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A Novel Fingerprinting Method for Glycosides in Nakshatra Plants by HPTLC

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Abstract

Nakshatra plants are medicinally important and used from olden days onwards to treat various diseases. In the present study, a HPTLC method was developed for separation of glycosides in plants. The powdered drug of Nakshatra plant was extracted with ethanol and identification was done chemically by Molisch's, Fehling's, Bial's test and separation of glycosides was performed densitometrically at λ=254-700 nm using as a mobile phase, ethyl acetate: methanol: water (20:2.5:2.5). The results of qualitative analysis revealed the presence of carbohydrates, reducing sugars and pentose. HPTLC method achieved a good separation of glycosides at 254 nm, 304 nm, 354 nm, 404 nm with recorded peak areas and R_f values. The comparative common compound of sample glycosides was also done.

Keywords: Glycosides; HPTLC; Mobile phase; Nakshatra plants

Introduction

Nearly 80% of global population depends on plant derived medicines [1]. There has been a public demand for plant based health products in developed and developing countries [2] and 50% of modern clinical drugs originate from natural products [3]. Plant secondary metabolites (PSMs), the constituents of plant kingdom constituents, are the most widely distributed heterogenous group of substances. Among PSMs is, glycosides play numerous important roles in living organisms [4]. Glycosides are widely used in the manufacture of several products/ drugs by and also pharmaceutical industries for other purposes such as flavouring agents [5]. A large number of medicinal plants are now widely used all over the world for production of glycosidic compounds as both traditional and modern drugs and development of new drugs. These are formed by glycosylation of secondary metabolites. It is reported that species of Nakshtra plants containing different glycosidic compounds play an important role in ayurveda and are useful to different purposes in life [6]. Studies on Nakshtra plants for identification and classification of compounds having aldehyde groups present in plants as secondary metabolites revealed that these phytocompounds serve as building blocks in the synthesis of several commercially important products [7]. It is well known that human beings are always dominated by the presence of sun, moon and other planetary structures which play a vital role in Astrology and Nakshtra plants were named after respective planets and these plant species possess medicinal properties for treating various diseases [8,9]. Moreover, each plant of this group is believed to exert positive effect on the nature of human being thus increasing the positive energy level of that place and thus gives the vaastu balancing effect of that place [10]. These plant species were arranged according to Bentham and Hooker system of classification and reported from the flora of the Gujarat state in India [9].

Phytochemical evaluation was done in many of these plants to know moieties of flavonoids, terpenoids, phenolics and other alkaloids [11]. Available literature revealed that different glycosides from these plants may possess good therapeutic applications [12]. However, very limited information is available on glycosides in Nakshtra plants. In routine TLC (Thin layer chromatography) testing, the detection is done by spray method and the R_f value is not accurate [13]. Hence, this study is designed to identify glycosides present in these plants and to isolate glycosides from leaves of various Nakshtra groups of plants using HPTLC.

Materials and Methods

Materials

The leaves of Nakshatra plants growing in Anantapur district of Andhra Pradesh state in India were collected. The chemicals used for extraction, testing and chromatography were of analytical grade. The solutions and reagents were prepared using distilled water. TLC plates were purchased from MERCK (25 TLC aluminum sheets 20 × 20 cm Silica gel 60 F 254).

Methods

Collection of plant leaves and extraction: The freshly collected leaves were cleaned under water and dried for 4 to 8 days at room temperature and powdered using a mixer-grinder. The powdered sample (3 gm) or test was extracted with 40 mL of 70% EtOH on a rotary shaker (120 thaws / min) for 8 hours. Five mL of 70% lead acetate was added to the filtrate and centrifuged at 5000 rpm for 10 min. The supernatant was further centrifuged by adding 3 mL of 6.3% Na_2CO_3 at 10000 rpm for 10 min (approximately 3 mL of Na_2CO_3 for 20 mL of filtrate). The retained supernatant was dried and redissolved in 4 mL of chloroform. The residue dissolved in chloroform was tested for presence of carbohydrates. Testing for carbohydrates was carried out by Molisch's, Fehling's and Bial's tests [14].

