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PRELIMINARY PHYTOCHEMICAL EVALUATION AND HPTLC FINGERPRINTING ANALYSIS OF *SIMAROURBA GLAUCA*

Deepa Dahar* and Aruna Rai

Department of Botany, Smt. C. H. M. College (Affiliated to University of Mumbai), Ulhasnagar 421003, Thane, Maharashtra, India.

***Corresponding Author: Deepa Dahar**

Department of Botany, Smt. C. H. M. College (Affiliated to University of Mumbai), Ulhasnagar 421003, Thane, Maharashtra, India.

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ABSTRACT

Compounds derived from natural sources have been gaining importance since past few decades because of the vast chemical diversity they offer. This has led to phenomenal increase in the demand of herbal medicines which necessitates ensuring the quality, safety and efficacy of herbal drugs. The present investigation deals with phytochemical analysis and HPTLC fingerprinting of leaf, stem and bark of *Simarouba glauca* from Simaroubaceae family. The phytochemical analysis by spot detection tests revealed the presence of tannins, alkaloids and phenols. Flavonoids, terpenoids and cardiac glycosides were found to be present only in some plant parts. Phlobatannins, saponins, steroids and anthocyanides were found to be absent in all the plant parts using different solvents. TLC analysis of methanolic extracts of some plant parts revealed the presence of flavonoids, glycosides and alkaloids, whereas terpenoids were found to be present in all parts. HPTLC fingerprint profile along with their Rf values and percentage proportion were recorded, which will help in identification and quality control of leaf, stem and bark of *Simarouba glauca*. The results provide justification for the use of the plants in folk medicine to treat various infectious diseases.

KEYWORDS: *Simarouba glauca*, Simaroubaceae, Phytochemicals, HPTLC fingerprinting, Medicinal plant.

INTRODUCTION

Among the remedies used, plant drugs constitute an important part. A number of scientific investigations have highlighted the importance and the contribution of medicinal plants towards the development of new drugs. Standardization and evaluation of these drugs is a very crucial parameter and is mostly based on pharmacological, phytochemical and allied approaches including various instrumental techniques like chromatography, microscopy and many other.[1] This will help to develop the drugs with fewer side effects, lower costs and better compatibility. The nature of phytoconstituents determines the pharmacological action of the plant material. Hence, the plant species may be considered as a biosynthetic laboratory for a magnitude of primary metabolites (carbohydrates, proteins and fats) as well as secondary metabolites (alkaloids, terpenoids, saponins, flavonoids, glycosides etc.) which exert a definite physiological effect.

The necessity for detection of secondary metabolites in plant samples has led to the formulation of a wide range of 'spot' tests and reagents for selective metabolite group. In many cases, the employment of a single test or reagent often proves insufficient. Due to the wide structural variation in metabolites, the use of different reagents and tests that interact with different structural groups is necessary to ascertain the detection of a specific compound group.

High performance thin layer chromatography (HPTLC) based methods could be considered as a good alternative, as they are being explored as an important tool in routine drug analysis. Characteristic HPTLC finger printing of a particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, purification, characterization and identification of marker chemical compounds of the species. The results obtained with HPTLC are not only reported as peak data but can also be presented and communicated as images.[2] HPTLC offers better resolution and estimation of active constituents can be done with reasonable accuracy in a shorter time.[3]

Simaroubaceae family has been the subject of many studies regarding its chemical constitution and numerous compounds have been isolated and their structure has been elucidated; among these are the quassinoids, alkaloids, triterpenes, steroids, coumarin, anthraquinones, flavonoids and other metabolites.[4] Quassinoids can be considered a taxonomic marker of the Simaroubaceae family, since it is the most abundant group of natural substances formed by oxidative

degradation of triterpene derivatives. Species from the Simaroubaceae family are known for their medicinal properties and are used traditionally for the treatment of malaria, and also as anthelminthic, antitumor, anti-inflammatory, antiviral, anorectic, tonic, insecticide and amoebicide.[5]

Simarouba glauca commonly known as 'The Paradise Tree' or 'King Oil Seed Tree' or 'Laxmitaru Tree', is a versatile multipurpose evergreen tree having a height of 7-15 meter. It is a poly-gamo-dioecious tree and a potential source of biodiesel.[6] Tree trunk is 50-80 cm in diameter. It produces bright green leaves 20-50 cm in length, small white flowers and small yellow reddish fruits.[7] The bark and leaf extract of *S. glauca* is well known for its different types of pharmacological properties such as haemostatic, anthelmintic, antiparasitic, antidysenteric, antipyretic and anticancerous.[8]

MATERIAL AND METHODS

Collection of Plant Material: Fresh plant material of *Simarouba glauca* (leaf, stem and stem bark) was collected from the Hiranandani garden (Powai), Mumbai. Plant material was first washed and then air dried followed by oven drying at temperature 80^{o}C. All the dried samples were powdered and then stored in air tight containers for further use.

