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Dr. Vinay Kumar Verma
Assistant Professor, P.G. Deptt. of Dravyaguna, Major S. D. Singh P.G. Ayurvedic College, Fatehgarh, Farrukhabad, Uttar Pradesh, India

Abhishek Kumar
Associate Professor, Sheat College of Pharmacy, Varanasi, Uttar Pradesh, India

KN Dwivedi
Professor, Deptt. of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India

Correspondence
Dr. Vinay Kumar Verma
Assistant Professor, P.G. Deptt. of Dravyaguna, Major S. D. Singh P.G. Ayurvedic College, Fatehgarh, Farrukhabad, Uttar Pradesh, India

Phytochemical and pharmacognostic study on Heartwood of *Chirabilva* (*Holoptelea integrifolia* Planch.)

Dr. Vinay Kumar Verma, Abhishek Kumar and KN Dwivedi

Abstract
In Ayurveda, a number of medicinal plants are used since long. *Chirabilva* (*Holoptelea integrifolia* Planch.) is one of them having immense medicinal properties. Heartwood of this plant was studied for its standardization. Pharmacognostic study revealed the organo-leptic characters of heartwood. Powder microscopy showed the presence of lignified tissues and vascular bundles, calcium oxalate crystals, hemicellulose-endospermic wall, medullary rays with pitted cells. T.S. of heartwood of *Chirabilva* also shows vessels, medullary rays with pitted cells and hemicellulose-endospermic wall. Phytochemical study on heartwood revealed the presence of proteins, glycosides, flavonoids, alkaloids, tannin and phenolic compounds. In TLC, two peaks were present having Rf value 0.46 and 0.90. HPTLC shows a number of peaks indicating the presence of several compounds.

Keywords: heartwood**,** *Chirabilva*, pharmacognostic study, phytochemical study, *Holoptelea integrifolia* Planch.)

Introduction
Medicinal plants were used in India since long for the treatment of several diseases described in Ayurveda, the ancient science of life. *Chirabilva* (*Holoptelea integrifolia* Planch.) is one such plant which is first described in *Charaka Samhita* [1]. Synonyms of *Chirabilva* in Sanskrit are *Putigandha, Putika* while in Hindi; it is called *Chirabil, Chiramil or Papri* [2]. English name of this plant is Indian Elm while trade name is *Kanju* [3, 4].

Botanical Description: A large, spreading, glabrous, deciduous tree. Bark grey, pustular, exfoliating in somewhat corky scales, leaves elliptic-ovate, acuminate, base rounded or sub-chordate. Flowers greenish-yellow, polygamous, in short racemes or fascicles on the leafless branches. Fruit sub-orbicular samara with membranous wing. Seed one, flat. Unpleasant odors present on cutting the bark & crushing the leaves. The wood is light yellow, lustrous, interlocked- grained, medium and even-textured, moderately heavy & strong. There is no distinct heart wood. It is distributed throughout the greater part of India up to an altitude of 2000 ft. and also on the roadside. Also found in tropical & subtropical region of Asia & Africa [5]. (Fig.1)

Fig 1: *Chirabilva* (*Holoptelea integrifolia* Planch.) tree

Heartwood: It is the central tough part of stem found in large old trees. The heartwood (also called *duramen*) is composed of dead cells with their walls heavily impregnated with various compounds such as resins, gums, tannins, pigments or phenolic compounds and hence become unsuitable for conduction but medicinally useful. As the growth process continues the rings of sapwood (also called *alburnum*) bordering the heartwood keeps on converting into heartwood. Distinction between sapwood and heartwood is not sharp as in case of *Holoptelea integrifolia* Planch [6].

Parts Used: Bark, Leaf, Seed, Heart wood.

Dosage: Decoction - 50 to 100 ml, Bark powder -1-3 g.

