Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2016; 5(6): 199-204
Received: 29-09-2016
Accepted: 30-10-2016

Ravindra C Sutar
Department of Pharmacology, Sanjivani College of Pharmaceutical Education and Research, Kopargaon. At-Sahajanandnagar, Post-Shinganapur Kopargaon, Dist-Ahmednagar, Maharashtra, India

Sudhir Singh Gangwar
Department of Pharmacy, GSVM Medical College, Kanpur, Uttar Pradesh, India

RN Thakur
Department of Pharmacy, GSVM Medical College, Kanpur, Uttar Pradesh, India

Ranjana Sharma
Department of Pharmacy, GSVM Medical College, Kanpur, Uttar Pradesh, India

S Parimala Krishnan
Department of Pharmacy, Annamalai Nagar, Annamalai University, Tamil Nadu, India

Correspondence
Ravindra C Sutar
Department of Pharmacology, Sanjivani College of Pharmaceutical Education and Research, Kopargaon. At-Sahajanandnagar, Post-Shinganapur, Kopargaon, Dist-Ahmednagar, Maharashtra, India

HPTLC finger printing analysis of the tannins from *Holoptelea integrifolia* (Roxb.) Planch Leaves

Ravindra C Sutar, Sudhir Singh Gangwar, RN Thakur, Ranjana Sharma and S Parimala Krishnan

Abstract
Objective: The present study was conducted to identify the tannins from petroleum ether (PEHI) and methanol extracts (MHI) of medicinally and economically useful leaves of *Holoptelea integrifolia* (Roxb.) Planch using High Performance Thin Layer Chromatography (HPTLC) technique.
Methods: Preliminary phytochemical screening was done and HPTLC studies were carried out. CAMAG HPTLC system equipped with Linomat V applicator (Switzerland). Densitometric scanning was performed with Camag TLC scanner IV in the reflectance absorbance mode at 540 nm and operated by Win CATS software (1.4.6 Camag) with the help of tungstant lamp.
Results: Preliminary phytochemical screening of petroleum ether extract of *Holoptelea integrifolia* showed the presence of steroids, terpenoids, alkaloids, glycosides, flavonoids, proteins, tannins and carbohydrates while methanolic extract of *Holoptelea integrifolia* showed the presence of steroids, alkaloids, flavonoids, tannins, proteins and carbohydrates. HPTLC finger printing of tannins of petroleum ether extract of leaves revealed eleven polyvalent phytoconstituents (11 peaks) and corresponding ascending order of R_f values in the range of 0.20 to 0.75, while methanol extract of leaves showed twelve polyvalent phytoconstituents (12 peaks) and corresponding ascending order of R_f values in the range of 0.12 to 0.73.
Conclusions: With the results of preliminary phytochemical analysis and above R_f values we have concluded the presence of tannins in both the extracts.

Keywords: *Holoptelea integrifolia* (Roxb.) Planch leaves, Phytochemical Screening, tannins, HPTLC Fingerprinting

Introduction
Natural remedies from medicinal plants are found to be safe and effective. Many plant species have been used in folklore medicine to treat various ailments. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries [1]. Standardisation of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material may be useful for proper standardisation of herbals and it's formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plant products using modern controlled technique and applying suitable standards [2]. High performance thin layer chromatography (HPTLC) is a valuable tool for reliable identification. It can provide chromatographic fingerprints that can be visualized and stored as electronic images [3]. *Holoptelea integrifolia* belongs to the family ulmaceae commonly called as Indian Elm and commonly used in India by the tribal people for it' s medicinal properties. The mucilaginous bark is boiled and the juice squeezed out and applied to rheumatic swellings [4]. In traditional system of medicine, bark and leaves of *Holoptelea integrifolia* are used as bitter, astringent, acrid, thermogenic, antiinflammatory, digestive, carminative, laxative, anthelmintic, depurative, repulsive, urinary astringent and in rheumatism [5, 6]. The plant *Holoptelea integrifolia* is used traditionally for the treatment of inflammation, gastritis, dyspepsia, colic, intestinal worms, vomiting, wound healing, leprosy, diabetes, hemorrhoids, dysmenorrhoea and rheumatism [7]. In this present study the preliminary phytochemical screening of *Holoptelea integrifolia* leaf extract has been done to identify the chemical constituents and HPTLC fingerprinting of *Holoptelea integrifoli* extracts has been performed which may be used as markers for quality evaluation and standardization of the drug