PREPARATION OF PLANT EXTRACTS

Extracts for Phytochemical Screening by Spot Detection Tests

Powdered plant material of leaf, stem and stem bark of *Simarouba glauca* (3.0 g) was refluxed using 30 ml of different solvents like distilled water (DW), ethanol, methanol and benzene in a conical flask covered with a funnel for 1 hour at 60^{o}C in a water bath. The mixture was cooled and then filtered using normal filter paper. The extracts obtained were then used to perform the tests.

Phytochemical Screening

Qualitative chemical tests were carried out using aqueous, ethanol, methanol and benzene extracts of the plant samples using standard procedures[9][10][11] (Table 1) to screen for the presence of secondary metabolites.

Extracts for Thin Layer Chromatography (TLC)

Extracts were prepared by refluxing 3.0 g of leaf, stem and stem bark of *Simarouba glauca* powder with 30 ml of 80% methanol in a conical flask covered with a funnel. The refluxing was carried out in a water bath at 60^{o}C for 1 hour. The solvent mixture was cooled and then filtered using normal filter paper. The filtrate was evaporated to dryness. The residue obtained after evaporation was reconstituted with the extraction solvent to obtain the stock solution.

Screening of Extracts Using Thin Layer Chromatography

The extracts prepared were subjected to thin layer chromatography. The chromatographic separation was performed using secondary metabolite specific screening solvent systems.[9][12][13] Two solvent systems were used for each metabolite group. The list of solvent systems used is as mentioned in table 2. Ten micro liters of each extract was hand loaded on pre-coated Merck TLC silica gel 60_{F254} plates using calibrated glass capillaries.

Post development in the solvent system, air dried plates were first observed under UV 365nm and UV 254nm for the detection of any compounds that show fluorescence without derivatisation. After observing the plates under UV, the plates were subjected to derivatisation using different reagents. The plates were dipped in the derivatisation reagent for 2-3 seconds.

The reagents used for the derivatisation are as mentioned in table 3. After drying, the plates were observed under UV 366nm and UV 254nm. In the case of reagents that require heating for colour development, the plates were heated in an oven at 120^{o}C temperature for 5 minutes. The plates were observed under UV light, both before and after heating.

Preparation of Plant Extracts for HPTLC Analysis

One gram of dried plant powder (leaf, stem and bark) of *Simarouba glauca* was weighed and phytoconstituents were extracted in 10 ml of methanol by vortexing for 1-2 minutes, left to stand overnight at room temperature (25±2^{o}C). The extracts were filtered through Whatman filter paper and the filtrate was used for HPTLC assay.

Assay

The HPTLC analysis was carried out following the method of Wagner and Bradt.[12] For the present study, CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner III, REPROSTAR III with 12 bit CCD camera for photo-documentation was used. The software used was WinCATS-4.

Analysis work was carried out on HPTLC equipment, CAMAG made (Muttenz, Switzerland) which consist of Linomat-V sample applicator fitted with a 100 µL syringe (Hamilton, Switzerland), CAMAG TLC visualizer, CAMAG TLC Scanner 3 and WinCATS software. Analysis was performed by using TLC precoated silica gel 60_{F254} aluminium plates with 200 µm thickness (E. Merck, Mumbai, India). 10 µl of samples were applied to the plate using the Linomat-V sample applicator fitted with a 100 µL syringe, samples were spotted in the form of the bands of 8 mm width. The slit dimension was kept at 6.0 mm × 0.45 mm, scanning speed (20 mm/s) was employed. The plates were developed in glass twin trough chamber (10 cm × 10 cm). The optimized chamber saturation time for mobile phase was 20 minutes at room temperature (25±2°C) at relative humidity of 60±5%. The chamber was pre-

saturated with mobile phase, Toluene: Chloroform: Ethyl alcohol (4:4:1). The developed plates were dried by hot air to evaporate solvents from the plate. The plates were photo documented in visible light, UV light of 254nm and UV light of 366nm. The plates were derivatised using anisaldehyde-sulphuric acid reagent and dried at 120^{o}C in hot air oven for 20 minutes. Densitometric scanning was performed on CAMAG TLC Scanner III which was operated by win CATS software.

