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Pharmacognostical and Phytochemical Evaluation of root bark of *Premna integrifolia* Linn

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Abstract
Premna integrifolia L. is an important medicinal herb contains apparent therapeutic properties. The present study focused on *P. integrifolia* L. root bark used in phytochemical and pharmacognostical evaluation. Pharmacognostical tool are helpful in authentication of root bark of *P. integrifolia* L. in crude form and also formulation containing it, as an ingredient in powdered form. Qualitative, quantitative phytochemical and heavy metal analysis, screening by thin layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) finger printing of various concentrations of hydroalcoholic extract were carried out.

Keywords: *P. integrifolia* L*;* Root bark; HPTLC; Successive extract; Qualitative parameters

Introduction
P. integrifolia L. (Syn: *P. serratifolia* L.; *P. obtusifolia* R.Br.), belongs to Verbenaceae family and it comprises of 200 species among which 30 species are available in India. Itisa large, thorny, deciduous shrub or a tree, up to 9 m height, distributed along the Indian coast and in the plains of Assam and in Khasi hills [1,2]. The roots and root bark of ***P. integrifolia*** L. are extensively used in Ayurveda. Considerable confusion exists regarding the correct botanical identity of "*Agnimanthah*'. The common Sanskrit name '*Agnimanthah*' exist for both ***P. integrifolia*** L. and *Clerodendron multiflorum* (Burm.f.) O. Kentze (Syn. *C. phlomidis* L.f.) both belong to the family Verbenaceae. *Laghu Agnimantha* is *C. multiflorum* while *Vrddha Agnimantha* is ***P. serratifolia*** [3]. *C. multiflorum* is an official drug to be used in Ayurveidic formulations and *P. integrifolia* is an official substitute [4]. Various parts of plant like leaves, stem, stem barks, root, root barks and wood are used to treat different diseases [5]. The presence of alkaloids like premnine [6], flavonoids-luteolin[7], sterol and triterpene in *P. integrifolia* root was reported [8]. *P. integrifolia* have hypolidemic[9], anti-diabetic [10], anti-inflammatory [11] and immuno-modulatory [12] activities were reported. In Ayurvedic preparation of root is one of the ingredients *Dasamulakwatha, Chyanprashavleh, Haritakiavleh, Ayushyavardhaktel, Narayanetel* etc., used in treatment of different ailments [13].

Materials and Methods
P. integrifolia root barks were collected from Indian Medical Practitioners Co-operative Pharmacy and Stores (IMPCOPS), Chennai, Tamil Nadu, India. Authentication of root barks by C. Arunachalam, Research Officer (Botany), Captain Srinivasa Murthy Regional Ayurveda Drug Development Institute, Chennai and the voucher specimen (00641/2014) was deposited in the Botany Department. The root bark was washed under running tap water to remove dust, air dried, crushed into powder using pulvarizer and stored in an air tight container.

Pharmacognostical and Physiochemical Evaluation
Morphological study of an anatomical sectioning [14] and powder microscopy (Olympus BX 51 Fluorescence microscope and Nickon D 7000 Camera) was conducted by standard procedures [15-17]. Assessment of pH, total ash, acid insoluble ash, loss on drying at 105˚C, water soluble extractives in powder of *P. integrifolia* root bark [18].

Preliminary Phytochemical Screening
The root bark powder (10 g/100 mL) was subjected to successive extraction by cold maceration method using solvents such as petroleum ether as an initial solvent, followed by chloroform, ethyl acetate, methanol and water for 24 hours in 3 times. At the end of extraction, each extract was passed through Whatman No.1 filter paper.

The filtrate was evaporated to dryness. The polarity of solvents were gradually increased and ranged from non polar to polar. Wide polarity range of compounds were extracted and subjected to screening of phytoconstituents. Qualitative analysis such as alkaloid, triterpenoid, coumarin, steroid, tannin, saponin, flavonoid, quinone, flavanone, anthocyanin, anthraquinone, phenol, protein, carbohydrate, glycoside, amino acid, acid and furan were carried out in successive extraction of root bark of *P. integrifolia* using standard procedure [19].