Separation of glycosides by HPTLC

Sample application: Sample solutions for HPTLC analysis were applied by means of a CAMG Linomat V automated with 6 mm. The glycosides were separated using EtOAc-MeOH-H_2O (20:2.5:2.5) as the mobile phase. After chromatograph development, the zones were quantified by linear scanning at 254 to 700 nm by the increment of

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50 nm with a CAMAG TLC scanner 3 with a deuterium source in the resolution mode 100 µm for step, slit dimension settings of length 5.00 and width 0.45 mm Micro and scanning speed 20 mm/s. The peak areas and Rf values were determined using CATS TLC software (version 4.x, CAMAG TLC, Software) [5,15].

Instrumentation

HPTLC Instrument	CAMAG Company
Sample Application Instrument:	Linomat V
Syringe Volume	100 µL
HPTLC plate Size	10 ×10 cm plate
Sample application volume	5 µL
Band length	6 mm
Distance between bands	10 mm
Chromatography development: Chamber	20 × 10 cm
Mobile Phase	EtOAc-MeOH-H_2O (20: 2.5: 2.5)
Saturation Period	60 min
Scanner	Scanner III
Wave length	254-700 nm
Wave length increment	50 nm
Scanning Speed	20 mm/s
Slit dimension	5.00 × 0.45 mm Micro
Resolution	100 um/step
Determination	CATS TLC software

Results and Discussion

The chemical structure of secondary plant product is glycosylated to form glycosides without exception more complex than that of primary products [16]. In nature glycosides are formed by interaction of nucleotide glycosides with alcohol, phenol, steroid, triterpenoid and flavonoids etc. and joined by glycosidic linkage [17]. These are present in flowers, fruit pigments and leaves show their action as anti-inflammatory, anti-diarrheal, anti-allergic, anti-thrombotic, anti tumour [4]. HPTLC is the widely used technique for the identification of botanical glycosides from plants. HPTLC has many advantages like multiple separations and detection procedures [6]. Hence, it is useful in qualitative analysis of plant products. For making green society, our chromatographers now prefer to develop environmental friendly, useful chromatographic systems for analysis. HPTLC is a powerful technique used for detection and potential quantitation of drugs and compounds in clinical samples. Nowadays, the interest in study of natural product is growing rapidly, especially as a part of drug discovery programs [18]. The results of screening of the carbohydrate in Nakshatra plants are presented in Table 1. Different tests conducted with the samples revealed the presence of carbohydrates (all), reducing sugars (Amala, Jamun, Khair, Pimpal, Palas, Roal, Rui) and pentoses (Jamun, Palas, Arjun, Bakul, Savar, Kadamba, Neem) in moderate concentrations in Nakshatra plants. Further, glycosides were also noticed. It was concluded that the Nakshatra plants have secondary metabolite of glycosides of different groups. They are reported to be effective in reducing blood lipid, in assimilating cholesterol, inhibiting thrombosis, dilating the coronary artery, and as antioxidatives etc. Different groups of glycosides present in the plant extracts were separated by High Performance TLC. The pinkish violet colour confirmed the presence of glycosides in the given samples. From the results obtained after densitometric scanning, good separation was observed at wavelengths of 254 nm, 304 nm, 354 nm, 404 nm were shown in Table 2. Vet shows 9 peaks at 254 nm, similarly mango shows 6 peaks at 254 nm, savar and arjun shows 9 peaks at 404 nm. Payar, pimpal, umber, jai and palas shows 8 peaks at 404 nm phanas shows 4 peaks at 404 nm, only roal shows 8 peaks at 354 nm, kadam, nagakeshar and amla show 5 peaks at 304 nm while jamun, neem, beal and moha 4 peaks at 304 nm, khair, rui, vad and chandan show 3 peaks and velu and shami show 2 peaks at 304 nm in Table 3. The corresponding HPTLC densitograms of Nakshatra plants were represented in Figure 1a-1x. The most appropriate mobile phase used for the separation is EtOAc-MeOH-H_2O (20: 2.5: 2.5). The concentration of glycosides was also assessed by determining the percent area and Rf values which are shown in Table 3. The peaks obtained from Nakshtra plant extracts when used for comparative analysis revealed that the Rf 0.83 is common for found in the extracts of prayer, pimple, jai, umber, neem and Velu as shown in Figure 2. Similarly, another glycoside with Rf value of 0.82 is common by foumnd in arjun, roal, mango, nagakeshar and shami represented in Figure 3. Thus, present HPTLC studies confirmed the presence of active glycosides in the ethanolic extract of Nakshatra plant species. The active glycosides combine with proteins to form glycoproteins, with lipids to form glycolipids which involve are in membrane formations, transportation and signal transduction and hormonal regulation of several biological functions.