Materials and Methods

Plant material

Leaves of *Holoptelea integrifoila* were collected in the Month of August from the agri-cultural fields of Tirunelveli district, Tamilnadu. The plant was identified and leaves of *Holoptelea integrifolia* were authenticated and confirmed from Dr.V.Chel ladurai, Research Officer, Botany, C.C.R.A.S. (Retired), Govt. of India by compairing morphological features (leaf and stem arrangement, flower /inflorescence arrangement, frui t and seed morphology etc.). The collected plant material was shade dried to retain itsvital phytoconstituents and then subjected to size reduction for further extraction process.

Preparation and Extraction of Plant material

Preparation of Petroleum ether and methanol extract

The powder of *Holoptelea integrifoila* leaves was charged in to the thimble of a Soxhlet apparatus and extracted using petroleum ether. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50 ^{0}C to get petroleum ether extract. The extract was finally air dried thoroughly to remove all traces of the solvent and the percentage yield was calculated. The perfectly dried extract was then stored in an air tight container in a refrigerator below 10^{o}C. After obtaining the petroleum ether extract the marc was pressed and it is air dried and again it was extracted using methanol. Appearance of colourless solvent in the siphon tube was the indication of exhaustive extraction and based on that the further extraction was terminated. The extract was then transferred into the previously weighed empty beaker and evaporated to a thick paste on the water bath, maintained at 50 ^{0}C to get semi solid mass of methanol extract. The extract was stored in an airtight container in a refrigerator below 10 ^{o}C.

The Petroleum ether and Methanol extracts of *Holoptelea integrifolia* leaves were subjected to the following investigations,

1. Preliminary phytochemical screening.
2. HPTLC Fingerprinting of Tannins

Phytochemical screening

The phytochemical investigation of the different leaf extracts of *Holoptelea integrifolia* was carried out with standard protocol [8]. The results were presented in Table 1.

HPTLC Fingerprinting

HPTLC studies were carried out following the method of Harborne [9] and Wagner *et al* [10].

HPTLC instrumentation and Chromatographic conditions

The sample solutions were spotted in the form of bands of width 8.0 mm with a Camag microlitre syringe on precoated silica gel aluminium plate 60F254 (20 cm × 10 cm with 250 µm thickness; E. Merck, Darmstadt, Germany, supplied by Anchrom Technologists, Mumbai) using a Camag Linomat V (Switzerland). The plates were activated at 120°C for 20 min prior to chromatography. A constant application rate of 1.0 µl/s was employed and space between two bands was 5 mm. The slit dimension was kept at 6.0mm × 0.45 mm and 10 mm/s scanning speed was employed. The slit bandwidth was set at 20 nm, each track was scanned thrice and baseline correction was used. The mobile phase for tannins consisted of toluene-ethyl acetate-formic acid in the volume ratio of 6:4:0.3 (v/v) and $FeCl_3$ was used for derivatization. 20 ml of mobile phase was used per chromatography. Linear a scending develop-ment was carried out in 20 cm x 10 cm twin trough glass chamber (Camag, Muttenz, Switzerland) saturated with filter paper whatman no: 1 in the mobile phase. The optimized chamber saturation time for mobile phase was 20 min at room temperature (25 ^{0}C ± 2) at relative humidity of 60% ± 5. The length of chromatogram run was 8.0 cm. Subsequent to the scanning, TLC plates were dried in a current of air with the help of an air dryer. Densitometric scanning was performed with Camag TLC scanner IV in the reflectance absorbance mode at 540 nm and operated by Win CATS software (1.4.6 Camag) with the help of tungstant lamp. Subsequent to the development; TLC plate was dipped in dragendorff reagent followed by drying in oven at 110 ^{o}C. Concentrations of the compound chromatographed were determined from the intensity of diffusely reflected light. Evaluation was carried out by comparing peak areas with linear regression [11-19].