Preparation of Hydro Alcoholic Extract (HAE) of Root Bark

HAE was prepared at room temperature by cold percolation method. Each extract was prepared by taken 1x10 g/100 mL and the root bark was defatted with petroleum ether followed by ethanol: water 50:50; 60:40; 70:30; (v/v) respectively for 72 hours with frequent shaking. These extracts were passed through Whatman No.1 filter paper. The filtrate was evaporated to dryness. Thin layer chromatography (TLC) and high performance thin layer chromatography (HPTLC) was carried out for each extract.

Standardization of TLC and HPTLC Finger Printing

The different ratio of HAE i.e. 50:50; 60:40; 70:30 (v/v) was spotted on aluminum plate pre-coated with Silica gel-60TG, F254 of 0.2 mm thickness using Linomat applicator. Different ratio of extracts and various volumes (10µL, 15µL, 20µL) were applied on TLC plate and developed a chromatogram using the solvent system containing toluene: ethyl acetate: formic acid (40:60:10) and dried. The plates were kept under UV 254 and 366 nm and also dipped in 5% vanillin-sulphuric acid reagent, the plates were heated in a hot air oven at 105°C until the color of the spots appeared [20]. The same was subjected to CAMAG HPTLC system and the Rf values and finger printing data were recorded using Win cats software [21].

Results

P. integrifolia is a shrub and entirely margined (Fig.1), dried mature root bark showed flat, curved, re-curved, 0.3-1 cm in thickness, up to 12 cm in length and up to 5 cm in width, outer surface rugged and exfoliating, externally silver-grey, inner surface hard, wrinkled and brownish, dirty grey fractured surface, odour nil and taste astringent (Fig. 2). T.S of of root bark showed outer periderm consists of thin walled rectangular, tangentially elongated 4 to 6 rows of tabular cells, followed by dead phloem consists of collapsed parenchyma cells filled with tannin content and embedded with round to oval starch grains, cluster and rosette crystals of calcium oxalate; inner periderm consists of thin walled rectangular, tangentially elongated lignified cells, followed by polygonal parenchymatous cortex with discontinuous band of stone cells, the inner zone of secondary phloem constitutes the non-collapsed, thin walled, radially running phloem parenchyma, at places group of sclerenchymatous fibers were seen (Fig. 3). Powder microscopy showed dirty grey fractured surface, showed fragment of polygonal lignified cork cells in surface view and tangential cells in sectional view, fragment of tangential longitudinally cut medullary ray associated with fibres, fragment of parenchyma cells filled with tannin content and a few starch grains, fragment of fibres having pegged, forked with sharp ends, a few fragments of idioblast cells, stone cells, numerous rosette crystals of calcium oxalate and a few starch grains round to oval, simple and compound, having 2-4 components (Fig.4).

Physiochemical evaluation of root bark of *P. integrifolia* was showed in Table.1. Ash content (21.97%), acid-insoluble ash (0.29%), alcohol- soluble extractive (10.69%), water-soluble extractive (6.98%), pH (5.1) and loss on drying at 105°C was found to be10.3%. Phytochemical screening was done for activity guided fractionation and the results were depicted in Table 2 & 3. HPTLC profile of different ratio of HAE 50:50, 60:40, 70:30 (v/v) in toluene: ethyl acetate: formic acid (40:60:10) at 10µL was shown in Table 4 & Fig. 5. HPTLC finger printing of different ratios were illustrated in Fig. 6-11. The HAE of 50:50 (v/v) contains 8 compounds, 60:40 (v/v) contains 9 compounds and 70:30 (v/v) contains 7 compounds using deuterium lamp at 254 nm. In case of 366 nm, the different ratios of HAE of 10µL at 50:50 (v/v) contains 10 compounds, 60:40 (v/v) contains 10 compounds and 70:30 (v/v) contains 8 compounds using scanner 3, Camag HPTLC instrument under mercury lamp appear in blue colour.

Discussion

Macro-microscopic method of authentication is the first and fundamental step for standardization of herbal formulation. Botanically *P. serratifolia* L. and *C. phlomidis* L. are different species called by similar vernacular name *Agnimantha*. It was observed that macroscopic and microscopic features of dried root bark of *P. integrifolia* L. will be helpful in confirmation of its botanical identity even it is in dried form. Though plants can easily be identified in its fresh form by taxonomic identification, the same is difficult while it is dried as many features of plant parts changes on drying. In case plant drugs are purchased in crude form from market the morphology and microscopic study will be helpful in its identification of botanical source.

