



PHARMACOGNOSTICAL AND PHYTOCHEMICAL ESSAY OF BHANDIRA (*Clerodendrum infortunatum* L.)

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ABSTRACT

The globalisation of Ayurveda and tremendous growth in the population has led to high demand of bio resources. So many medicinal plants mentioned in the Ayurvedic classics are on the verge of extinction. So, there is a need to include new medicinal plants which are less explored to the mainstream. One such plant is *Bhandira* (*Clerodendrum infortunatum* L.). The drug *Bhandira* (*Clerodendrum infortunatum* L.) is a softly tomentose perennial shrub. *Bhandira* is categorized into the family Lamiaceae. The root of the plant is widely used by folklore practitioners for different disorders. The drug is not much explored in Ayurveda. The pharmacognostic and phytochemical study of the drug reveals the important components of the drug. Hence, present study was undertaken to evaluate pharmacognostic and phytochemical study of the drug *Bhandira* (*Clerodendrum infortunatum* L.). In pharmacognostic study the macroscopic and microscopic studies were done. Ash analysis and preliminary phytochemical study also was done. In phytochemical study HPTLC and TLC was carried out. Preliminary phytochemical study of the drug *Bhandira* (*C.infortunatum* Linn) showed the presence of Carbohydrates, Flavonoids, Steroids, Resins, Phenolics, Tannins and Triterpenoids. Ash analysis of root of *Bhandira* (*C.infortunatum* Linn) showed the presence of Fluoride, Phosphate and Sodium among the inorganic constituents. In HPTLC documentation *C.infortunatum* root extract showed 7 peaks at 254nm, 8 peaks at 366nm and 11 peaks at 550nm.

Keywords: *Bhandira*, *Clerodendrum infortunatum* L, Pharmacognostic study, Phytochemical study.

INTRODUCTION

The drug *Bhandira* (*Clerodendrum infortunatum* Linn.) is a softly tomentose¹ perennial shrub attaining a height of around 1m and possessing pinkish white or light purple coloured flowers². It was classified under the family Verbinaceae. But recently it is categorised into the family Lamiaceae³. *Bhandira* is *laghu*, *tikta*, *ushna*, *Kaphavata shamaka* and has *krimighna* action⁴. It is indicated in *Kushta*, *Amavata*, *Jwara*, and *Krimi*⁵. The plant is widely used by folklore practitioners around Alappuzha district of Kerala. Mainly root and leaf of the plant is used by the folklore practitioners for different disorders. Hence the present study was undertaken to evaluate pharmacognostic and phytochemical study drug *Bhandira* (*Clerodendrum infortunatum L.*). *Clerodendrum infortunatum L* is a large shrub, tomentose. Stem and branches quadrangular. Leaves: opposite, up to 25x20cm, ovate or suborbicular, shortly acuminate at apex, cordate at base, denticulate or serrate, pubescent above, pubescent or tomentose beneath; petiole up to 12 cm long. Flower: large terminal panicles. Calyx: deeply 5 lobbed, much enlarged in fruit; lobes ovate-acuminate. Corolla: white; tube 2.5cm long; lobes lanceolate, subequal. Drupes: bluish black when ripe, enclosed in accrescent calyx. Distribution and Habitat⁹ - Degraded forest areas, moist evergreen forests and plain lands. Found all over India, Srilanka, Myanmar, Andaman & Nicobar Islands, Thailand, Malaysia, and Bangladesh.

In this study Pharmacognostical analysis of *Bhandira* (*Clerodendrum infortunatum L*) root was done by macroscopic study and microscopic study, Physicochemical study of *Bhandira* (*Clerodendrum infortunatum L*) root, Phytochemical study on *Bhandira* (*Clerodendrum infortunatum L*) root by Systemic extraction and chemical analysis of the extract, Ash analysis, Chromatographic methods by TLC and HPTLC.