Ayurvedic Properties
Rasa: *Tikta, Kashaya* ***Guna:*** *Laghu. Ruksha*
Virya: *Usna* ***Vipaka:*** *Katu*
Doshakarma: *Kapha Pitta Shamaka*
Rogaghnata**:** *Kaphapaittikavikara, Shotha, Agnimandya, Chardi, Udararoga, Shula, Gulma, Arsha, Krimi, Raktavikara, Prameha, Kushtha, Medoroga.*
Karma: *Shothahara, Deepana, Anulomana, Plihasarkara, Bhedana, Krimighna, Raktashodhaka, Pramehghna, Kushthaghna, Lekhana* [7, 8].

Actions and Uses: The bark and leaves are bitter, astringent, acrid, thermogenic, anti-inflammatory, digestive, carminative, laxative, anti-helminthic, depurative, revulsive and urinary astringent. They are useful in inflammations, acid gastritis, dyspepsia, flatulence, colic, intestinal worms, vomiting, wounds, vitiligo, leprosy, filariasis, diabetes mellitus, obesity, hemorrhoids and rheumatism. Seeds are useful in infected ulcers and as a deodorant for foul smell of body.

Chemical Constituents: Two triterpenoid fatty acid esters Holoptelin-A and B, 2-amino naphthaquinone, fiedelin, epifriedelinol, β-sitosterol and its β-D-glucose (stem bark); β-sitosterol 2α, 3α-dihydroxyelan-12-en-28-oic acid and hederagenin (heartwood); hexacosanol, octacosanol, β-sitosterol and β-amyrin (1eaves); carbohydrates, pigments, oils, acids, glycosides, sterols, tannins, proteins, free amino acids, major fatty acids- palmitic acid, oleic acid, myristic, stearic, linoleic and linolenic acids; and steroids-β-sitosterol and stigmasterol (dried seeds); histamine and 5- hydroxy tryptamine (pollens) were present in different parts [9-11].

Pharmacognosy is the study of identification of drugs derived from natural sources which are crude or primary type. The American Society of Pharmacognosy defines pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources." It is derived from the Greek word *pharmakon* meaning “a drug” and *gignosco* meaning “to acquire a knowledge.”It also contains the knowledge of history, distribution, cultivation, collection, preservation and uses of crude drugs [12, 13].

The concept of standardization and quality control of drug can be found in ancient ayurvedic texts. In those days, the physician himself identified, checked the drugs based on habitat, morphology, taste, color, texture and uses as medicine [14].

But in modern times, these tests and tools are not sufficient to control the quality. Hence, the World Health Assembly (WHA 42.43-1989) has emphasized the need to ensure the quality of medicinal plant products by using modern quality control techniques and applying suitable standards. Assessment of complete and accurate physicochemical value of ayurvedic drug not only provides scientific basis of its quality but also helps in globalization of ayurveda. Under these circumstances, pharmacognosy and phytochemistry are necessary for authentication of crude [15].

In the present work, preliminary phytochemical and pharmacognostic study of heartwood of *Holoptelea integrifolia* Planch has been done.

- Macroscopic study of powder of Heart wood of stem.
- Microscopic study of powder of Heart wood of stem.
- Transverse section of Heart wood of stem.
- Phytochemical study of powder of Heart wood of stem.
- TLC of extract.
- HPTLC of extract.

Material and Methods

- **Plant Material:** Wood of *Holoptelea integrifolia* Planch was collected from forests of Mirzapur near Varanasi in January, 2016. Heartwood was obtained after removing most of the bark (Fig.2).

Fig 2: Heartwood of *Chirabilva*

- Sample of the collected raw drug was kept in the museum of the department of Dravyaguna, Faculty of Ayurveda, IMS, BHU, with specimen accession no. DG/17-18/140.

Preliminary pharmacognostic characteristics

Macroscopic characteristic of drug

Materials
1. Coarse powder of *Chirabilva* (Ht. wd.)
2. Petri dish.

Method
5 gm coarse powder of sample was taken in a Petri dish and examined with naked eye.