Table 1: Tests used for secondary metabolite detection using aqueous, ethanol, methanol and benzene extracts.

Sr. No.		Tests	Observations
1.	Tannins	Add 2 ml of ferric chloride or 1 ml of lead acetate solution into 1 ml of extracts	Blue black colouration or white precipitate
2.	Phlobatannins	Add few drops of 1% aqueous HCl to 1 ml of extract	Deposition of red precipitate
3.	Flavonoids	Add 1 ml of aqueous NaOH and 1ml of 10% HCl into 1 ml of extract	Yellow colouration disappears on standing
4.	Terpenoids	Add 2 ml of chloroform to 1 ml of extract and few drops of H_2SO_4 from sides of the test tube	Reddish brown colouration
5.	Saponins	Mix 1ml of extract with 2 ml of distilled water and shake vigorously for a persistent froth	Formation of emulsion
6.	Cardiac glycosides	Add 1 ml of extract to 2 ml of glacial acetic acid containing one drop of ferric chloride solution. Add 1 ml of concentrated H_2SO_4	Greenish blue colouration
7.	Alkaloids	a. Add 1 ml of Dragendorff's reagent to 1 ml of extract b. Add 1 ml of Mayer's reagent to 1 ml of extract	Turbid orange colour Whitish yellow or cream precipitate
8.	Anthocyanides	Add 5 ml of dilute HCl to 1 ml of extract	Pale pink colouration
9.	Phenols	Add 2 ml of 10% lead acetate to 1 ml of extract	Brown precipitate
10.	Steroids	Add 5 ml of chloroform to 1 ml of extract. From this solution take 1 ml and add few drops of concentrated H_2SO_4	Brown ring formation

Table 2: Solvent systems used for the chromatographic separation of secondary metabolites.

Secondary Metabolites	Solvent systems	
	System 1 (*Wagner and Bladt, 1996*)	**System 2**
Alkaloids	Toluene: Ethyl acetate: Diethylamine (70: 20: 10)	Methanol: Ammonia (200: 3) (*Harborne, 1998*)
Flavonoids	Ethyl acetate: Formic acid: Glacial acetic acid: Water (100: 11: 11: 26)	Toluene: Ethyl acetate: Formic acid (70: 30: 1) (*Reich and Schibli, 2007*)
Glycosides	Ethyl acetate: Methanol: Ethanol: Water (81: 11: 4: 8)	Ethyl acetate: Pyridine: Water (5: 1: 4) (*Harborne, 1998*)
Saponins	Chloroform: Glacial acetic acid: Methanol: Water (64: 32: 12: 8)	Ethyl acetate: 96% Ethanol: Water: Ammonia (65: 25: 9: 1) (*Reich and Schibli, 2007*)
Terpenoids	Toluene: Ethyl acetate (93: 7)	Hexane: Ethyl acetate (1: 1) (*Harborne, 1998*)

Table 3: TLC derivatisation reagents used for secondary metabolite detection.

Secondary metabolites	Spray reagent (*Wagner and Bladt, 1996*)	
	Spray 1	**Spray 2**
Alkaloids	Dragendorff reagent	Iodine vapours
Flavonoids	Natural products-Polyethylene glycol reagent	10% Ethanolic ferric chloride
Glycosides	Diphenylamine-aniline-phosphoric acid reagent	Carr price reagent
Saponins	Liebermann-Burchard reagent	Vanillin-Sulphuric acid reagent
Terpenoids	Anisaldehyde-Sulphuric acid reagent	10% Ethanolic Sulphuric acid

RESULTS AND DISCUSSION

Phytochemical Analysis

The results of the phytochemical screening of distilled water (DW), ethanolic, methanolic and benzene extract of *Simarouba glauca* leaf, stem and bark using spot detection tests are presented in table 4. Tannins, alkaloids and phenols were found to be present in leaf, stem and stem bark of *S. glauca* in all the extracts. Flavonoids were found to be absent only in benzene extracts of leaf, stem and stem bark whereas terpenoids were found to be present in leaf and bark extracts prepared using DW, ethanol and methanol. Only DW extract of stem showed the presence of terpenoids. Cardiac glycosides were found to be present in the leaf extracts prepared using all the solvents; however stem extracts gave positive response only with ethanol and benzene extracts. Stem bark showed presence of cardiac glycosides in all the extracts except distilled water.