Results and Discussion

Preliminary phytochemical analysis of petroleum extract of *Holoptelea integrifolia* showed the presence of steroids, terpenoids, alkaloids, glycosides, flavonoids, proteins, tannins and carbohydrates while methanolic extract of *Holoptelea integrifolia* showed the presence of steroids, alkaloids, flavonoids, tannins, proteins and carbohydrates (Table 1).

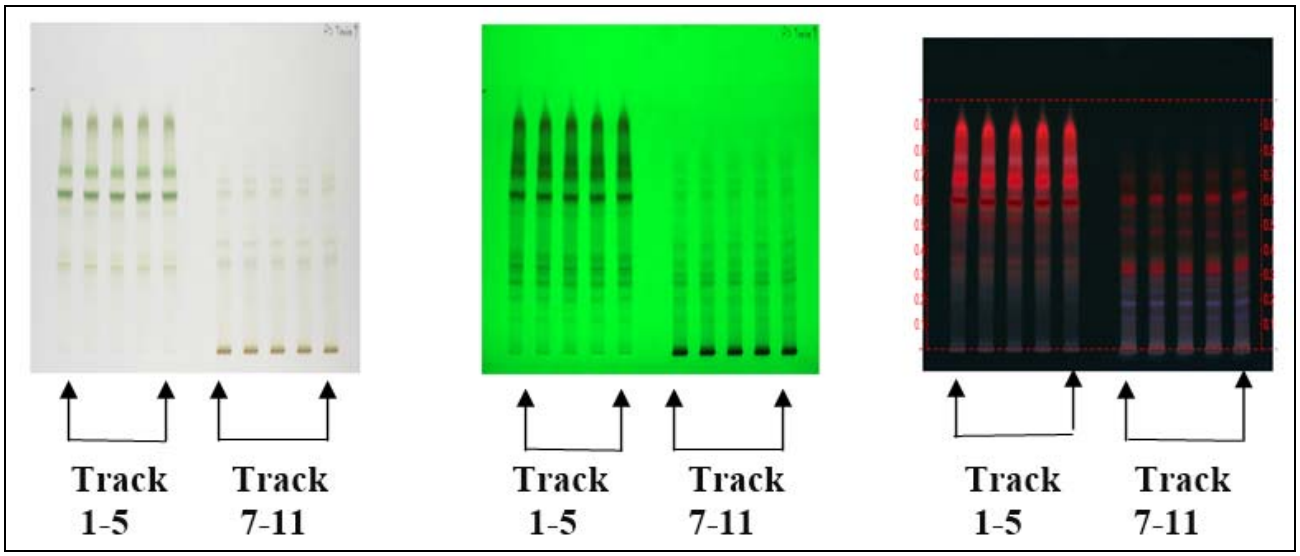
The chromatograms shown in fig.1 indicate that all sample co nstituents were clearly separa-ted without any tailing and diffuseness.

Table 1: Preliminary phytochemical screening of petroleum ether and methanol extracts of *Holoptelea integrifolia* (Roxb) planch leaves

Extracts / Constituents	Test performed	Pet. ether	Methanol
Test for Steroids	1. Salkowski test Liebermann-Burchard test	++ ++	+ +
Test for Triterpenoids	1. Salkowski test	++	-
Test for Phytosterol	1. Liebermann-Burchard test	++	++
Test for Phenolic compound		-	+
Test for Glycosides	1. Balget's test Keller-Killiani test Legals test	++ +	+ +
	2. Borntrager's test	+ +	+ +
Tests for Saponin	1. Foam Test	-	-
Tests for Carbohydrates	1. Molisch's test	++	++
	2. Barfoed's test	++	++

