

E-ISSN: 2278-4136  
P-ISSN: 2349-8234  
JPP 2016; 5(4): 215-219  
Received: 27-05-2016  
Accepted: 28-06-2016

**Ravindra C Sutar**

Department of Pharmacology,  
Sanjivani College of  
Pharmaceutical Education and  
Research, Kopargaon. At-  
Sahajanandnagar, Post-  
Shinganapur (Pin code- 423603),  
Tal- Kopargaon, Dist-  
Ahmednagar, Maharashtra,  
India.

**Deepak S Musmade**

Department of Pharmaceutical  
Chemistry, Sanjivani College of  
Pharmaceutical Education and  
Research, Kopargaon. At-  
Sahajanandnagar, Post-  
Shinganapur (Pin code- 423603),  
Tal- Kopargaon, Dist-  
Ahmednagar, Maharashtra,  
India.

**Agasti L Ware**

Department of Pharmaceutical  
Chemistry, Sanjivani College of  
Pharmaceutical Education and  
Research, Kopargaon. At-  
Sahajanandnagar, Post-  
Shinganapur (Pin code- 423603),  
Tal- Kopargaon, Dist-  
Ahmednagar, Maharashtra,  
India.

**Correspondence****Ravindra C Sutar**

Department of Pharmacology,  
Sanjivani College of  
Pharmaceutical Education and  
Research, Kopargaon. At-  
Sahajanandnagar, Post-  
Shinganapur (Pin code- 423603),  
Tal- Kopargaon, Dist-  
Ahmednagar, Maharashtra,  
India.

## HPTLC finger printing analysis of the alkaloids from *Holoptelea integrifolia* (Roxb.) Planch leaves

Ravindra C Sutar, Deepak S Musmade and Agasti L Ware

**Abstract**

**Objective:** The present study was conducted to identify the Alkaloids 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 tungstane 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, proteins and carbohydrates. HPTLC finger printing of alkaloids of petroleum ether extract of leaves revealed eight polyvalent phytoconstituents (8 peaks) and corresponding ascending order of R<sub>f</sub> values in the range of 0.05 to 0.72. while methanol extract of leaves showed six polyvalent phytoconstituents (6peaks) and corresponding ascending order of R<sub>f</sub> values in the range of 0.11 to 0.67.

**Conclusions:** With the results of preliminary phytochemical analysis and above R<sub>f</sub> values we have concluded the presence of alkaloids in both the extracts.

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

**1. Introduction**

*Holoptelea integrifolia* belongs to the family ulmaceae commonly called as Indian Elm and commonly used in India by the tribal people for its medicinal properties. The mucilaginous bark is boiled and the juice squeezed out and applied to rheumatic swellings [1]. 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 [2, 3]. 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 [4]. 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* extract has been performed which may be used as markers for quality evaluation and standardization of the drug

**2. Materials and Methods****2.1 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. Chelladurai, Research Officer, Botany, C.C.R.A.S. (Retired), Govt. of

India by comparing morphological features (leaf and stem arrangement, flower /inflorescence arrangement, fruit and seed morphology etc.). The collected plant material was shade dried to retain its vital phytoconstituents and then subjected to size reduction for further extraction process.

**2.2 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 °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 °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 °C to get semi solid mass of methanol extract. The extract was stored in an airtight container in a refrigerator below 10 °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 Alkaloids

### 2.3 Phytochemical screening

The phytochemical investigation of the different leaf extracts of *Holoptelea integrifolia* was carried out with standard protocol [5]. The results were presented in Table 1.

### 2.4 HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out following the method of Harborne [6] and Wagner *et al* [7].

#### 2.4.1 Sample Preparation

Petroleum ether and methanol extracts obtained were evaporated under reduced pressure using rotovac evaporator. Each extract residue was re-dissolved in 5 ml of chromatographic grade Petroleum ether and methanol, which was used for sample application on pre-coated silica gel 60F254 aluminium sheets.

### 2.5 Developing Solvent System

A number of solvent systems were tried, for extracts, but the satisfactory resolution was obtained in the solvent toluene-ethyl acetate-dimethylamine in the volume ratio of 7:2:1 (v/v).