Materials and Methods

Collection of Sample: The botanically identified and authenticated samples of *Clerodendrum infortunatum* Linn roots were collected from Alappuzha district in Kerala. Herbarium sample collected and deposited in PG dept. of Dravyaguna Vijnana(AAMC/DG2020/08).

Place of work: Pharmacognostical and Chromatographic study was carried out at Centre of Medicinal Plants & Research Centre, Kottakkal, Malappuram District, Kerala. Phytochemical study was carried out in P G Department of Dravyaguna Vijnana, Alvas Ayurveda Medical College. Moodbidri.

A) Pharmacognostical study

1. Macroscopy of *Clerodendrum infortunatum* Linn Root - The external features of the test sample – *Clerodendrum infortunatum* Linn Root was documented and the organoleptic characters were noted.
2. Microscopy of *C.infortunatum* Linn Root - *C.infortunatum* Linn Root was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section with a sharp blade and the sections were stained with saffranine. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.
3. Powder microscopy of *C.infortunatum* Linn Root - Fine powder of *C.infortunatum* Linn Root was mounted in Glycerine as well as with phloroglucinol and con.Hcl. Gently heat the mixture and was observed under Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

B) Physicochemical study

Physical Standards: The air dried roots of *Clerodendrum infortunatum L.* were powdered finely and subjected to various analysis such as Determination of moisture content¹⁰, Determination of total ash¹¹, Determination of acid insoluble ash¹², Determination of water soluble ash¹³, water soluble extractive value¹⁴, ethyl alcohol soluble extractive value¹⁵, methanol soluble extractive value¹⁶, chloroform soluble extractive value¹⁷, Acetone soluble extractive value¹⁸, Petroleum ether soluble extractive value¹⁹. The extractive value in

various solvents and ash value are important in identification and standardization of single drugs.

C) Preliminary phytochemical study²⁰

Various tests were conducted to know the presence of Proteins, Carbohydrates, Tannins, Saponins, Flavonoids, Steroids, Alkaloids, Triterpenoides, Starch, Resins, Phenolics, Elagic acid.

Ash Analysis²¹: The air-dried powdered drug was taken in a crucible and heated in an electric Bunsen burner to make the ash. Then it was diluted with distilled water, boiled and filtered. The solution was tested to know the presence of Carbonates, Fluorides, Chlorides, Sulphates, Chromate, Phosphate, Potassium, Sodium, Aluminium, and Calcium.

D) Chromatographic studies

1. Thin layer chromatography²²: TLC is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation. Visualisation by Observe the plate under UV light at 254nm and 366nm and 550nm and then record the Rf value and the colour of the resolved bands. Toluene: Ethyl acetate: Formic acid (7: 3: 0.1).

Developing reagent used was Phenol reagent (FCR).

2. HPTLC Fingerprint profile: After development the air-dried plate scanned at 254nm, 366nm and 550nm after derivatising with anisaldehyde sulphuric acid reagent in CAMAG TLC SCANNER 3 with win CATS software.

Results

Macroscopic feature of *C.infortunatum* Linn. Root: The colour of the root were earthy brown and has longitudinal striations. Root were about 12- 15cm long and 1-2cm in diameter.(Fig 1)

Microscopic features of *C.infortunatum* Linn. Root: T.S of the root is irregular circular in outline, showed cork with phellogen layers. Medullary rays were present in the middle. Powder microscopy of the root of *Bhandira* showed cork cell fragments, bordered pitted vessel fragments, cortical parenchyma cells, fibres, medullary rays, starch grains, sclerieds.

Physicochemical studies

1. Determination of moisture content: Percentage of moisture content of the drug *C.infortunatum* Linn. - 6.82%. (Table no .1)
2. Determination of the total ash: Percentage of total ash of the drug *C.infortunatum* Linn – 4.6 %. (Table no.2)
3. Determination of Acid insoluble ash: Percentage of Acid insoluble Ash of the drug *C.infortunatum* Linn - 1.12% . (Table no.3)
4. Determination of water soluble ash: Percentage of Water soluble Ash of the drug *C.infortunatum* Linn - 2.2%. (Table no.4)
5. Determination of Extractive values (Table no 5).