Conclusion

A simple, precise, accurate and reproducible HPTLC method was successfully developed for analysis of medicinal products in Nakshatra plants containing glycosides. This method enabled us to detect and quantify the number of glycosides in various medicinal products such as extracts and formulations. The method can be applied by pharmacists to estimate glycosides in their products as a routine control method and also to keep a check on the variations from batch to batch. The ethanol extract of Nakshatra plants containing glycosides is found to be better when compared to extraction with other solvents and can be used for further formulations. From the HPTLC studies, it has been found that the mixture of ethyl acetate, methanol and water used as mobile phase contains not a single compound but a mixture of compounds confirming that the pharmacological activity shown by them is due to the cumulative effect of all the compounds in composite

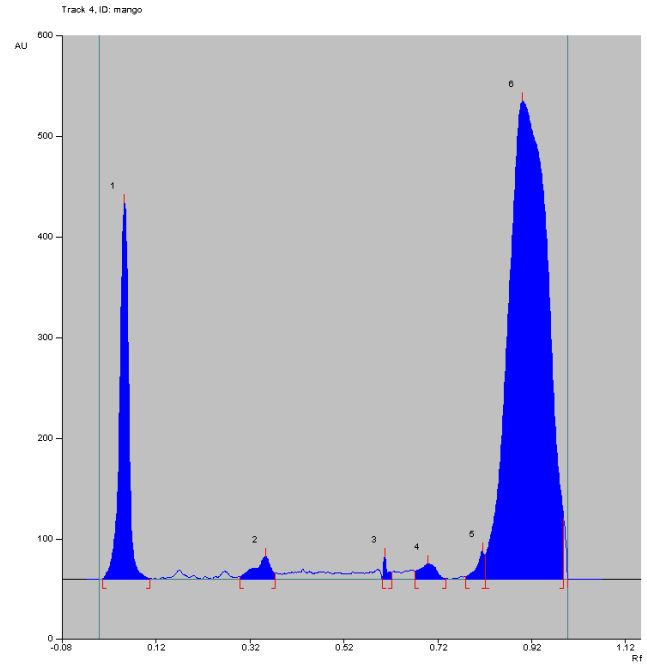

Figure 1a: Densitogram obtained from mango.

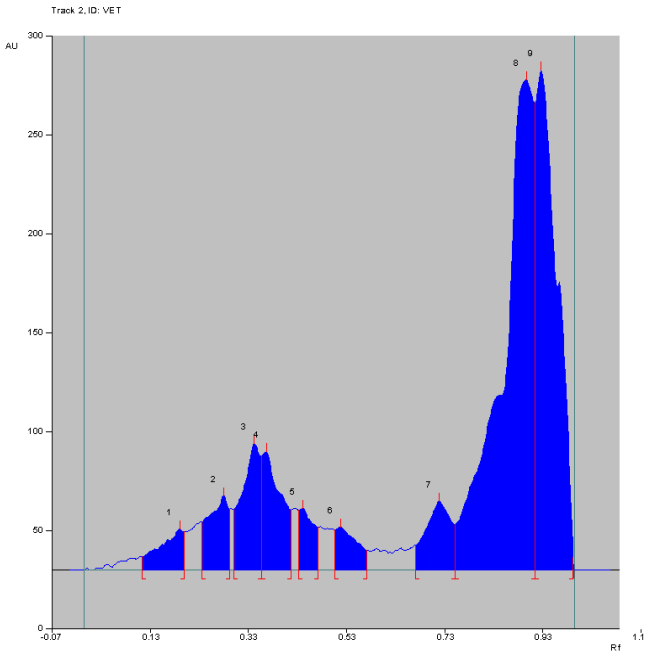

Figure 1b: Densitogram obtained from Vet.