Table 1: Macroscopic characteristic of powder of Ht. wd. of *Chirabilva*

S. No.	Parameters	Observation of fruit
1	Nature	Coarse powder
2	Colour	Yellowish
3	Odour	Mild foetid
4	Taste	Sweet, bitter and astringent
5	Texture	Rough & fibrous
6	Size	Uneven sized coarse particles

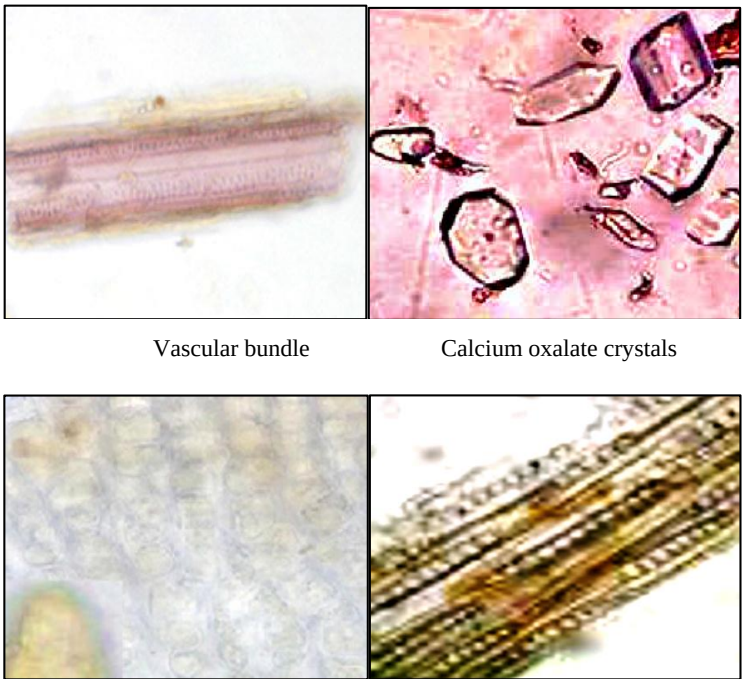
Microscopic characteristic of drug

The coarse powder of Ht. wd. of *Chirabilva* was pulverized in to fine powder. The powder was investigated for its microscopic characteristics.

Materials
1. Fine powder of Ht. wd. of *Chirabilva* (Fig. 3)
2. Chloral hydrate
3. Plain water
4. Microscope
5. Slide and Cover slip
6. Watch glass

Fig 3: Fine powder of heartwood

Method: 5 gm powder of heartwood of *Chirabilva* was boiled separately with chloral hydrate solution in small quantity. Cleaved powder was removed in three separate watch glasses respectively and stained with one drop each of Phloroglucinol and conc. HCl. A little of the treated powder was mounted in dil. Sulphuric Acid and the slides were observed under microscope at low power (Fig. 4).



Vascular bundle Calcium oxalate crystals

Hemicellulose-Endospermic wall Medullary rays with pitted cells

Fig 4: Powder microscopy of heartwood of *Chirabilva* (*Holoptelea integrifolia* Planch.)

This process was repeated with Dil. Hcl and Dil. Iodine+ Conc. H_2SO_4 and after mounting, the slides were observed. The observations and their interpretations are tabulated below.

Table 2: Microscopic characteristics of powdered heartwood of *Chirabilva* (*Holoptelea integrifolia* Planch.)

S. No.	Reagents	Observations	Characterstics
1	Phlorogucinol + conc. HCL	Pink	Lignified tissues and vascular bundles
2	Dil. Hydrochloric acid	Soluble	Calcium oxalate crystals
3	Dil. Iodine + conc. Sulphuric acid	Blue	Hemicellulose-Endospermic wall
4	Phlorogucinol + conc. HCL	Yellow fibers	Medullary rays with pitted cells

Transverse section of Ht. Wd. of *Chirabilva*: T.S. of Ht. Wd. of *Chirabilva* shows vessels, medullary rays with pitted cells and hemicellulose-endospermic wall (Fig. 5).