Phytochemical study

1. Preliminary phytochemistry: The different phytochemical components present in *C.infortunatum* Linn Carbohydrates, Flavonoids, Steroids, Resins, Phenolics, Tannins and Triterpenoids. (Table no.6)
2. Ash analysis: Ash analysis of *C.infortunatum* Linn showed the presence of Fluoride, Phosphate and Sodium. (Table no.7)

Thin Layer Chromatography-The photo documentation of the results viewed at 254 nm, 366 nm, 540 nm. (Fig no.) And HPTLC densitometric scan at 254nm (Fig no.4), at 366nm (Fig no.4), at 540nm (Fig no.4) were documented.

DISCUSSION

The drug *Bhandira* (*Clerodendrum infortunatum* Linn.) is a softly tomentose perennial shrub attaining a height of around 1m and possessing pinkish white or light purple coloured flowers. *Bhandira* has no references in *Brihatrayis*, *laghutrayis* or any *nighantus*. P V Sharma is the first one to describe the plant *Bhandira* in his book *Dravyaguna Vijnana*. *Bhandira* has *Tikta rasa*, *Laghu ushna sara guna* and *Kaphavatahara* and *Pitharechaki* action. It is useful in *Krimi*, *Jwara*, *Kushta*, *Amavata*, *Madhumeha*, *Dushtavrana*. The root, leaf of the plant is widely used by folklore practitioners for different ailments.

Pharmacognostical study: Macroscopic Study reveals that the colour of the root was earthy brown and has longitudinal striations. Root were about 12- 15cm long and 1-2cm in diameter. Rootlets are thin and long.

Roots were found to be hard and woody. T.S of the root is irregular circular in outline, showed cork with phellogen layers. Medullary rays were present in the middle. Stone cells are found to be oval and thick walled. Phloem was parenchymatous and filled with starch grains, xylem vessels are pitted in nature with abundant starch grains. Powder microscopy of the root of Bhandira showed cork cell fragments, bordered pitted vessel fragments, cortical parenchyma cells, fibres, medullary rays, starch grains, scleroids, prismatic crystals of calcium oxalate and stone cells.

Phytochemical study: Preliminary phytochemical study of the drug *Bhandira* (*C.infortunatum* Linn) showed the presence of Carbohydrates, Flavonoids, Steroids, Resins, Phenolics, Tannins and Triterpenoids. Ash analysis of root of *Bhandira* (*C.infortunatum* Linn) showed the presence of Fluoride, Phosphate and Sodium among the inorganic constituents. These chemical analyses need more estimation to know the actual number of inorganic constituents present in the plant. **Chromatographic study:** HPTLC and TLC documentation of drug had been carried out. In HPTLC documentation *Clerodendrum infortunatum* root extract showed 7 peaks at 254nm, 8 peaks at 366nm and 11 peaks at 550nm. This data indicates the presence of number of phyto components in the root of *Clerodendrum infortunatum* Linn.

CONCLUSION

The drug *Bhandira* is well known for its folklore use among various folklore practitioners and also mentioned in some of contemporary Ayurvedic texts. The preliminary phytochemical study of the root extract of *Bhandira* (*C.infortunatum* L.) shows the presence of Carbohydrates, Flavonoids, Tannins, Triterpenoids, Steroids, Resins and Phenolics. The percentage of hot water extract was higher than all other extracts. Ash analysis of the drug *Bhandira* (*C.infortunatum* L.) showed the presence of Fluoride, Phosphate and Sodium. The HPTLC of the drug *Bhandira* (*C.infortunatum* L.) root extract showed 7 peaks at 254nm, 8 peaks at 366nm and 11 peaks at 550nm.