S. No.	Nakshatra	Botanical Name	Common Name	Family	Molish's Test	Fehling's Test	Bial's Test
1	Aswini	*Strychnus nuxvomica*	Kuchla, Kajara	*Loganiaceae*	+	+	Nil
2	Bharani	*Emblica officinalis*	Aamla	*Euphorbiaceae*	+	++	Nil
3	Krutika	*Ficus glomeata*	Umber	*Articaceae*	+	+	Nil
4	Rohini	*Eugenia jambolana*	Jamun	*Myrtaceae*	+	++	+
5	Mruga Shirsha	*Acacia catechu*	Khair	*Mimosaceae*	+	++	Nil
6	Ardraa	*Santalum album*	Chandan	*Santalaceae*	+	+	Nil
7	Punarvasu	*Bambus aerandinasia*	Velu	*Graminae*	+	+	Nil
8	Pushya	*Ficus religiosa*	Pimpal	*Articaceae*	+	++	Nil
9	Ashiesha	*Messu aferrea*	Naagkeshar	*Guttiferae*	+	+	Nil
10	Magha	*Ficus bengalensis*	Vad	*Articacea*	+	+	Nil
11	Purvaphalaguni	*Butea frondosa*	Palas	*Fabaceae*	+	++	+
12	Uttaraphalguni	*Ficus infectoria*	Payar	*Articaceae*	+	+	Nil
13	Hasta	*Jasminum auriculatum*	Jaai	*Oleaceae*	+	+	Nil
14	Chitra	*Aegle marmalos*	Bael	*Rutaceae*	+	+	Nil
15	Swati	*Terminalia arjuna*	Arjun	*Combretaceae*	+	+	+
16	Vishakha	*Adhatoda vasica*	Elephant apple	*Guttiferae*	+	+	Nil
17	Anuradha	*Mimus opselengi*	Bakul	*Sapotaceae*	+	+	+
18	Jeshta	*Salmaliam albarica*	Saavar	*Malvaceae*	+	+	+
19	Mul	*Veteria indica*	Roal	*Dipterocarpaceae*	+	++	Nil
20	Purvaashadha	*Calamus roteng*	Vet	*Palmae*	+	+	Nil
21	Uttaraashadha	*Artocarpus integrifolia*	Phanas	*Articaceae*	+	+	Nil
22	Shravan	*Calotropis gigantea*	Rui	*Asclepediaceae*	+	++	Nil
23	Dhanishta	*Prosopis spicigera*	Shami	*Mimosaceae*	+	+	Nil
24	Shattaraka	*Mitrigyna parvifolia*	Kadamba	*Rubiceae*	+	+	+
25	Purvabhadrapada	*Mangifera indica*	Amba	*Anacardiaceae*	+	+	Nil
26	Uttarabhadrapad	*Azadirachta indica*	Neem	*Meliaceae*	+	+	+
27	Revati	*Madhuca indica*	Moha	*Sapotaceae*	+	+	Nil

Note: += indicates the presence

Table 1: Qualitative tests for carbohydrates in Nakshatra plants.