The pH value slightly acidic in nature for *P. integrifolia* root bark powder. The percentage of total ash, acid insoluble ash, loss on drying at 105˚C, water soluble extractive and alcohol soluble extractive were found to be negligible. Steroids were present in successive extracts of petroleum ether to alcoholic extract. Quinones, flavanones and anthocyanins were seen in chloroform to alcohol extractives. Coumarins, phenols, carbohydrates and glycosides were observed in ethyl acetate to alcohol extracts. Triterpenoids, alkaloids, tannins and furan were present only in alcoholic extracts. In aqueous extracts some of the compounds such as triterpenoids, coumarins, proteins, carbohydrates, glycosides and amino acids were absent and all other phytochemicals were present. Hydroalcoholic extracts of various ratios (50:50, 60:40, 70:30 v/v) showed all phytoconstituents except anthoquinones, proteins and acids. HPTLC analysis showed a more number of components in hydroalcoholic extracts of 60:40 (v/v) ratio. As per Ayurveda, *P. integrifolia* root barks are used for *Dasamula* drug preparations and used to treat various ailments. Based on the components present in the root bark may help researcher to find the mechanism of action of that plant.

Table 1: Estimation of Physiochemical Parameters

S. No.	Parameters	Results
1.	pH	5.1
2.	Ash Value (%)	21.97
3.	Acid-insoluble Ash (%)	0.292
4.	Alcohol-soluble Extractive (%)	10.69
5.	Water-soluble Extractive (%)	6.98
6.	Loss on Drying at 105°C (%)	10.30

[Values are mean ± SD (n=3)]

Table 2: Phytochemical Screening in Successive Extracts

S. No.	Phytochemical	Petroleum Ether	Chloroform	Ethyl Acetate	Methanol	Ethanol	Water
1.	Alkaloids	-	-	-	+	+	+
2.	Triterpenoids	-	-	-	+	+	_
3.	Coumarins	-	-	+	+	+	_
4.	Steroids	+	+	+	+	+	+
5.	Tannins	-	-	-	+	+	+
6.	Saponins	-	-	-	+	+	+
7.	Flavonoids	-	-	-	+	+	+
8.	Quinones	-	+	+	+	+	+
9.	Flavanones	-	+	+	+	+	+
10.	Anthocyanins	-	+	+	+	+	+
11.	Anthoquinones	-	-	-	-	-	_
12.	Phenols	-	-	+	+	+	+
13.	Proteins	-	-	+	-	-	_
14.	Carbohydrates	-	-	+	+	+	_
15.	Glycosides	-	-	+	+	+	_
16.	Amino Acids	-	-	-	-	+	_
17.	Acid	+	-	-	-	-	+
18.	Furan	-	-	-	+	+	+

Table 3: Phytochemical Screening of HAE

S. No.	Phytochemical	50:50 v/v	60:40 v/v	70:30 v/v
1.	Alkaloids	+	+	+
2.	Triterpenoids	+	+	+
3.	Coumarins	+	+	+
4.	Steroids	+	+	+
5.	Tannins	+	+	+
6.	Saponins	+	+	+
7.	Flavonoids	+	+	+
8.	Quinones	+	+	+
9.	Flavanones	+	+	+
10.	Anthocyanins	+	+	+
11.	Anthoquinones	-	-	-
12.	Phenols	+	+	+
13.	Proteins	-	-	-
14.	Carbohydrates	+	+	+
15.	Glycosides	+	+	+
16.	Amino Acids	+	+	+
17.	Acid	-	-	-
18.	Furan	+	+	+

Table 4: HPTLC Finger Printing of Different Ratios of HAE - 10µl

S. No	Mobile phase: Toluene: ethyl acetate: formic acid (40:60:10)		
		254 nm	366 nm
1.	HAE-50:50 v/v	7	7
2.	HAE-60:40 v/v	9	8
3.	HAE-70:30 v/v	7	8