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Figures

Fig 1) macroscopy of Bhandira (*Clerodendrum infortunatum L.*)

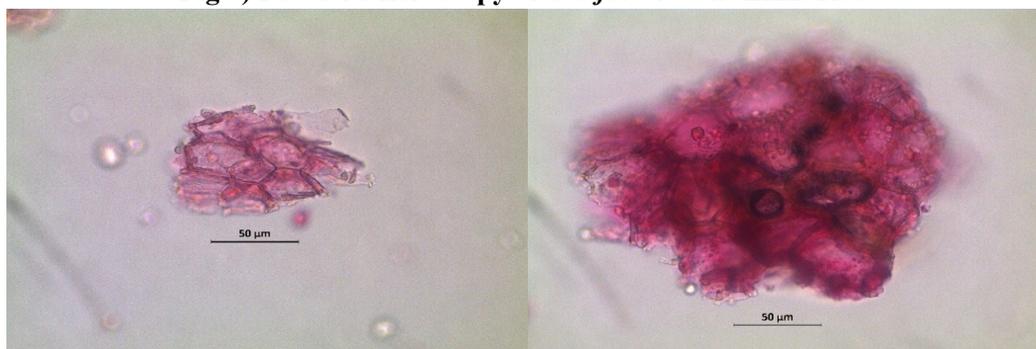


Fig 2 Microscopy of *C.infortunatum* Linn Root

mr- medullary rays, **x-** xylem, **ph-** phloem, **epi-** epidermis, **cc-** cork cell, **st-** stone cells, **pm-** parenchyma.