	3. Fehling's test	++	++
	4. Benedict's test	++	++
Test for Alkaloids	1. Mayer's Reagent	+	+
	2. Hager's Reagent	+	+
	3. Dragendorff's Reagent	+	+
Tests for Flavonoids	1. Ferric-chloride test	++	+
	2. Shinoda test	++	+
Test for Tannins	1. $FeCl_3$ Solution	+	+
	2. Gelatin test	+	+
Test for Proteins	1. Millon's test	+	+
	2. Xanthoproteic test	+	+
	3. Biuret test	+	+
	4. Ninhydrin test	+	+
Test for Gums and mucilage		-	-
Test for Starch		+	_

Tannin Confirmation



Chromatogram at visible | Chromatogram at 254 nm | Chromatogram at 366 nm

Track 1-5: Petroleum ether extract
Track 7-11: Methanol extract
Note: There was no data available for track 6

Fig 1: HPTLC fingerprint profile of tannins of leaf extract of *Holoptelea integrifolia* Detection of tannins in PEHI, MHI

It was observed that track 1-5 shows petroleum ether extract and track 7-11 shows methanol extract and 3D plot of tannins of *Holoptelea integrifolia* leaf in Fig 3. The chromatograms in Fig. 4 and Fig. 5 shows separation of constituents.

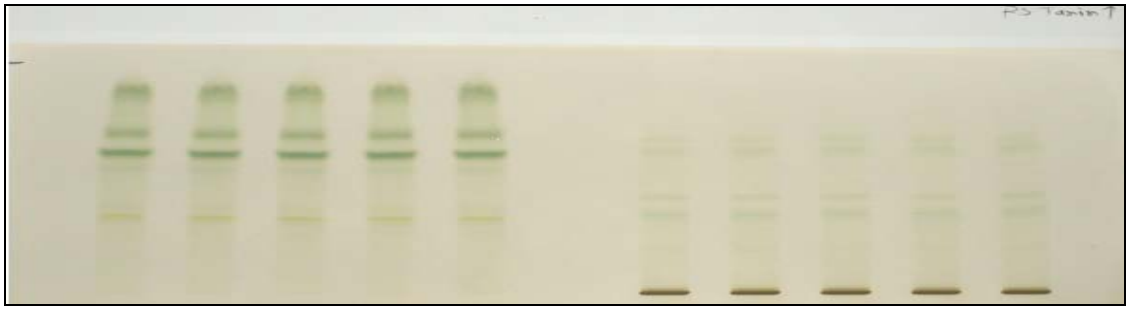


Fig 2: Tannins confirmation at visible derivatisation with $FeCl_3$

It was observed that there is a separation of different phytoconstituents, in PEHI and MHI.

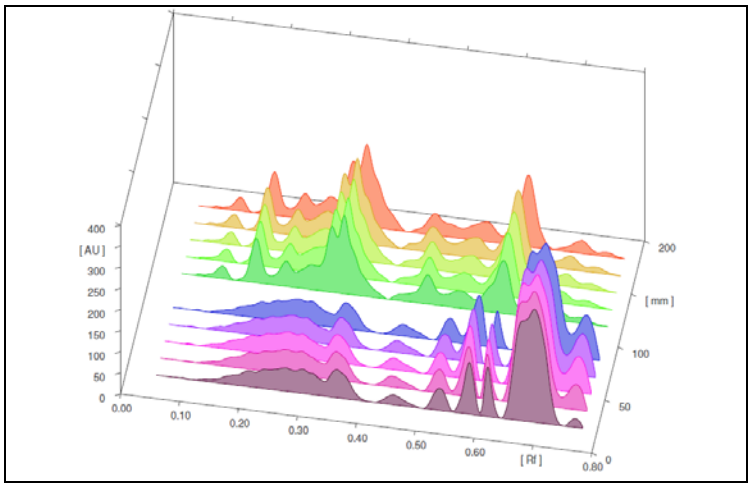


Fig. 3: 3-D plot of Fingerprint of tannins of *Holoptelea integrifolia* leaf

Table 2: R_f values for tannins in petroleum ether extract of *Holoptelea integrifolia* leaf