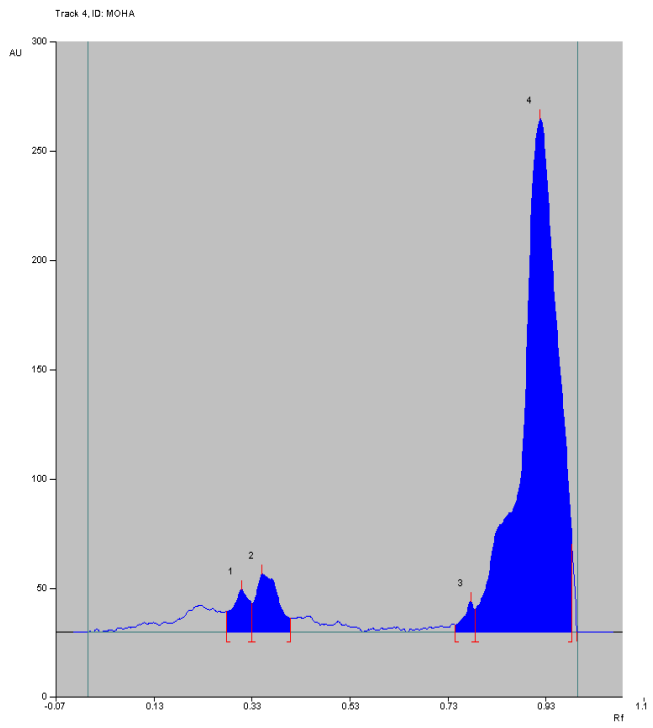

Figure 1c: Densitogram obtained from Moha.

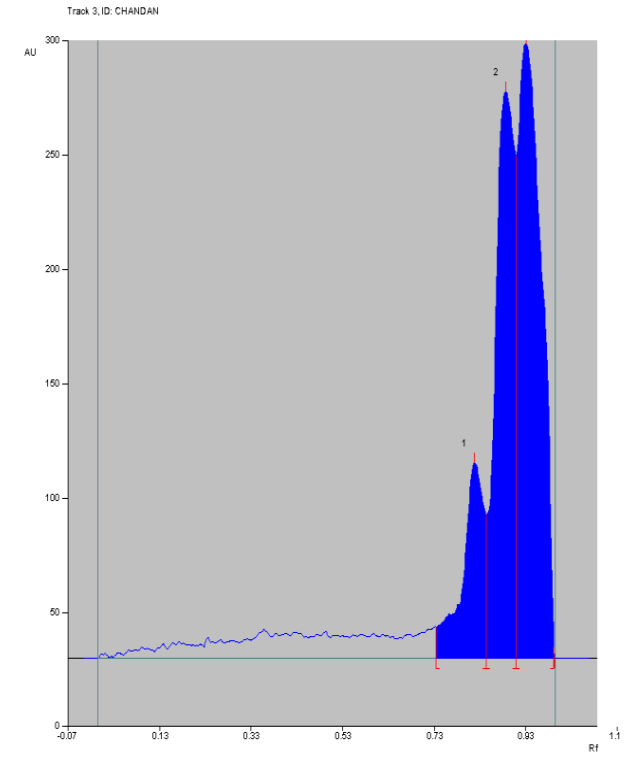

Figure 1e: Densitogram obtained from Chandan.

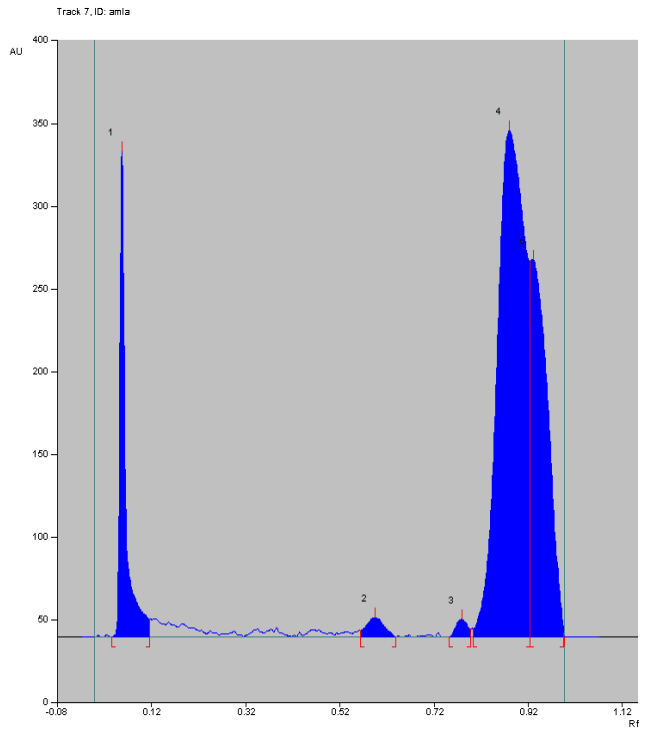

Figure 1d: Densitogram obtained from Amla.