Fig 1: Photograph of *P. integrifolia*

Fig 2: Dried root bark of *P. integrifolia*

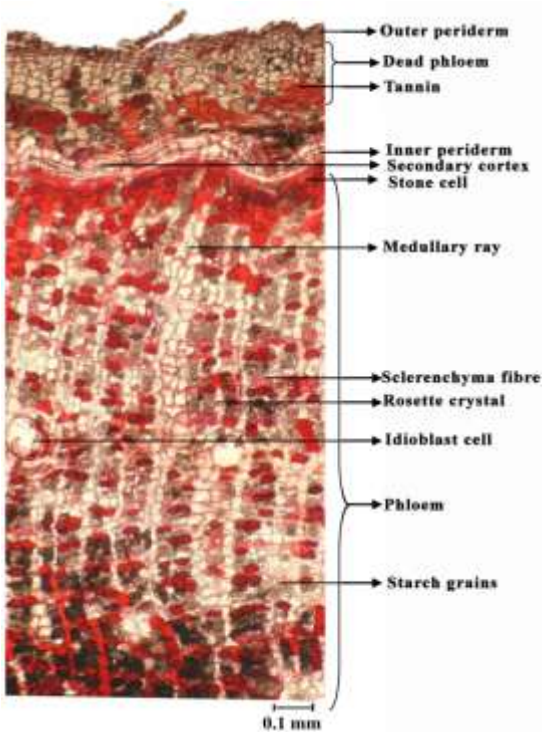

Fig 3: Transverse section of *P. integrifolia* L. root bark

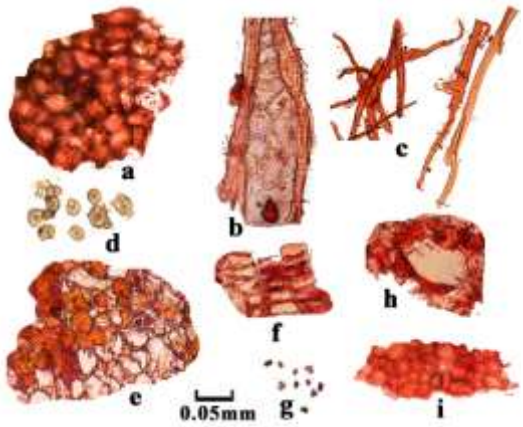

Fig 4: Powder Microscopy of *P. integrifolia* L.
a. cork cells in surface view; **b**. tangentially cut medullary ray associated with fibres **c**. fibres **d**. rosette crystals of calcium oxalate **e**. parenchyma with tannin content **f**. lignified cork in sectional view **g**. starch grains **h.** idioblast cell **i**. stone cells

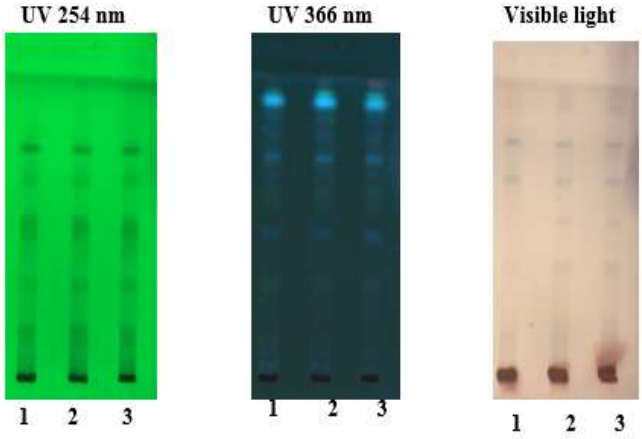

Fig 5: TLC finger print profile of different ratio of HAE (50:50, 60:40, 70:30 v/v) in Toluene: ethyl acetate: formic acid (40:60:10) at 10 µL
Track 1:50:50 (v/v); Track 2: 60:40 (v/v); Track 3: 70:30 (v/v