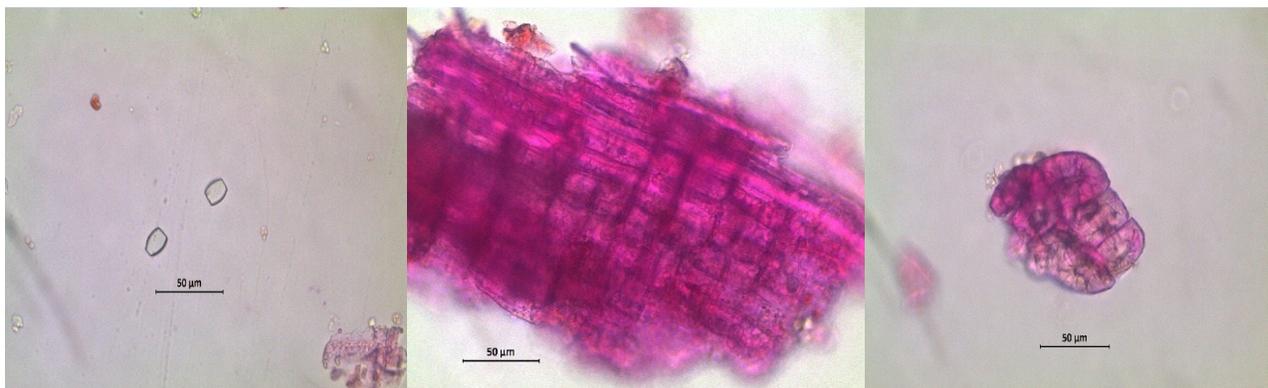


Fig 3) Powder Microscopy of *C.infortunatum* Linn. Root



a) cork cells with fragments

b) cortical parenchyma cells



b) prismatic crystal of calcium

d) fibers and medullary rays

e) groups of stone cells

Fig 4 TLC



TLC at 250NM



366nm



550NM

Fig 5 HPTLC at 254nm

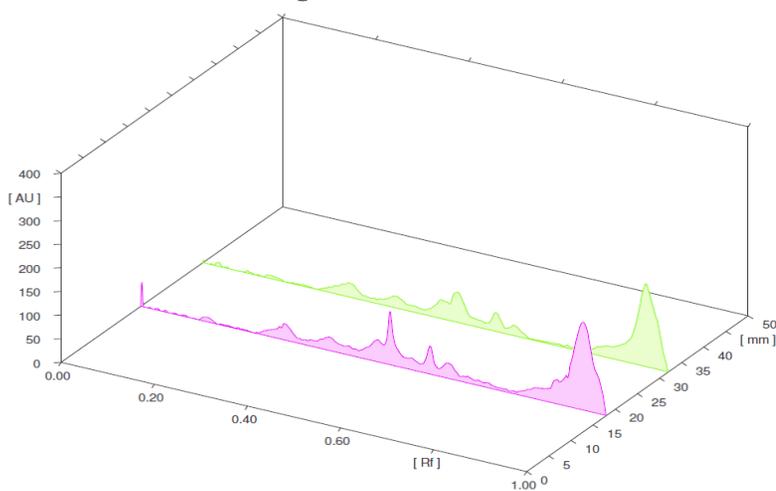


Fig 6 HPTLC at 366nm

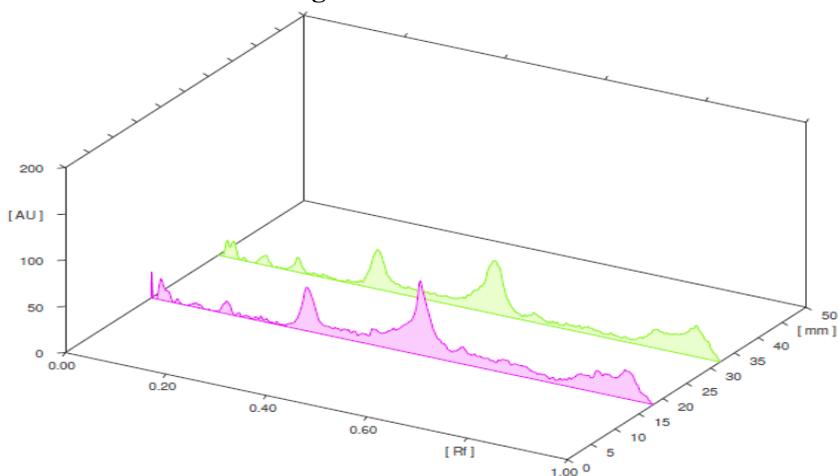
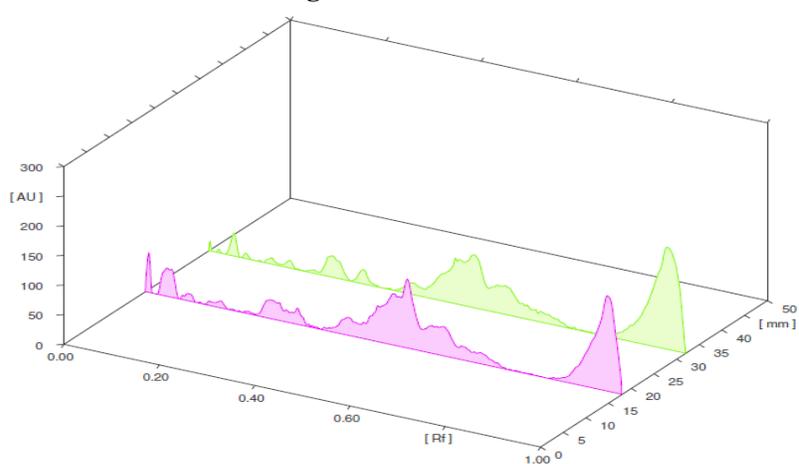


Fig7 HPTLCat550nm



Tables

Table 1: Moisture content of the drugs:

Name of Drug	Weight of Drug	Loss of weight after drying	Moisture
<i>C.infortunatum</i> Linn.	5 gm	0.341 gm	6.82 %

Table 2: Percentage of total ash:

Name of Drug	Weight of Drug	Weight of Ash	Total Ash
<i>C.infortunatum</i> Linn.	5 gm	0.230 gm	4.6 %

Table 3: Percentage of Acid insoluble ash:

Name of Drug	Weight of Drug	Weight of acid insoluble	Acid insoluble ash (% w/
<i>C.infortunatum</i> Linn.	5 gm	0.056 gm	1.12%