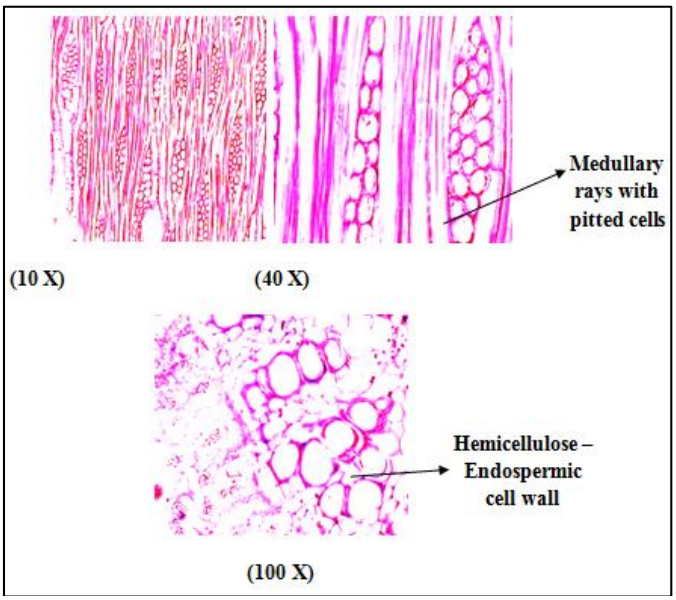


Fig 5: Transverse section of Ht. wd. of *Chirabilva*

Standardization of *Chirabilva*

The parameters which were used for evaluation are nature, odour, colour, taste and texture, determination of hydro-alcoholic extractive value, determination of total ash, acid insoluble ash, water soluble ash, fluorescence analysis of the drug, determination of foreign matter etc [16].

Procedure for different parameters

Determination of hydro-alcoholic extractive value

Hydro-alcoholic extract of air dried 100 gm coarse powder of the heartwood of *Chirabilva* was extracted with Ethanol: Distilled water (50:50), 450 ml each, with continuous heat extraction with Soxhlet apparatus and filtered (Fig. 6).

Fig 6: Soxhlet apparatus during extraction process

Fig 7: Hydro-alcoholic Extract

The extract was concentrated to get dry residue and stored in the desiccators and weighed which was 10.6 g. The percentage of hydro-alcoholic extract was calculated with reference to the air dried drug. This extract was used for subsequent experiments (Fig. 7).

Total extractive value of drug = 10.6%

Determination of total ash

2 gm of air dried powder of heartwood of *Chirabilva* was accurately weighed in a silica dish and incinerated at a temperature not exceeding 450 ^{0}C until free from carbon, cooled and then weighed again (Fig. 8). The total ash value of the drug = 6.45%

Fig 8: Total Ash

Determination of Acid-insoluble ash

The ash was boiled for 5-10 minutes with 25 ml of 2M HCl and then the insoluble matter was collected in a Gooch crucible, then washed with hot water, ignited and weighed. (Fig.9) The percentage of acid insoluble ash was then calculated with reference to the air dried drug. Acid-insoluble ash value of drug =0.25%

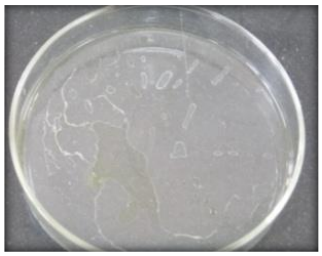


Fig 9: Acid insoluble Ash

Determination of Water-soluble Ash

The ash was boiled for 5-10 minutes with 25 ml of water, and then the insoluble matter was collected in a Gooch crucible and then washed with hot water, ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the total ash and the difference in weight represents the water soluble ash. The percentage of water soluble ash was then calculated with reference to the air dried drug. Water-soluble Ash value of drug= 6.2%

Fluorescence analysis of the drug

Many crude drugs show fluorescence when the sample is exposed to UV radiation. Evaluation of crude drugs based on fluorescence in day light is not much used, as it is usually unreliable due to the weakness of fluorescent effect. For this, fluorescence lamps fitted with suitable filters which eliminate visible radiation from the lamp and transmit UV radiation of

different wavelengths usually of 254 nm and 366 nm. The crude sample of Ht. wd. of *Chirabilva* had blue fluorescence.