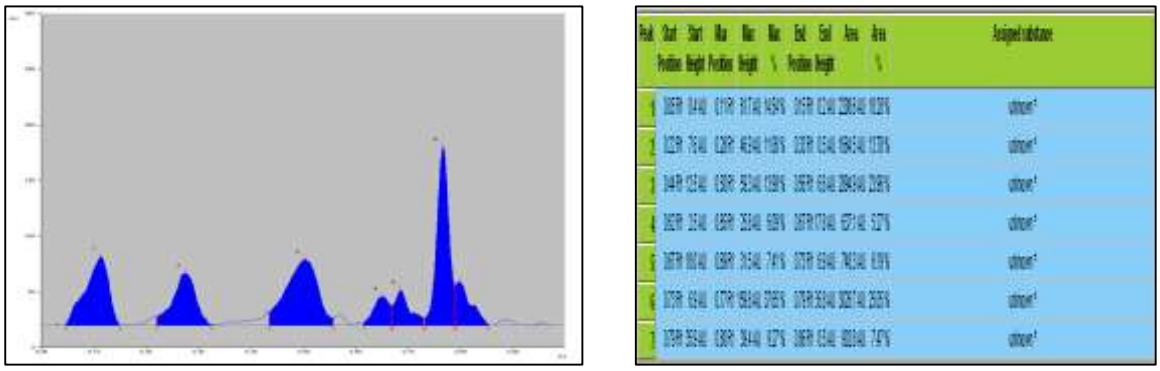

Fig 6: HPTLC Finger printing of HAE under 254 nm (50:50 v/v)-Track-1

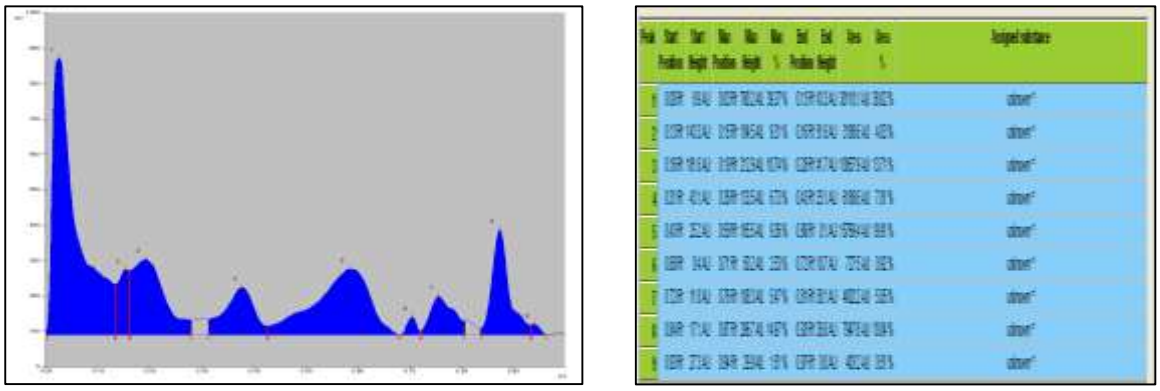

Fig 7: HPTLC Finger printing of HAE under 254 nm (60:40 v/v)-Track-2

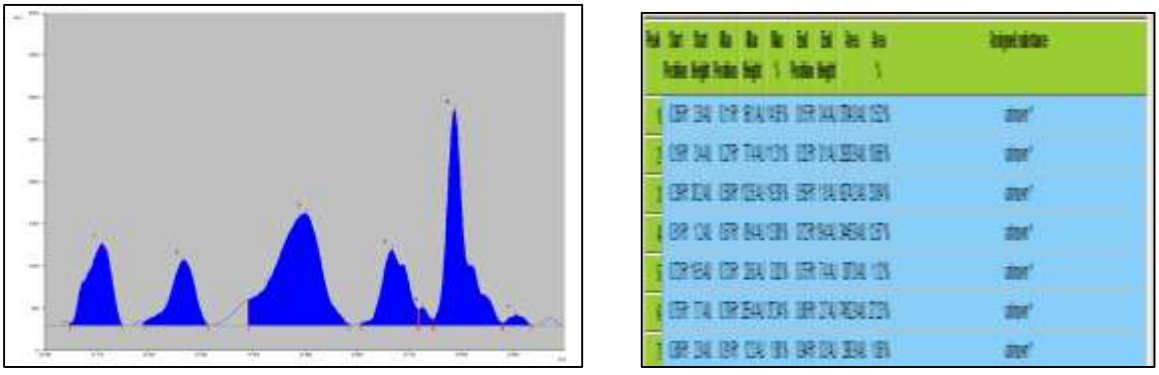

Fig 8: HPTLC Finger printing of HAE under 254 nm (70:30 v/v)-Track-3

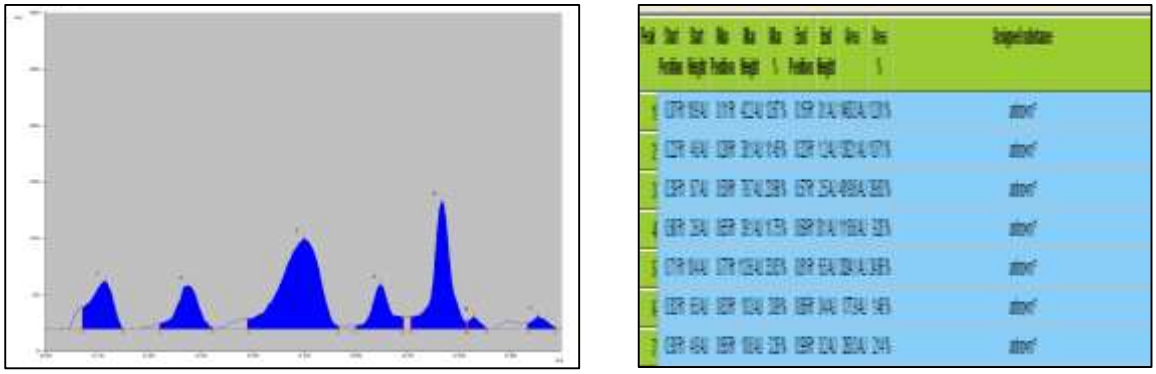

Fig 9: HPTLC Finger Printing of HAE under 366 nm (50:50 v/v)-Track-1

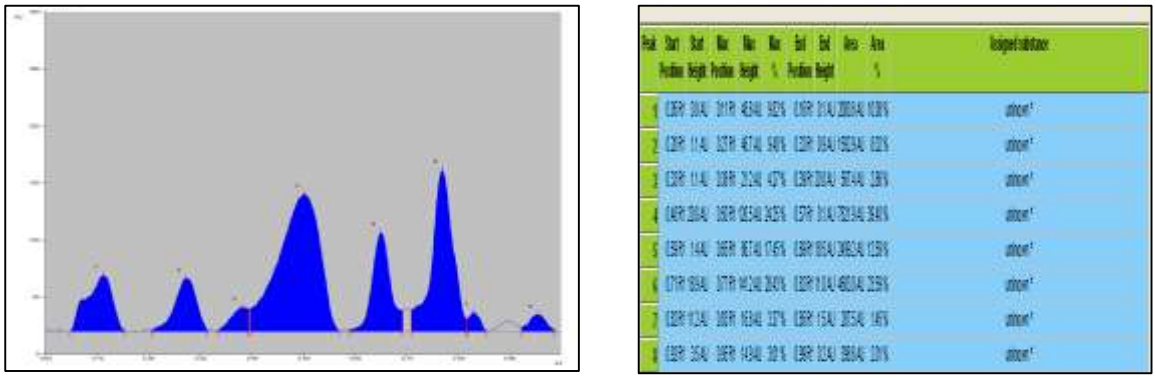

Fig10: HPTLC Finger Printing of HAE under 366 nm (60:40 v/v)-Track-2