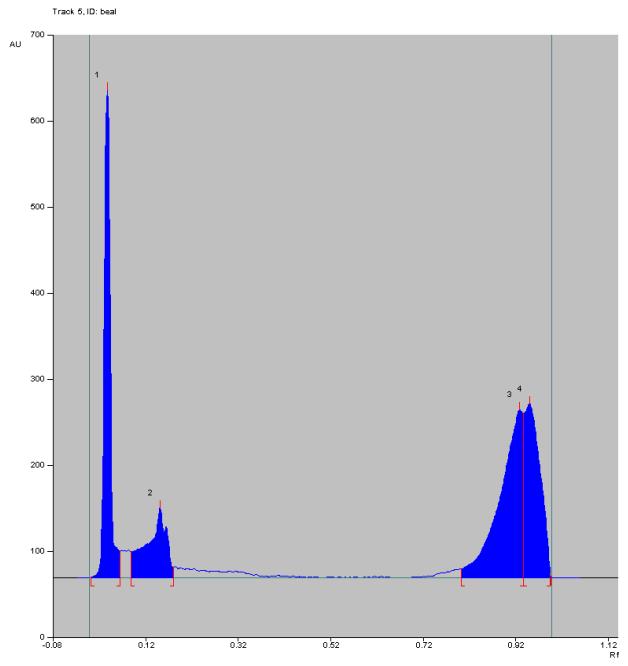

Figure 1f: Densitogram obtained from Beal.

Botanical Name	Hindi/Marathi Name	UV-Visible at 254-700 nm
Mangifera indica	Amba	254
Calamus roteng	Vet	254
Madhuca indica	Moha	304
Bambusa erandinasia	Velu	304
Santalum album	Chandan	304
Messua ferrea	Naagkeshar	304
Ficus bengalensis	Vad	304
Azadirachta indica	Neem	304
Calotropis gigantea	Rui	304
Aegle marmalos	Beal	304
Eugenia jambolana	Jamun	304
Emblica officinalis	Aamla	304
Prosopis spicigera	Shami	304
Acacia catechu	Khair	304
Mitrigyna parvifolia	Kadamba	304
Mimusops elengi	Bakul	304
Veteria indica	Roal	354
Artocarpus integrifolia	Phanas	404
Terminalia arjuna	Arjun	404
Ficus infectoria	Payar	404
Butea frondosa	Palas	404
Ficus religiosa	Pimpal	404
Strychnus nuxvomica	Kuchla, Kajara	404
Salmalia malbarica	Saavar	404
Jasminum auriculatum	Jaai	404
Ficus glomeata	Umber	404

Table 2: Optimum UV-Vis ranges (254 to 700 nm) for separation of glycosides in Nakshatra plants.