Determination of foreign matter

100 g of Ht. wd. of *Chirabilva* was accurately weighed and spread in a thin layer and sorted the foreign matter into groups either by visual inspection or by using a magnifying lens (6X or 10X). The remainder of the sample was shifted through a 250 no. sieve; dust was regarded as mineral admixture. The portions of this sorted foreign matter weighed 0.02 g (less than 0.04gm). The content of drug was then calculated in g/100gm of air dried sample which was 99.98g.

Observation

Table 3: Certificate of analysis of heartwood of *Chirabilva*

S. No.	Parameters	Observation
I	Physical tests	
	Nature	Coarse powder
	Colour	Yellowish
	Odour	Mild foetid
	Taste	Sweet, Bitter and astringent
II	Foreign matter	Nil
III	Fluorescence	Blue
IV	Ash value (% w/w)	
	Total ash	6.45
	Acid insoluble ash	0.25
	Water soluble ash	6.2

Table 4: Percentage yield of Extracts of Cirabilva

Extracts	Nature of Extract	Weight (g)/100g of drug	% Yield w/v
Hydro-alcohol	Viscous	10.6	10.6

Preliminary screening of phytochemicals

The preliminary phytochemical studies were performed for testing the different chemical groups present in the drug based on various studies [17, 18, 19, 20]. 10% (w/v) solution of extract was taken unless otherwise mentioned in the respective individual test.

General screening of various extracts of the plant material was carried out for qualitative determination of the groups of organic compounds present in them.

Result

Table 5: Phytochemical screening of hydro-alcoholic extract of Heart wood of *Holoptelea integrifolia* Planch.

S. No.	Chemical Tests	Hydro-alcoholic Extract
1	Carbohydrates	-
2	Proteins	+
3	Amino acids	-
4	Glycosides	+
5	Flavonoids	+
6	Alkaloids	+
7	Tannins and Phenolic Compounds	+
8	Steroids	-
9	Vitamins	-

Thin layer chromatography (TLC) of heartwood of *Chirabilva* extracts

TLC or thin layer chromatography is a type of planar chromatography. TLC is routinely used by researchers in the field of phyto-chemicals, biochemistry etc. to identify the components in a compound mixture like alkaloids, phospholipids, amino acids etc. It is a semi quantitative method of analysis and its sophisticated version or highly precise quantitative version is high performance thin layer chromatography (HPTLC). Similar to other chromatographic methods, TLC is also based on the principle of separation. The separation depends on the relative affinity of compounds towards stationary and mobile phase. The compounds under the influence of mobile phase (driven by capillary action) travel over the surface of stationary phase. During this movement the compounds with higher affinity to stationary phase travel slowly while the others travel faster.

Thus, separation of components in the mixture is achieved. Once separation occurs, individual components are visualized as spots at respective level of travel on the plate. Their nature or characters are identified by means of suitable detection techniques [21].

Materials

1. Extracts from lab.
2. Solvent system- Methanol: Dichrolomethane (1:19).
3. TLC plate.
4. Developing tanks.
5. Spraying agent: 10% sulphuric acid.
6. Heating oven.
7. UV Lamp Detector.

Procedure

1. The stationary phase is applied onto the plate uniformly and then allowed to dry and stabilize.
2. A thin mark is made at the bottom of the plate with a pencil to apply the sample spots.
3. Then samples solutions are applied on the spots marked on the line at equal distances.
4. The mobile phase Methanol: Dichrolomethane (1:19) poured into the TLC tanks to a level few centimeters above the tanks bottom.
5. Then the plate prepared with sample spotting is placed in TLC tanks such that the side of the plate with sample line is towards the mobile phase. Then, the chamber is closed with a lid.
6. The plate is immersed such that sample spots are well above the level of mobile phase but not immersed in the solvent for development. Sufficient time is allowed for development of spots. Then the plates are removed and allowed to dry. The sample spots are visualized in suitable UV light chamber (Fig. 10).

