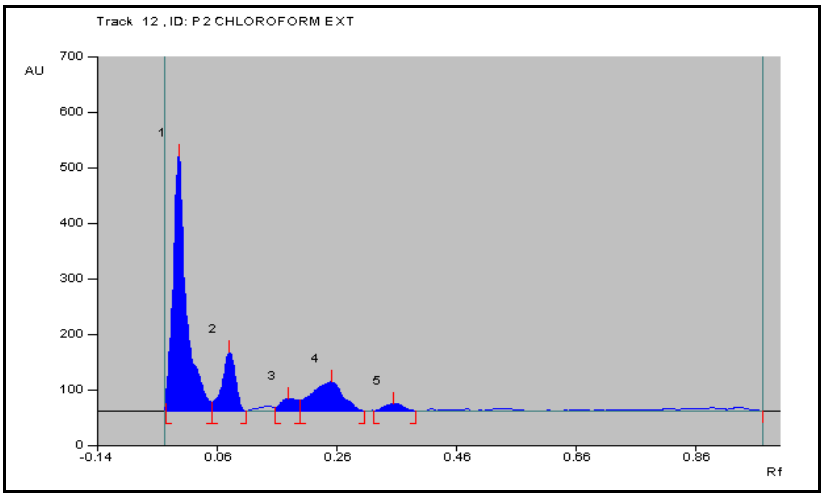

Fig.12:HPTLC chromatogram of chloroform extract at 554 nm (after derivatization in iodine chamber)

Table 9:Peak list and *Rf* value of the chromatogram of chloroform extract at 554 nm (after derivatization in iodine chamber)

S.No	Peak	End Rf
1	1	0.05
2	2	0.11
3	3	0.2
4	4	0.31
5	5	0.39

3. Petroleum ether Extract

e) At 366nm-

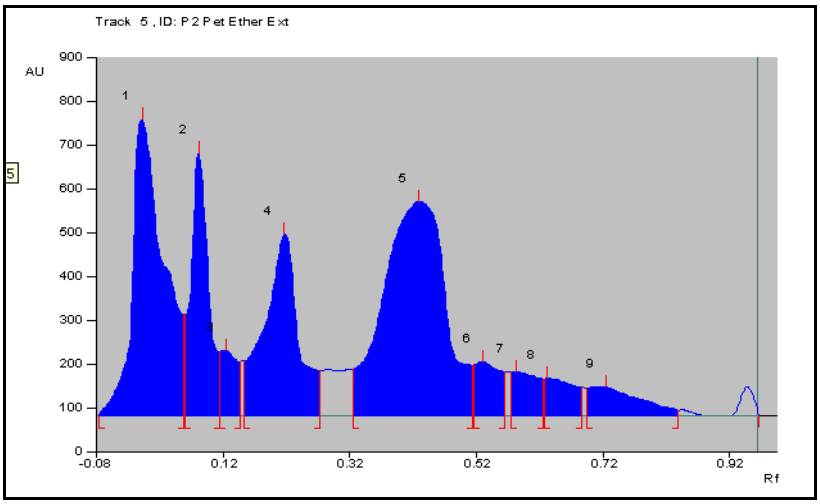

Fig.13:HPTLC chromatogram of petroleum ether extract at 366nm

Table 10:Peak list and *Rf* value of the chromatogram of petroleum ether extract at 366nm

S.No	Peak	End Rf
1	1	0.06
2	2	0.12
3	3	0.15
4	4	0.28
5	5	0.52
6	6	0.57
7	7	0.63
8	8	0.69
9	9	0.84

f) At 554nm-

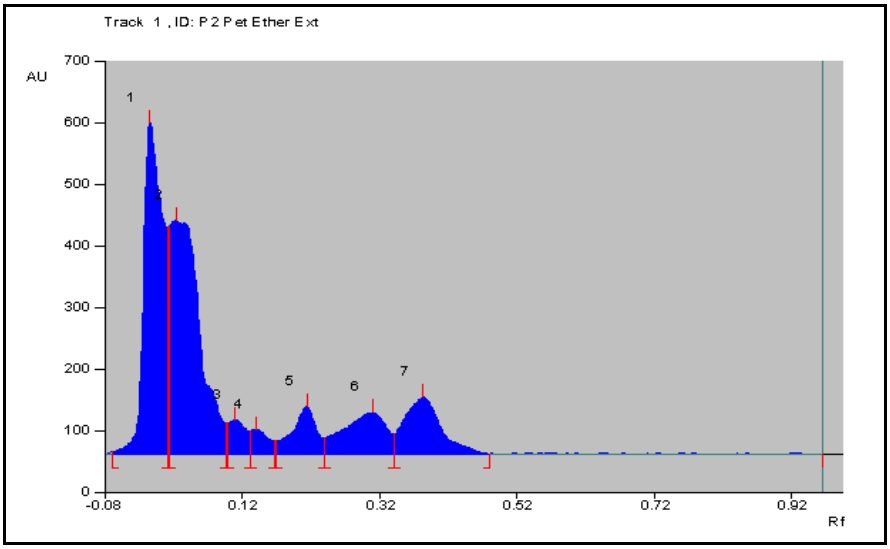

Fig.14:HPTLC chromatogram of petroleum ether extract at 554 nm (after derivatization in iodine chamber)