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.11	0.1	0.20	40.5	3.59	0.21	38.9	1415.3	5.53	AutoGenerated8
2	0.23	48.3	0.26	58.4	5.18	0.28	54.2	1676.4	6.55	AutoGenerated4
3	0.28	54.3	0.28	57.3	5.08	0.32	33.3	1576.7	6.16	AutoGenerated7
4	0.32	33.5	0.34	66.6	5.91	0.40	0.2	1884.6	7.37	unknown *
5	0.41	0.2	0.44	27.9	2.48	0.49	0.1	808.8	3.16	AutoGenerated12
6	0.49	0.5	0.52	54.8	4.86	0.54	13.1	1074.9	4.20	AutoGenerated15
7	0.54	13.1	0.57	126.6	11.23	0.59	2.9	2089.1	8.17	AutoGenerated14
8	0.59	0.7	0.60	99.6	8.83	0.62	0.6	1039.1	4.06	AutoGenerated1
9	0.63	0.5	0.66	243.0	21.55	0.67	238.4	4239.2	16.57	AutoGenerated6
10	0.67	238.7	0.68	267.2	23.69	0.73	40.5	7769.1	30.37	unknown *
11	0.73	40.8	0.75	85.6	7.60	0.77	8.3	2009.5	7.85	unknown *

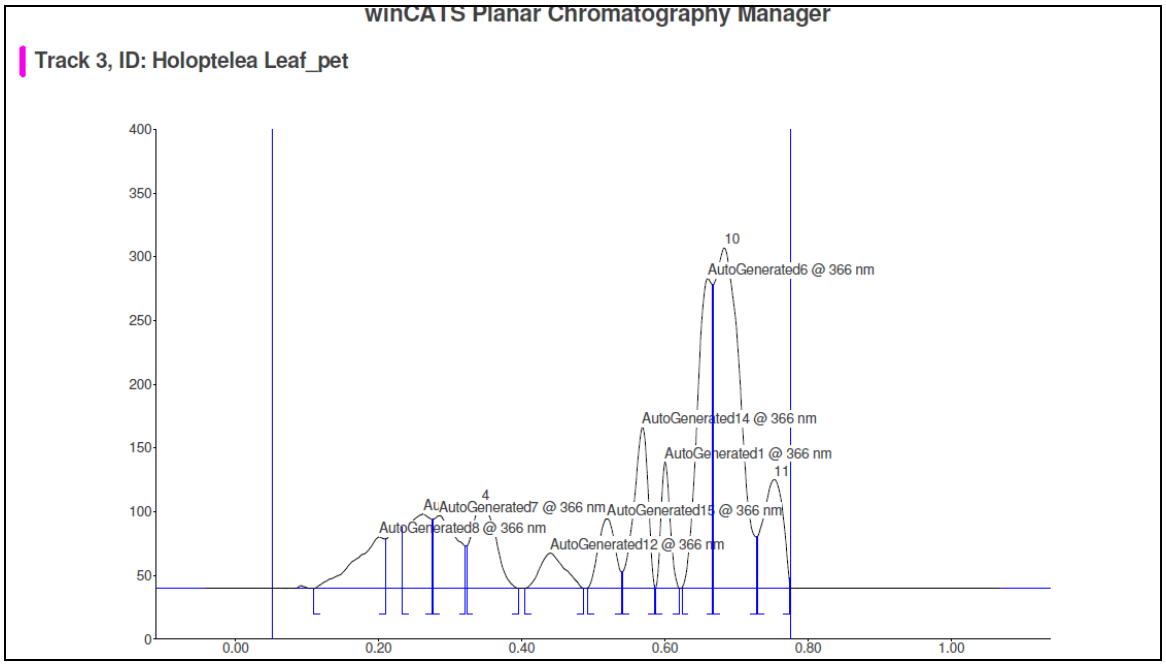


Fig 4: Chromatogram for tannins in petroleum ether extract of *Holoptelea integrifolia* leaf Fingerprinting study of tannins of PEHI at 366 nm

Fingerprinting study of PEHI at 366 nm shows eleven R_f between the range of 0.20- 0.75. R_f0.68 has 23.69% concentration in Table 2, Figure 4.