Our results highlight that all the extracts prepared using different solvents contains phytochemicals like tannins, flavonoids, terpenoids, cardiac glycosides, alkaloids and phenols. However, flavonoids and terpenoids of some plant parts were found to be absent in the extracts made using benzene. It may be due to poor solubility of these phytochemicals in benzene. It was suggested earlier that methanol solvent is best for the extraction of secondary metabolites from the six solvents used (distilled water, methanol, acetone, chloroform, ethyl acetate and hexane).[14]

Contrary to the present finding, phytochemicals like alkaloids, phenols, flavonoids, tannins, lignin, steroids, glycosides, saponins, terpenoids and anthraquinone were found to be present in methanolic and ethanolic extracts of the *S. glauca* leaves.[15] Similarly, presence of alkaloids, flavonoids, glycosides, phenols and saponins in various extracts of *S. glauca* leaf were also reported.[16] *Simarouba glauca* contains large number of compounds like alkaloids, flavonoids, carbohydrates, glycosides, phenolic compound, tannins, triterpenoids, cardinolides and saponins.[17] Presence of alkaloids, phenols, tannins and flavonoids in stem bark of *S. glauca* were also reported earlier.[18]

The results of thin layer chromatogram (TLC) for detection of different metabolites of *S. glauca* are presented in table 5. Saponins were found to be absent in all selected parts of the plant with both solvent systems. Glycosides and alkaloids were observed only in stem of *S. glauca* with both the solvent systems and also with both derivatisation reagents. Terpenoids were found to be present in leaf, stem and bark of *S. glauca* with both the solvent systems as well as with both derivatisation reagents. Flavonoids were found to be present in stem with both solvent systems and with both derivatisation reagents whereas leaf and bark showed presence of flavonoids only when ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26) was used as solvent system and natural product-polyethylene glycol reagent was used for derivatisation.

In conclusion, it can be stated that thin layer chromatography is comparatively more accurate in terms of detecting the presence of secondary metabolites from plant extracts. While spot detection tests can be used for preliminary screening of extracts, a more definitive result can be obtained from thin layer chromatography due to the better interaction of metabolites and reagents after separation. However, the choice of solvent system and more importantly the choice of derivatisation reagent can affect the result of a TLC.

HPTLC Fingerprinting of *Simarouba glauca*

HPTLC fingerprinting profile of *Simarouba glauca* leaf, stem and bark with distinct band pattern before and after derivatisation reagent anisaldehyde sulphuric acid are shown in photoplate 1 and 2. Rf values of *Simarouba glauca* bark, leaf and stem are tabulated in table 6, 7 and 8 respectively. HPTLC densitograph of methanolic extract of bark, leaf and stem of *S. glauca* are depicted in figure 1, 2 and 3 respectively.

Before derivatisation when plates were observed under visible light, many prominent bands were observed in tracks 1, 2, 3 (bark extract) and tracks 4, 5 (leaf extract) (Photoplate 1.a). However, under UV 254nm several distinct bands were observed in all the tracks (Photoplate 1.b). Similarly, when plates were observed under UV 366nm, all tracks showed prominent bands, tracks 6 and 7 (stem extract) showed many distinct bands with blue fluorescence (Photoplate 1.c).

After derivatising the plates with anisaldehyde-sulphuric acid reagent, when observed under visible light many prominent bands were seen in all the tracks (Photoplate 2.a) and under UV 366nm also many distinct fluorescence bands were observed in all the tracks. Tracks 6 and 7 showed green fluorescence bands which indicates that there might be presence of allylic alcohol[19] (Photoplate 2.b).