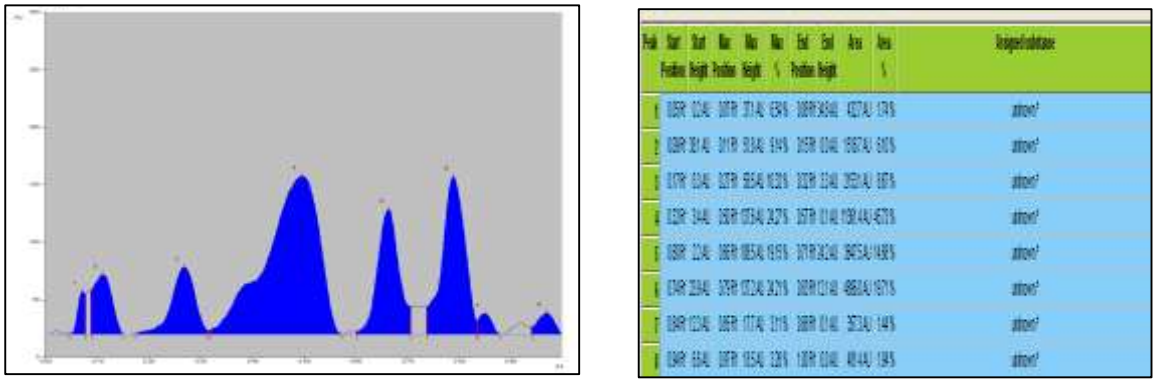

Fig11: HPTLC Finger Printing of HAE under 366 nm (70:30 v/v)-Track-3

Conclusion

Findings of phytoconstituents and pharmacognostical studies will be helpful in authentication of root bark of *P. integrifolia* L. in crude form and also formulation containing it, as an ingredient in powdered form. Physiochemical evaluation directly related to the geographic variation the components present in it. Hence further study is focused on the hydro alcoholic extract of 60:40 (v/v) to find out its efficacy in Ayurvedic fraternity.