### 2.6 HPTLC instrumentation, Chromatographic conditions and detection of spots

The sample solutions were spotted in the form of bands of width 8.0 mm with a Camag microlitre syringe on pre-coated 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 fingerprinting of alkaloids consisted of toluene-ethyl acetate-dimethylamine in the volume ratio of 7:2:1 (v/v) and Dragendorff reagent was used for derivatization of alkaloids. 20 ml of mobile phase was used per chromatography. Linear ascending development 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 °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 °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 [8-16].

### 3. Results and Discussion

Preliminary phytochemical analysis 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, proteins and carbohydrates (Table 1).

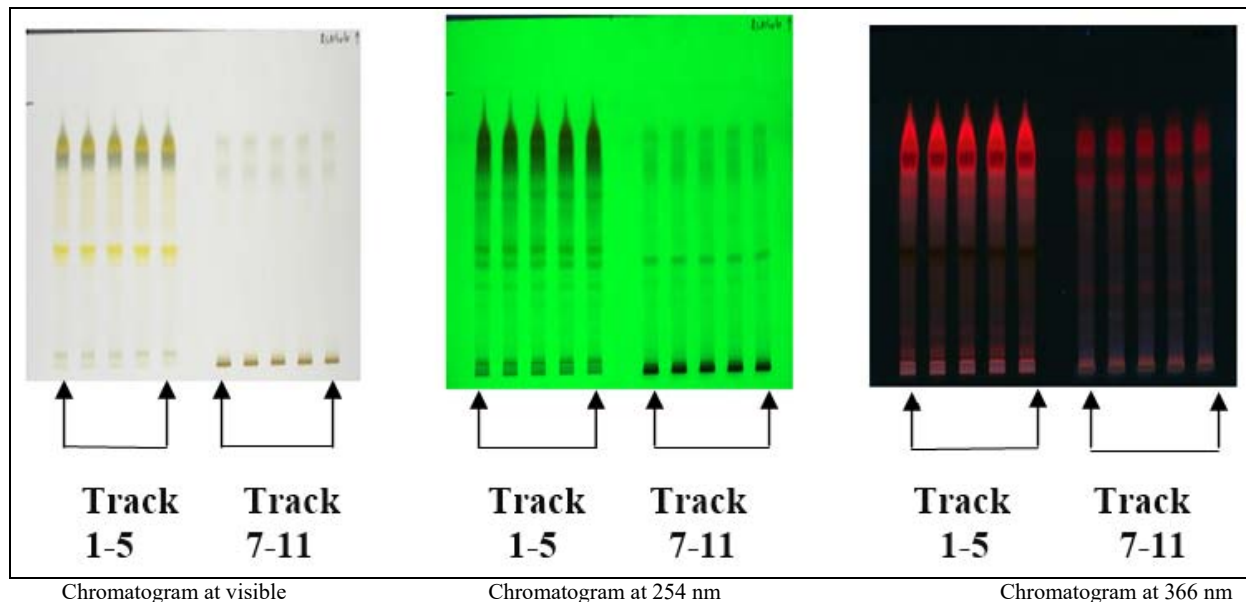
The chromatograms shown in fig.1 indicate that all sample constituents were clearly separated without any tailing and diffuseness.

**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	+	+
Tests for Saponin		2. Borntrager's test	+	+
		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 <sub>3</sub> 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		+	

### 3.1 Alkaloid Confirmation



**Fig 1:** HPTLC fingerprint profile of alkaloids of leaf extract of *Holoptelea integrifolia*

### 3.2 Detection of Alkaloids in PEHI, MHI at 366 nm

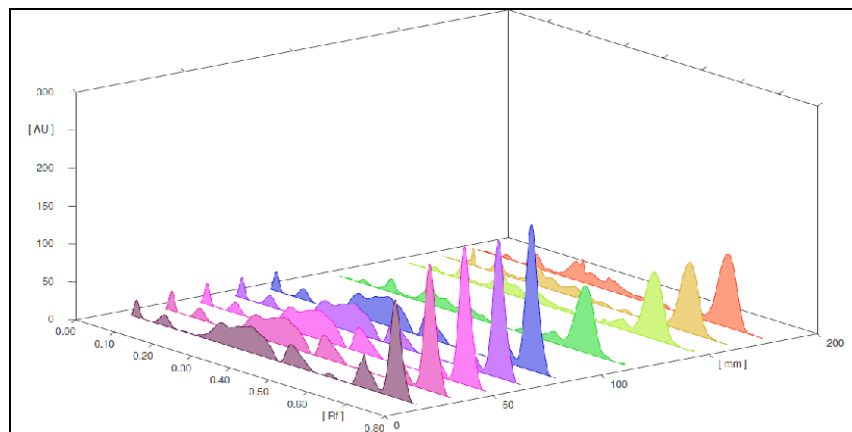
It was observed that track 1-5 shows petroleum ether extract and track 7-11 shows methanol extract and 3D plot of