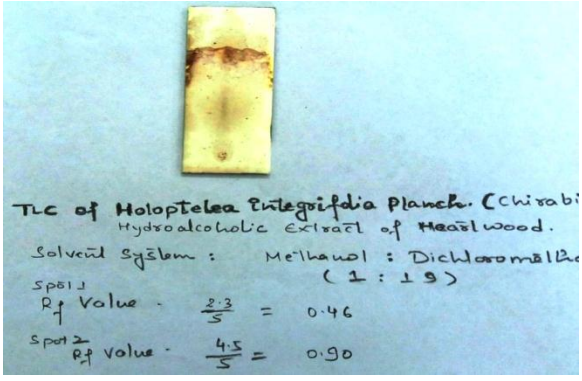


Fig 10: TLC of *Chirabilva* Extract

Results

$$\text{Rf value} = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent}}$$

For Spot 1- Rf Value = $\frac{2.3}{5}$ = 0.46

For Spot 2- Rf Value = $\frac{4.5}{5}$ = 0.90

The hydro-alcoholic extract of heartwood of *Chirabilva* was prepared. A large number of solvent systems were tried to achieve a good resolution. Finally, the solvent system Methanol: Dichloromethane in (1:19) ratio was selected for TLC of hydro-alcoholic extract. Two bands appeared for after spraying with Conc. H_2SO_4 for this solvent system at Rf 0.46, 0.90 respectively on TLC plate.

HPTLC- Methodology

0.3 g of extract was dissolved in 2 ml of Absolute Methanol and heated on a hot plate. 10µl of the above extract were applied on HPTLC plate of silica gel of 3.0 x 10.0 cm dimension, 60 F 254 (Manufacturer E. MERCK KGaA) to a bandwidth of 7 mm using Linomat 5 TLC applicator.

The plate was developed in Methanol: Dichloromethane (1:19). The developed plates were visualized in UV 254 and 366 and scanned under UV 254 and 366 nm. R_f value of the spots and densitometric scan were recorded (Table 6). HPTLC was done at Centre of Experimental Medicine & Surgery, IMS, BHU. HPTLC fingerprint of extract of Ht. Wd. of Chirabilva is shown in Fig. 11. 3-D image of Rf peaks are shown in Fig. 12. Rf. peaks and area under curve of these peaks are shown in Fig. 13 and Fig. 14 respectively.

Table 6: Showing different Rf value and area % of HPTLC.

Peak	Start Rf	Start Height	Max. Rf	Max. Height	Max %	End Rf	End Height	Area	Area %
1	0.00	0.7	0.03	113.5	4.26	0.06	0.2	1819.2	2.04
2	0.08	8.9	0.12	765.5	28.79	0.14	719.6	24210.1	27.11
3	0.14	720.4	0.15	735.6	27.67	0.16	513.0	11102.9	12.43
4	0.17	520.2	0.23	558.9	21.02	0.35	90.4	36857.8	41.27
5	0.35	90.4	0.37	98.6	3.71	0.47	27.6	5058.3	5.66
6	0.55	25.4	0.60	37.4	1.41	0.60	35.6	1111.4	1.24
7	0.61	36.1	0.63	77.4	2.91	0.64	74.8	1294.8	1.45
8	0.64	74.9	0.67	87.0	3.27	0.70	69.1	2936.9	3.29
9	0.70	69.2	0.71	70.6	2.66	0.77	11.6	2041.3	2.29
10	0.78	11.6	0.82	69.1	2.60	0.87	0.6	1719.8	1.93
11	0.89	0.3	0.92	18.1	0.68	0.95	9.7	396.0	0.44
12	0.95	9.9	0.98	26.9	1.01	1.02	3.8	770.2	0.86