HPTLC densitograph of methanolic extract of *Simarouba* bark showed fourteen peaks (Figure 1) with Rf in the range of 0.00-1.0 and Rf 0.07 has maximum percentage of 9.56 (Table 6), *Simarouba* leaf showed thirteen peaks (Figure 2) with Rf in the range of 0.00-1.0 and Rf 0.83 has maximum percentage of 12.32 (Table 7) and *Simarouba* stem showed eighteen peaks (Figure 3) with Rf in the range of 0.01-1.0 and Rf 0.68 has highest percentage of 22.93 (Table 8) when observed at wavelength 366nm, maximum percentage indicating the presence of majority of phytochemicals in that region.

HPTLC fingerprinting analysis on various plants have been reported earlier by several researchers, using leaves and green and roasted beans of *Coffea arabica*,[20] leaves of *Bridelia montana*,[21] *Careya arborea* seeds[22] and

Gymnema sylvestre leaves,[1] but probably no report are available on HPTLC fingerprinting of *Simarouba glauca*.

In present study HPTLC fingerprint profile along with their Rf values and percentage proportion were recorded, which will help in identification and quality control of leaf, stem and bark of *Simarouba glauca*. The studies revealed that the solvent systems developed and the specific derivatising reagents used gave well resolved bands for secondary metabolites present in the raw material.

Conflicts of Interest: There are no conflicts of interest.

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Table 4: Qualitative analysis for the presence of secondary metabolites from leaf, stem and bark of *Simarouba glauca* extracts using different solvents.

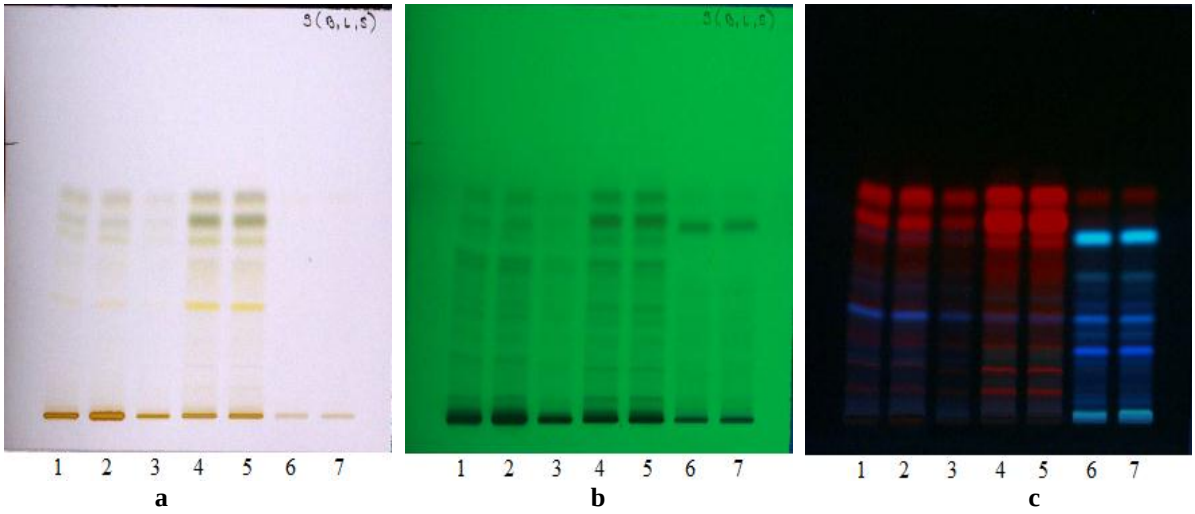
Secondary metabolites	DW extract			Ethanolic extract			Methanolic exract			Benzene extract		
	Leaf	Stem	Bark	Leaf	Stem	Bark	Leaf	Stem	Bark	Leaf	Stem	Bark
Tannins	+	+	+	+	+	+	+	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-	-	-	-	-	-	-
Flavonoids	+	+	+	+	+	+	+	+	+	-	-	-
Terpenoids	+	+	+	+	-	+	+	-	+	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac glycosides	+	-	-	+	+	+	+	-	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
Anthocyanides	-	-	-	-	-	-	-	-	-	-	-	-
Phenols	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	-	-	-	-	-	-	-	-	-	-	-	-

(+: phytochemical group detected; -: phytochemical group not detected)