Table 3: R_f values for tannins in methanol extract of *Holoptelea integrifolia* leaf

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.09	3.5	0.12	29.6	3.30	0.14	0.1	512.1	2.52	AutoGenerated11
2	0.14	0.1	0.18	104.8	11.69	0.20	20.7	1822.3	8.98	unknown *
3	0.20	20.8	0.23	60.1	6.71	0.24	36.3	1258.7	6.20	unknown *
4	0.25	36.5	0.27	58.9	6.57	0.28	57.5	1426.2	7.03	unknown *
5	0.28	58.0	0.31	156.0	17.41	0.32	131.5	2574.2	12.68	unknown *
6	0.32	131.8	0.33	184.7	20.61	0.40	1.9	4306.9	21.22	AutoGenerated2
7	0.40	0.3	0.47	69.5	7.75	0.50	27.7	2054.2	10.12	unknown *
8	0.50	27.8	0.53	41.0	4.57	0.55	24.8	1330.9	6.56	AutoGenerated15
9	0.55	24.7	0.60	125.3	13.97	0.64	6.9	3651.2	17.99	AutoGenerated1
10	0.64	7.1	0.66	24.4	2.72	0.67	20.5	420.3	2.07	AutoGenerated6
11	0.67	20.6	0.69	28.6	3.19	0.71	10.9	647.2	3.19	unknown *
12	0.72	11.0	0.73	13.5	1.51	0.76	1.3	291.3	1.44	AutoGenerated5

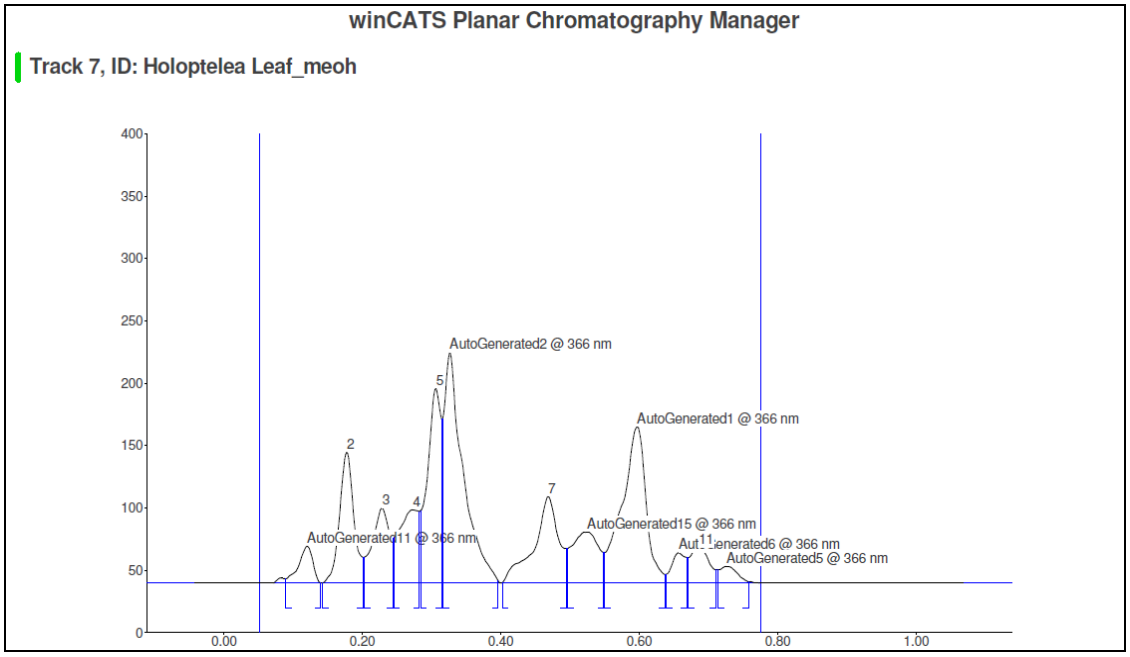


Fig 5: Chromatogram for tannins in methanol extract of *Holoptelea integrifolia* leaf

Fingerprinting study of tannins of MHI at 366 nm

Fingerprinting study of MHI at 366 nm shows twelve R_f between the range of 0.12- 0.73. R_f0.33 has 20.61% concentration in Table 3, Figure 5.