Fig. 11: HPTLC finger print of extract of Ht. wd. of *Chirabilva*

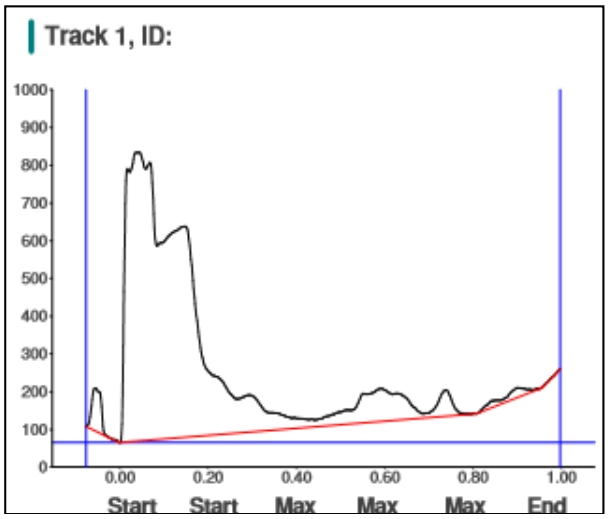


Fig 13: Showing Rf peaks of HPTLC

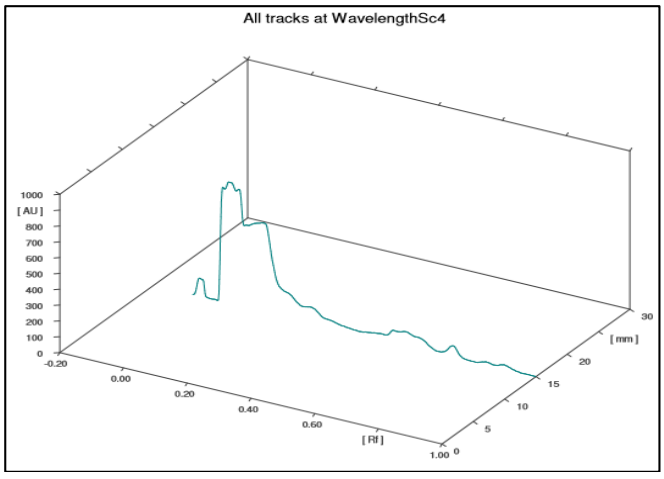


Fig. 12: 3D image of Rf peaks of HPTLC

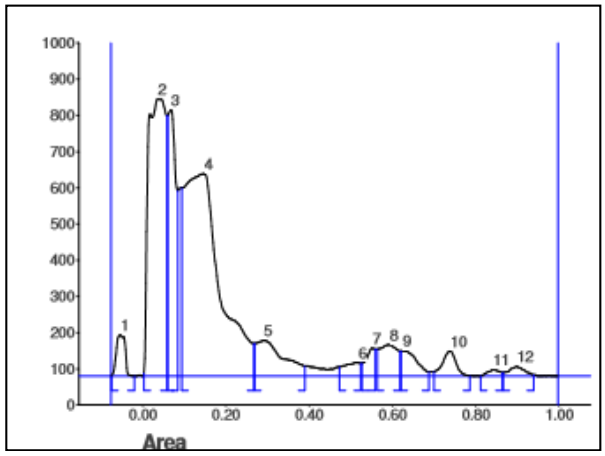


Fig 14: Showing area under curve of Rf values of HPTLC

Conclusion

Powder microscopy of heartwood of *Chirabilva* (*Holoptelea integrifolia* Planch.) showed the presence of lignified tissues and vascular bundles, calcium oxalate crystals, hemicellulose-endospermic wall, medullary rays with pitted cells. Transverse section also shows vessels, medullary rays with pitted cells and hemicellulose-endospermic wall. Phytochemical study on heartwood revealed the presence of proteins, glycosides, flavonoids, alkaloids, tannin and phenolic compounds. In TLC, two peaks were present having Rf value 0.46 and 0.90. HPTLC shows a number of peaks indicating the presence of several compounds.

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Conflict of interest: None

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