References

1. Anonymous, The Wealth of India, Raw Materials, Vol. VIII: Ph-Re; Publications & Information Directorate, CSIR, New Delhi, 1969, 240.
2. The plant list Version 1.1 Published on the internet, 2013. <http://www.theplantlist.org/tpl1.1/record/ kew-164980>; http://www.theplantlist.org/tpl1./record/kew -466112
3. Bapalal V. Some Contraversial Drugs in Indian Medicine, First Edition, Chaukhambha Orientalia, Varanasi, 1982, 186.
4. Anonymous, The Ayurvedic Formulary of India, Part-III, First Edition, Government of India, Ministry of Health and Family Welfare, Department of AYUSH, 2011, pp.419.
5. Rajendran R, Krishnakumar E. Anti-Arthritic Activity of *P. serratifolia* Linn. Wood against Adjuvant Induced Arthritis. Avicenna J Med Biotechnol. 2010; 2(2):101-6.
6. Basu NK, Joneja AN. Chemical investigation of *P. integrifolia*. Ind J Pharm. 1949; 11:191-3.
7. Dasgupta B, Sinha NK, Pandey VB, Ray AB. Major alkaloid and flavonoids of *P. integrifolia*. Planta Med. 1984; 50:281-3.
8. Basu NK, Dandiya PC. Chemical investigation of *P. integrifolia*. J Am Pharm Assoc Sci Edu. 1947; 36:389-91.
9. Khanna AK, Chander R, Kapoor NK. Hypolipidemic activity of *P. integrifolia* in rats. Fitoterapia, 1991; 62:271-4.
10. Kar A, Choudhary BK, Bandyopadhyay NG. Evaluation of a few Indian folk medicinal plants less known for their hypoglycemic activity. Ethnobotany. 1999; 11:18-21.

11. Barik BR, Bhowmik T, Dey AK, Patra A. Premnazole, an isoxazole alkaloid of *Premna integrifolia* and *Gmelina arborea* with anti-inflammatory activity. Fitoterapia 1992; 63: 295-9.
12. Gokani RH, Lahiri SK, Santani DD, Shah MB. Evaluation of immunomodulatory activity of *Clerodendrum phlomidis* and *Premna integrifolia* Root. Int J Pharmacol.2007; 3:352-6.
13. Anonymous, The Ayurvedic Pharmacopoeia of India. First Edition, Vol. III, Department of ISM and Homoeopathy, Ministry of Health and Family Welfare, Government of India, New Delhi, 2001, pp.3-4.
14. Brain KR, Turner TD. The practical evaluation of phyto pharmaceuticals, Wright-Scientechnica Bristol 1975, 4.
15. Anonymous, The Ayurvedic Pharmacopoeia of India, Part-I, Vol. VI, First Edition, Government of India, Ministry of Health and Family Welfare, Department of AYUSH, New Delhi, 2008, pp.233-42.
16. Narayana A, Namboodiri K, Kolammal AM. Pharmacognosy of Ayurvedic Drugs, Series 1, No 3, Kerala, 1953.
17. Sass JE. Elements of botanical microtechnique, Mc Graw Hill Book Co, INC New York, 1940, 222.
18. Quality Control Methods for Medicinal Plant Materials, WHO, Geneva, 1998.
19. Horborne JB. Phytochemicals method: A guide to modern techniques of plant analysis. (3rdedition) Champenan and Hall Co., New York. 1998, 1-302.
20. Gopu CL, Suyog A, Hiral M, Paradkar AR, Mahadik KR. Simultaneous determination of cinnamaldehyde, eugenol and piperine by HPTLC densitometric method. Phytochem Anal. 2008; 19(12):116-21.
21. Karthika K, Paulsamy S. TLC and HPTLC Fingerprints of Various Secondary Metabolites in the Stem of the Traditional Medicinal Climber, *Solena amplexicaulis.* Ind J Pharm Sci. 2015; 77(1):111-6.