Conclusion

It is observed in the above HPTLC studies that, PEHI and MHI contain a lot of polyvalent chemical constituents with different R_f values. The developed fingerprint analysis of leaf extract of *Holoptelea integrifolia* will help to isolate and identify new tannins which will offer a possibility to discover lead a molecule for drug development.

Acknowledgment

The authors wish to thank Mr. Prashant S. Hande, Application Specialist, Anchrom Lab, Anchrom Test Lab Pvt. Ltd. Mulund (E), Mumbai - 400081 for his excellent and generous help for analyzing the HPTLC data.

References

1. Bobbarala V, Bramhachari PV, Ravichand J, Reddy YHK, Kotresha D, Chaitanya KV. Evaluation of hydroxyl radical scavenging activity and HPTLC fingerprint profiling of *Aegle marmelos* (L.) Correa extracts. J Pharm Res. 2011; 4(1):252-255.
2. Sharma P, Kaushik S, Jain A, Sikarwar SM. Preliminary phytochemical screening and HPTLC fingerprinting of Nicotiana tobacum leaf. J Pharm Res. 2010; 3(5):1144-1145.
3. Johnson M, Mariswamy Y, Ganaraj WE. Chromatographic finger print analysis of steroids in *Aerva lanata* L by HPTLC technique. Asian pac j Trop Biomed. 2011; 1:428-433.
4. Nadkarni KM. Indian Materia Medica. Popular Prakashan Pvt Ltd. Mumbai, India. 1976, 651-652.
5. Kirtikar KR, Basu BD. Indian Medicinal Plants. Edn 3, Sri Satguru Publications, New Delhi, India, 2000; 3:2292-2294.
6. Prajapati ND, Purohit SS, Sharma AK. A Handbook of Medicinal Plants a Complete Source Book. Agrobias.

Jodhpur, India, 2003, 273.
7. Warrier PK, Nambiar VPK, Ramakutty C. Indian Medicinal Plants a compendium of 500 species, Orient longman private Limited, 1995; 3:162.
8. Khandelwal KR. Practical Pharmacognosy. Techniques and Experiments. 10th ed. Nirali Prakashan, Pune, India. 2006, 149-156.
9. Harborne JB. *Phytochemical methods;* 3rd edition, London: Chapman and Hall, 1998.
10. Wagner H, Baldt S. *Plant drug analysis;* Berlin: Springer; 1996. R.P.W. Scott, Encyclopedia of Chromatography, 10th edn, Marcel Dekker, USA, 2001, 252-254.
11. ICH/CPMP Guidelines Q2B, Validation of Analytical Procedures– Methodology. 1996.
12. Cazes J, Scott RPW. Chromatography Theory, Marcel Decker, NY, 2002, 443-454.
13. Reviewer Guidance, Validation of Chromatographic Methods, 1994.
14. Sethi PD. HPTLC: Quantitative Analysis of Pharmaceutical Formulations, CBS Publications, New Delhi, 1996, 162-165.
15. Heftman E. Chromatography Fundamentals and Applications of Chromatography and Related Differential Migration Methods. edn, Elsevier, Amsterdam. 2004; 69A(6):253-291.
16. British Pharmacopoeia, International edn, HMSO, Cambridge, 2002, II, Appendix 112 (IB).
17. Sherma J. Encyclopedia of Pharmaceutical Technology, 2nd edn, Marcel Dekker, USA, 2001, 252-254.
18. ICH/CPMP guidelines Q2A, Text on Validation of Analytical Procedures, 1994.
19. Rockville MD. USP 23, NF 19, Asian edn, United States Pharmacopeial Convention, 982, 1225.