alkaloids of *Holoptelea integrifolia* leaf in Fig 3. The chromatograms in Fig. 4 and Fig.5 shows separation of constituents.



**Fig 2:** Alkaloid confirmation at visible, derivatisation with modified Dragendorff reagent

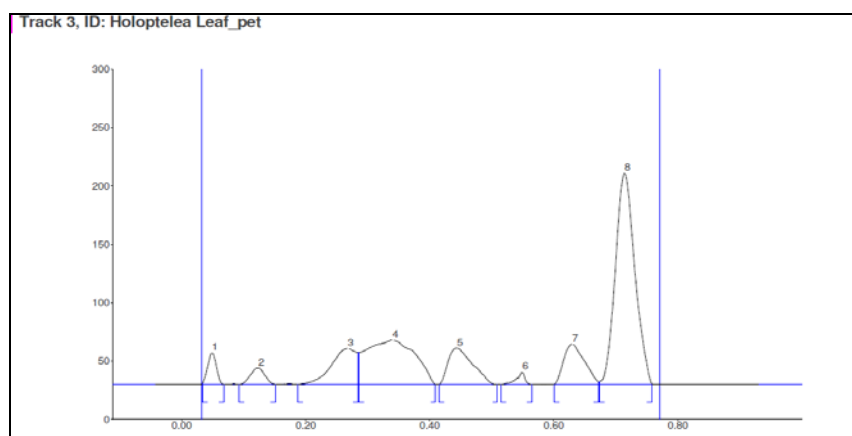
It was observed that there is a separation of different phytoconstituents, in PEHI and MHI.



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

**Table 2:** R<sub>f</sub> values for alkaloids 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	0.03	0.9	0.05	27.4	7.40	0.07	0.0	323.4	2.92	unknown *
2	0.09	0.0	0.12	14.5	3.92	0.15	0.2	282.7	2.55	unknown *
3	0.19	0.1	0.27	31.2	8.43	0.28	27.5	1054.7	9.51	unknown *
4	0.29	27.6	0.34	38.5	10.40	0.41	0.3	2522.5	22.75	unknown *
5	0.42	0.0	0.44	31.5	8.51	0.51	0.0	1039.5	9.38	unknown *
6	0.52	0.0	0.55	11.1	3.00	0.57	0.0	126.1	1.14	unknown *
7	0.60	0.1	0.63	34.9	9.43	0.67	2.5	977.9	8.82	unknown *
8	0.68	2.7	0.72	181.2	48.93	0.76	0.5	4759.4	42.93	unknown *



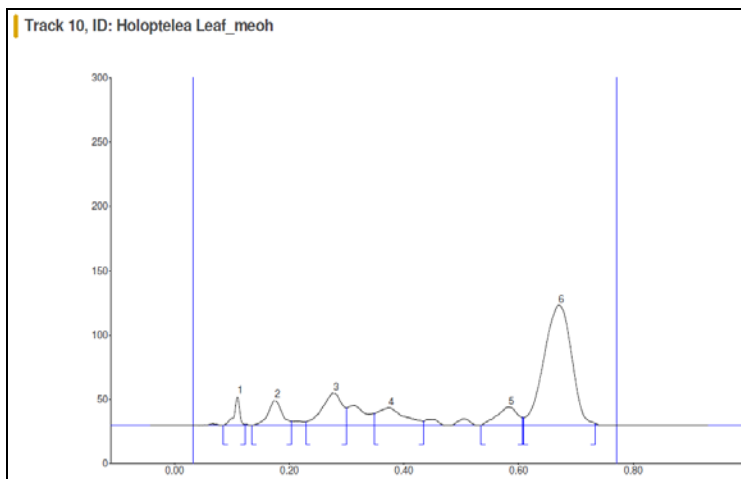
**Fig 4:** Chromatogram for alkaloids in petroleum ether extract of *Holoptelea integrifolia* leaf

### 3.3 Fingerprinting study of Alkaloids of PEHI at 366 nm

Fingerprinting study of PEHI at 366 nm shows eight R<sub>f</sub> Between the range of 0.05- 0.72. R<sub>f</sub>0.72 has 48.93% concentration in Table 2, Figure 4.