Table 11:Peak list and *Rf* value of the chromatogram of petroleum ether extract at 554 nm (after derivatization in iodine chamber)

S.No	Peak	End Rf
1	1	0.01
2	2	0.1
3	3	0.13
4	4	0.17
5	5	0.24
6	6	0.34
7	7	0.48

4. Aqueous Extract

g) At 366nm-

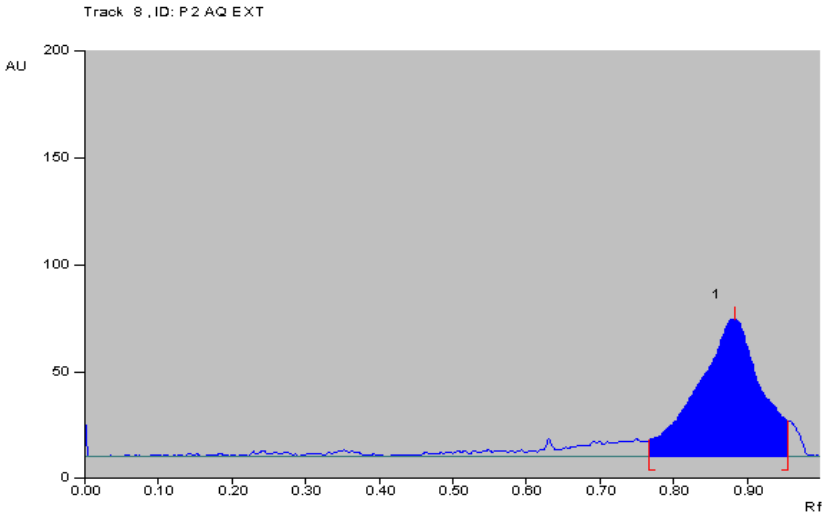

Fig. 15:HPTLC chromatogram of aqueous extract at 366nm

Table 12:Peak list and *Rf* value of the chromatogram of aqueous extract at 366nm

S.No	Peak	End Rf
1	1	0.95

h) At 554nm-

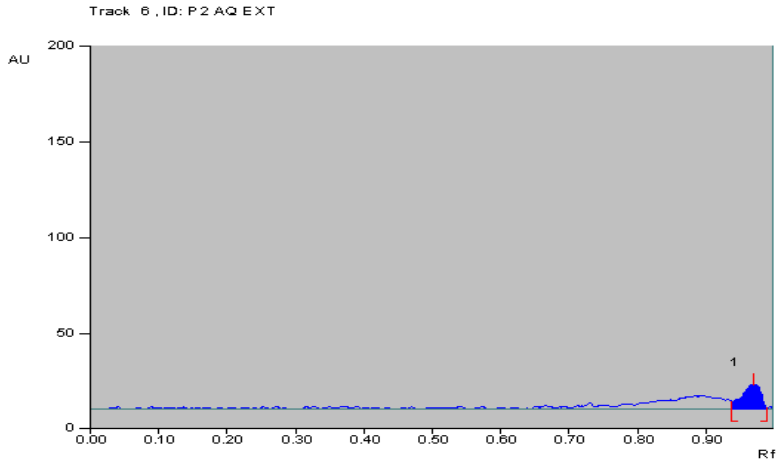

Figure 16-HPTLC chromatogram of aqueous extract at 554 nm (after derivatization in iodine chamber)

Table 13:Peak list and *Rf* value of the chromatogram of aqueous extract at 554 nm (after derivatization in iodine chamber)

S.No	Peak	End Rf
1	1	0.99

CONCLUSIONS

The macro and microscopical characters along with physicochemical and fluorescence characters of root powder *of Premna Herbacea* was used to establish the pharmacognostical standards and qualitative parameters as per pharmacopoeia and WHO guide lines. It can be concluded that the roots of contains triterpenoids as the chief constituent which could be bioactive. The comparative and multidisciplinary approach of the study of *Premna Herbacea* help in understanding its identification, taxonomical determination and medicinal importance in depth.

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