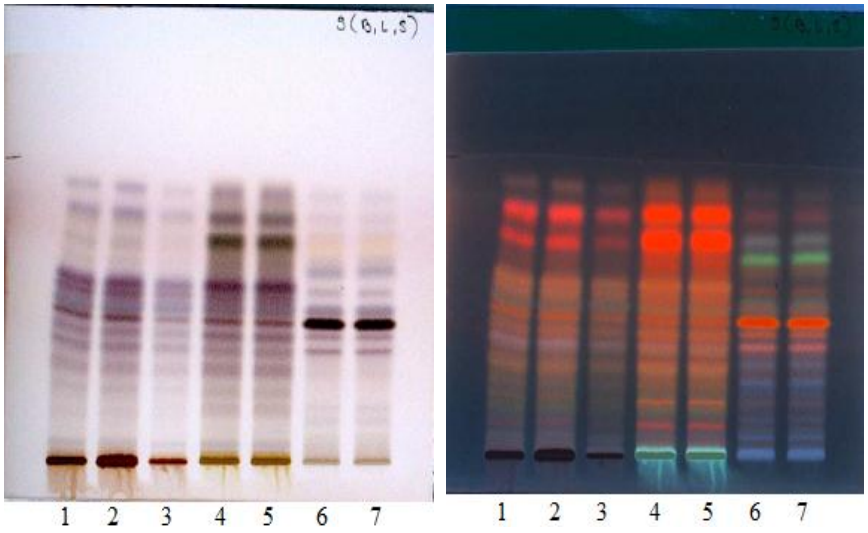
Table 5: Results of TLC analysis of *Simarouba glauca* leaf, stem and bark using methanolic extract.

Secondary metabolites	System 1						System 2					
	Spray 1			Spray 2			Spray 1			Spray 2		
	Leaf	Stem	Bark	Leaf	Stem	Bark	Leaf	Stem	Bark	Leaf	Stem	Bark
Flavonoids	+	+	+	-	+	-	-	+	-	-	+	-
Terpenoids	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	-	-	-	-	-	-	-	-	-	-	-	-
Glycosides	-	+	-	-	+	-	-	+	-	-	+	-
Alkaloids	-	+	-	-	+	-	-	+	-	-	+	-

(+: phytochemical group detected; -: phytochemical group not detected)



Track 1,2 and 3: bark extract, Track 4 and 5: leaf extract and Track 6 and 7: stem extract.
a. In visible light, b. Under UV 254nm, c. Under UV 366nm.
Photoplate 1: HPTLC plates of *Simarouba glauca* before derivatization



a **b**

Track 1,2 and 3: bark extract, Track 4 and 5: leaf extract and Track 6 and 7: stem extract.
a. In visible light, b. Under UV 366nm.
Photoplate 2: HPTLC plates of *Simarouba glauca* after derivatization

Table 6: HPTLC peak values of methanolic extract of *S. glauca* bark.

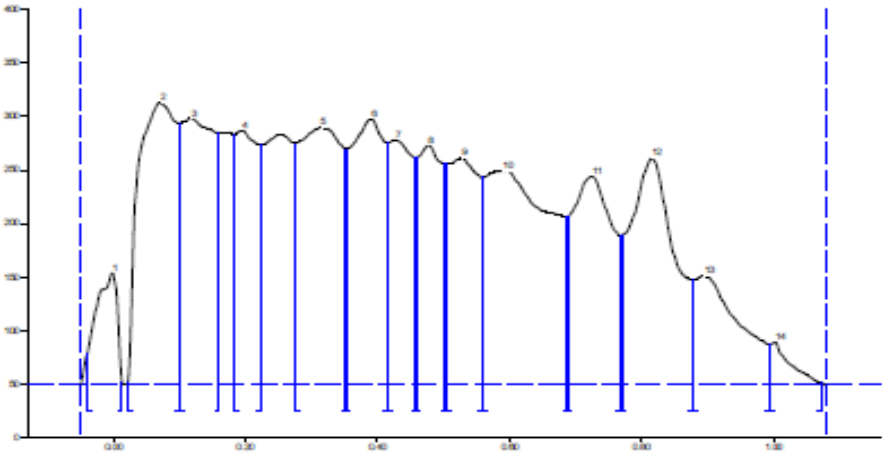
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.04	29.7	0.00	104.8	3.81	0.01	2.7	2406.0	2.25	Unknown*
2	0.02	2.6	0.07	263.3	9.56	0.10	244.2	10592.6	9.92	Unknown*
3	0.10	244.3	0.12	248.6	9.02	0.16	235.3	9209.6	8.62	Unknown*
4	0.18	234.2	0.20	237.3	8.61	0.22	224.0	6014.7	5.63	Unknown*
5	0.28	225.6	0.32	240.4	8.73	0.35	220.1	11153.5	10.44	Unknown*
6	0.35	220.2	0.39	248.1	9.00	0.42	226.0	9126.4	8.55	Unknown*
7	0.42	226.0	0.43	228.7	8.30	0.46	211.7	5997.6	5.62	Unknown*
8	0.46	211.8	0.48	223.4	8.11	0.50	206.0	6010.5	5.63	Unknown*
9	0.51	206.6	0.53	211.7	7.68	0.56	192.9	7160.0	6.70	Unknown*
10	0.56	193.2	0.59	199.5	7.24	0.69	157.2	14146.3	13.25	Unknown*
11	0.69	157.3	0.73	195.5	7.08	0.77	139.6	8661.0	8.11	Unknown*
12	0.77	139.7	0.82	211.6	7.68	0.88	97.8	10339.1	9.68	Unknown*
13	0.88	97.9	0.90	102.0	3.70	1.00	38.2	5101.5	4.78	Unknown*
14	1.00	38.2	1.00	40.9	1.48	1.07	2.3	882.3	0.83	Unknown*