**Table 3:** R<sub>f</sub> values for alkaloids in methanol extract of *Holoptelea integrifolia* leaf

winCATS Planar Chromatography Manager										
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.08	0.0	0.11	22.7	12.01	0.12	0.5	181.8	3.00	unknown *
2	0.13	0.1	0.17	19.6	10.38	0.20	3.5	393.2	6.49	unknown *
3	0.23	2.6	0.28	25.0	13.25	0.30	13.5	741.1	12.24	unknown *
4	0.35	9.3	0.37	13.9	7.34	0.43	3.3	540.8	8.93	unknown *
5	0.53	0.1	0.58	14.3	7.59	0.61	6.1	429.9	7.10	unknown *
6	0.61	6.0	0.67	93.3	49.42	0.73	1.7	3767.4	62.23	unknown *



**Fig 5:** Chromatogram for alkaloids in methanol extract of *Holooptelea integrifolia* leaf

### 3.4 Fingerprinting study of Alkaloids of MHI at 366 nm

Fingerprinting study of PEHI at 366 nm shows six  $R_f$  between the range of 0.11- 0.67.  $R_f$ 0.67 has 49.42% concentration in Table 3, Figure 5.

### 4. 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 *Holooptelea integrifolia* will help to isolate and identify new alkaloids, which will offer a possibility to discover lead a molecule for drug development.

### 5. 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.

### 6. References

- Nadkarni KM. Indian Materia Medica. Popular Prakashan Pvt Ltd. Mumbai, India. 1976, 651-652.
- Kirtikar KR, Basu BD. Indian Medicinal Plants. Edn 3, Sri Satguru Publications, New Delhi, India, 2000; 3:2292-2294.
- Prajapati ND, Purohit SS, Sharma AK. A Handbook of Medicinal Plants a Complete Source Book. Agrobias. Jodhpur, India, 2003, 273.
- Warrier PK, Nambiar VPK, Ramakutty C. Indian Medicinal Plants a compendium of 500 species, Orient longman private Limited, 1995, 3:162.
- Khandelwal K R. Practical Pharmacognosy. Techniques and Experiments. 10<sup>th</sup> ed. Nirali Prakashan, Pune, India. 2006, 149-156.
- Harborne JB. *Phytochemical methods*; 3<sup>rd</sup> edition, London: Chapman and Hall, 1998.
- Wagner H, Baldt S. *Plant drug analysis*; Berlin: Springer; 1996. R.P.W. Scott, Encyclopedia of Chromatography, 10<sup>th</sup> edn, Marcel Dekker, USA, 2001, 252-254.
- ICH/CPMP Guidelines Q2B, Validation of Analytical Procedures– Methodology, 1996.
- Cazes J, Scott RPW. Chromatography Theory, Marcel Decker, NY, 2002, 443-454.
- Reviewer Guidance, Validation of Chromatographic Methods, 1994.
- Sethi PD. HPTLC: Quantitative Analysis of Pharmaceutical Formulations, CBS Publications, New Delhi, 1996, 162-165.
- Heftman E. Chromatography Fundamentals and Applications of Chromatography and Related Differential Migration Methods. 6<sup>th</sup> edn, Elsevier, Amsterdam. 2004; 69A:253-291.
- British Pharmacopoeia, International edn, HMSO, Cambridge, 2002; II, Appendix 112 (IB).
- Sherma J. Encyclopedia of Pharmaceutical Technology, 2<sup>nd</sup> edn, Marcel Dekker, USA, 2001, 252-254.
- ICH/CPMP guidelines Q2A, Text on Validation of Analytical Procedures, 1994.
- USP 23, NF 19, Asian edn, United States Pharmacopoeial Convention, Rockville, M.D., 982, 1225.