Table 4: Percentage of Water-soluble ash:

Name of Drug	Weight of Drug	Weight of Water-soluble ash	Water soluble ash (%w/w)
<i>C.infortunatum</i> Linn.	5 gm	0.110 gm	2.2 %

Table 5: Percentage of extractive values of drugs in different solvents:

The Extractive Values In Different Solvents	Percentage of Extract of <i>C.infortunatum</i> Linn.
Water	17.2 %
Ethanol	1.04 %
Methanol	8.58 %
Chloroform	1.5 %
Petroleum Ether	1.04 %
Acetone	1.78 %

Table 6: Phytochemical components present in various extracts:

Components	Observation	<i>C.infortunatum</i> Linn.
Proteins		
a) Biuret's test	No red colour	Absent
b) Millon's test	No white precipitate	
Carbohydrates		
a) Benedict's test	A coloured precipitate	Present
b) Fehling's test	A red precipitate	
c) Molisch' test	A red violet ring	
Tannins	Green colour	Gallo tannins present
Saponins	No honeycomb structure	Absent
Flavonoids		
a) Shinoda test	Pink or reddish-brown colour	Present
Steroids		
a) Leibermann-Burchad	Greenish colour	Present
b) Salkowski reaction	Red colour is produced	
Alkaloids		
a) Dragendroff's test	No red precipitate	Absent
b) Mayer's test	No white/yellow precipitate	
Triterpenoides		Present

a) Leibermann-Burchad	Violet coloured ring	
Starch	No colour change	Absent
Resins	Turbidity present	Present
Elagic acid	No red colour	Absent
Phenolics	Bluish black colour is formed	Present

Table 7: Ash Analysis

Results of Ash analysis components	<i>C.infortunatum</i> Linn
Carbonate	Absent
Fluoride	Present
Chloride	Absent
Sulphate	Absent
Chromate	Absent
Phosphate	Present
Potassium	Absent
Sodium	Present
Aluminium	Absent
Calcium	Absent

Table 8: RF values at 250nm

Track	peak	Start rf	start height	max rf	max height	max%	end rf	end height	area	area %	assigned substance
1	1	0.25	2.3	0.31	34.5	9.42	0.34	10	1249.8	13.43	unknown *
1	2	0.36	10	0.4	28.6	7.79	0.43	17.7	1075	11.55	unknown *
1	3	0.47	13.9	0.51	47	12.81	0.52	38.7	1402.5	15.07	unknown *
1	4	0.52	39	0.54	112.9	30.79	0.56	22.5	1847.5	19.86	unknown *
1	5	0.6	18.6	0.62	59.6	16.26	0.64	15.2	971.8	10.44	unknown *
1	6	0.64	15.8	0.66	32.2	8.78	0.69	9.9	865	9.3	unknown *
1	7	0.81	4.3	0.9	51.9	14.15	0.9	47.4	1892.6	20.34	unknown *
2	1	0.28	10.8	0.31	29.9	13.56	0.34	9.7	1168.5	18.5	unknown *
2	2	0.39	11.4	0.42	25.3	11.46	0.44	12.7	791.9	12.53	unknown *
2	3	0.48	10.1	0.51	39.9	18.08	0.53	35	1202.7	19.04	unknown *
2	4	0.53	35.3	0.55	64	28.99	0.59	15.9	1834.2	29.04	unknown *
2	5	0.61	13.6	0.63	39.5	17.89	0.65	11.4	777.5	12.31	unknown *
2	6	0.65	11.5	0.67	22.1	10.03	0.7	2.5	542.4	8.59	unknown *