Table 7: HPTLC peak values of methanolic extract of *S. glauca* leaf.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.05	5.1	0.00	129.9	3.92	0.02	5.0	3046.3	2.15	Unknown*
2	0.03	3.2	0.08	264.7	7.99	0.09	259.0	7341.6	5.17	Unknown*
3	0.10	258.4	0.13	326.3	9.85	0.16	257.9	11028.0	7.77	Unknown*
4	0.16	258.2	0.20	332.7	10.04	0.25	243.2	14971.0	10.55	Unknown*
5	0.25	243.4	0.30	288.2	8.70	0.35	241.7	17109.0	12.06	Unknown*
6	0.35	241.7	0.39	256.0	7.73	0.43	229.5	12182.5	8.58	Unknown*
7	0.43	229.6	0.44	232.2	7.01	0.47	218.4	5433.3	3.83	Unknown*
8	0.47	218.5	0.48	219.5	6.62	0.51	190.6	5559.6	3.92	Unknown*
9	0.51	190.8	0.53	200.5	6.05	0.59	163.4	9017.0	6.35	Unknown*
10	0.59	163.5	0.67	232.7	7.02	0.68	229.1	11968.6	8.43	Unknown*
11	0.69	229.3	0.74	377.6	11.39	0.79	233.7	19985.8	14.08	Unknown*
12	0.79	234.0	0.83	408.3	12.32	0.99	42.5	23279.5	16.40	Unknown*
13	1.00	42.8	1.00	44.8	1.35	1.07	5.8	986.2	0.69	Unknown*

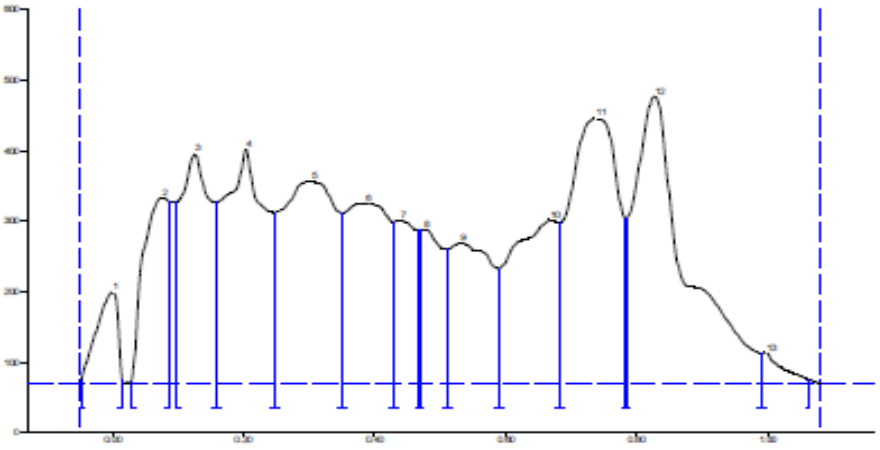
Table 8: HPTLC peak values of methanolic extract of *S. glauca* stem.

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	-0.05	1.6	0.01	55.1	2.24	0.03	0.6	1389.0	2.02	Unknown*
2	0.03	0.2	0.05	118.4	4.80	0.07	51.5	1650.2	2.40	Unknown*
3	0.07	51.6	0.09	78.4	3.18	0.11	65.0	1623.9	2.36	Unknown*
4	0.11	65.5	0.15	84.0	3.41	0.18	62.5	3021.5	4.39	Unknown*
5	0.18	62.8	0.21	91.4	3.71	0.22	85.5	2252.3	3.28	Unknown*
6	0.22	85.8	0.24	97.5	3.96	0.25	96.2	1689.5	2.46	Unknown*
7	0.25	96.2	0.28	121.2	4.92	0.30	100.8	3609.0	5.25	Unknown*
8	0.30	100.8	0.33	136.4	5.53	0.35	123.1	3729.3	5.42	Unknown*
9	0.37	123.6	0.40	182.4	7.40	0.42	129.6	4601.5	6.69	Unknown*
10	0.42	129.7	0.44	158.9	6.45	0.45	148.8	3130.6	4.55	Unknown*
11	0.45	150.0	0.46	156.9	6.37	0.48	61.5	1893.6	2.75	Unknown*
12	0.48	62.5	0.49	130.3	5.28	0.50	100.5	1862.0	2.71	Unknown*
13	0.51	100.8	0.54	155.9	6.33	0.60	103.9	7286.0	10.59	Unknown*
14	0.60	103.9	0.63	114.7	4.65	0.63	114.5	2216.8	3.22	Unknown*
15	0.64	114.2	0.68	565.2	22.93	0.78	80.2	19369.4	28.16	Unknown*
16	0.78	80.4	0.82	114.1	4.63	0.87	70.4	5300.8	7.71	Unknown*
17	0.87	70.5	0.89	74.3	3.01	0.99	25.1	3467.1	5.04	Unknown*
18	0.99	25.2	1.00	29.8	1.21	1.07	1.1	679.3	0.99	Unknown*



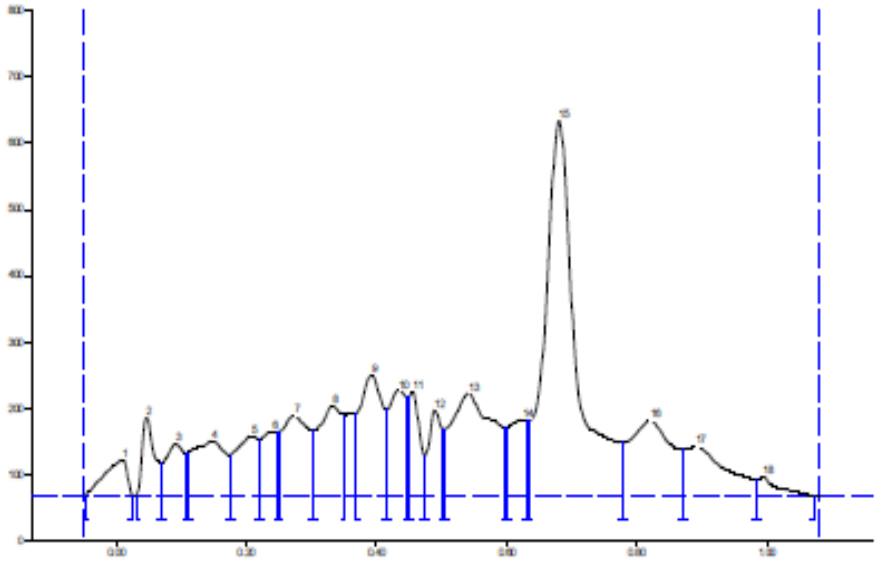
Track 3 ID: *S. glauca* bark extract

Figure 1: HPTLC densitograph of methanolic extract of *Simarouba glauca* bark showing different peaks of phytoconstituents.



Track 4 ID: *S. glauca* leaf extract

Figure 2: HPTLC densitograph of methanolic extract of *Simarouba glauca* leaf showing different peaks of phytoconstituents.



Track 6 ID: *S. glauca* stem extract

Figure 3: HPTLC densitograph of methanolic extract of *Simarouba glauca* stem showing different peaks of phytoconstituents.

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