Table 9: RF values at 366nm

track	peak	Start rf	start height	max rf	max height	max%	end rf	end height	area	area %	assigned substance
1	1	0.01	1.1	0.02	23	10.85	0.04	0.1	302.8	5	unknown *
1	2	0.13	1.4	0.15	13.7	6.49	0.17	0.7	194.9	3.22	unknown *
1	3	0.28	4	0.31	46.9	22.15	0.34	9.5	1259.1	20.79	unknown *
1	4	0.49	22.5	0.54	80.1	37.82	0.58	17.6	2892.3	47.76	unknown *
1	5	0.83	6.7	0.86	16.8	7.96	0.87	15.8	434.8	7.18	unknown *
1	6	0.93	20.6	0.94	31.2	14.73	0.98	9.9	972.5	16.06	unknown *
1	1	0.01	0.9	0.02	18.4	8.43	0.02	11.4	136.4	2.18	unknown *
2	2	0.03	12.2	0.03	18.6	8.49	0.04	1	175.8	2.81	unknown *
2	3	0.08	1.5	0.1	10.9	4.98	0.11	0	164.2	2.63	unknown *
2	4	0.12	0.5	0.16	15.9	7.26	0.18	0.4	242.6	3.88	unknown *
2	5	0.29	3.9	0.32	42.8	19.58	0.35	9.8	1238.3	19.81	unknown *
2	6	0.5	14.5	0.55	57.7	26.38	0.59	6.7	2349	37.58	unknown *
2	7	0.84	7.6	0.88	20.7	9.44	0.89	18.1	592.3	9.48	unknown *
2	8	0.92	20	0.95	33.8	15.44	0.99	7	1352	21.63	unknown *

Table 10: Rf Values at 550nm

track	peak	Start rf	start height	max rf	max height	max%	end rf	end height	area	area %	assigned substance
1	1	0.01	64.5	0.01	66.8	13.37	0.02	0	267.9	1.96	unknown *
1	2	0.03	0.5	0.05	47.5	9.51	0.07	1	1115.5	8.16	unknown *
1	3	0.07	0.2	0.09	12.5	2.5	0.11	0.1	206.5	1.51	unknown *
1	4	0.14	6.2	0.16	11.7	2.35	0.17	0	172.7	1.26	unknown *
1	5	0.23	1.1	0.26	31.6	6.34	0.29	17.3	1067.7	7.81	unknown *
1	6	0.31	17.7	0.32	27.7	5.55	0.34	2.7	390	2.85	unknown *
1	7	0.39	5.3	0.42	30.2	6.05	0.45	22.7	974.8	7.13	unknown *
1	8	0.46	26.2	0.52	86.5	17.33	0.54	82.5	3805.2	27.85	unknown *
1	9	0.54	83.4	0.55	116.2	23.26	0.58	36.6	2768.6	20.26	unknown *
1	10	0.58	37	0.63	49.7	9.95	0.66	19	2435.7	17.82	unknown *
1	11	0.69	16.1	0.7	18.9	3.79	0.73	6.8	461.3	3.38	unknown *
2	1	0.04	0.3	0.05	38.9	10.34	0.07	0.3	435	3.77	unknown *
2	2	0.12	0.2	0.13	10.9	2.89	0.15	2.9	154.3	1.34	unknown *
2	3	0.15	2.7	0.17	12.6	3.35	0.18	0.4	168.2	1.46	unknown *
2	4	0.23	3.8	0.26	36.1	9.6	0.3	0.3	1073.9	9.31	unknown *
2	5	0.3	0.1	0.32	24.1	6.42	0.35	2.5	446.6	3.87	unknown *
2	6	0.39	0.3	0.43	19.7	5.23	0.45	13.1	618.1	5.36	unknown *
2	7	0.45	13.2	0.52	76.8	20.45	0.54	72.7	3227.4	27.98	unknown *
2	8	0.54	73.2	0.56	89.6	23.83	0.59	37.9	2890.8	25.06	unknown *
2	9	0.6	41.6	0.62	49.2	13.08	0.65	23.6	1930.1	16.73	unknown *
2	10	0.69	13.8	0.7	18.1	4.81	0.76	1.5	589.9	5.11	unknown *

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