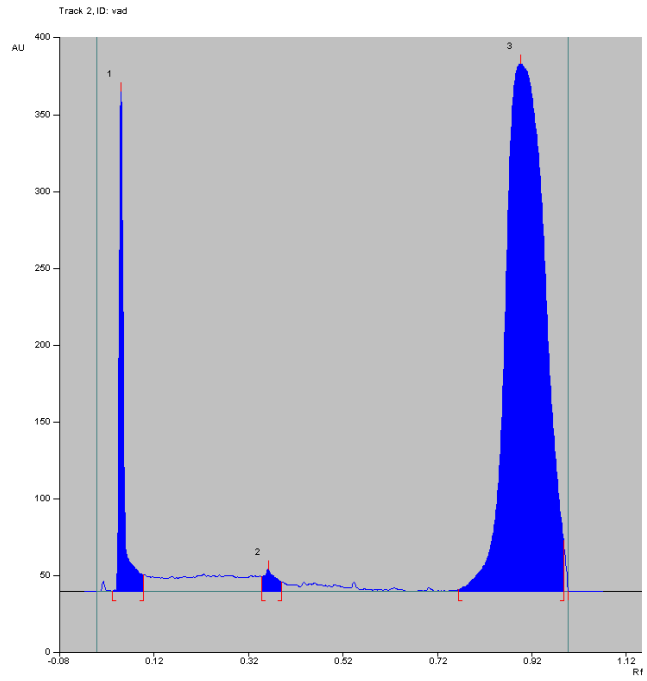

Figure 1g: Densitogram obtained from Vad.

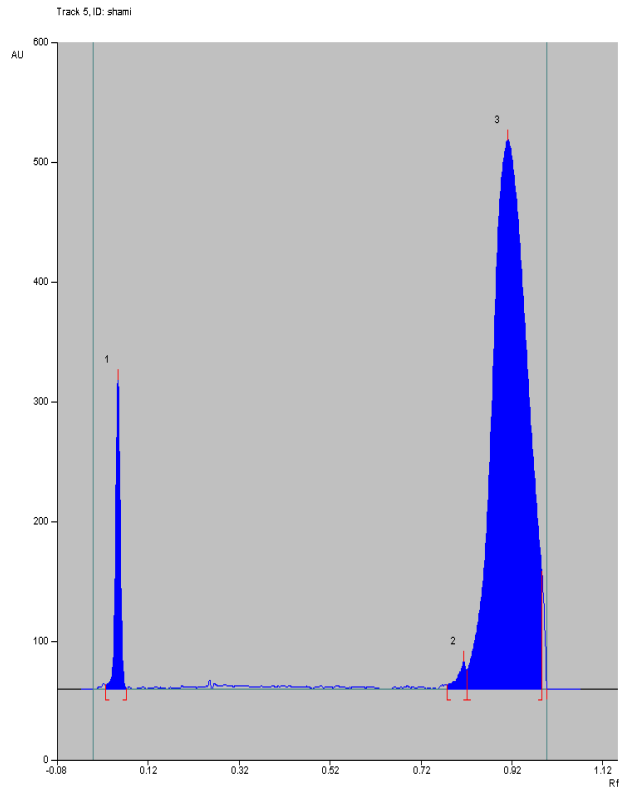

Figure 1h: Densitogram obtained from Shami.

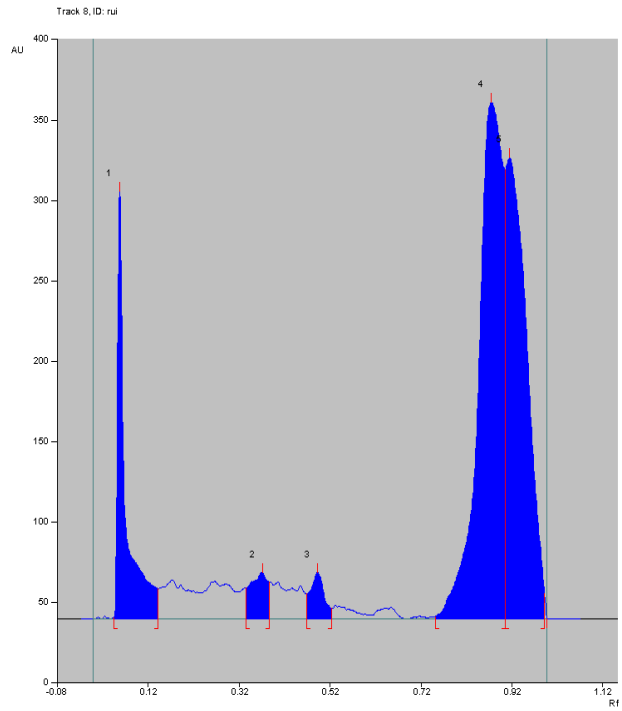

Figure 1i: Densitogram obtained from Rui.

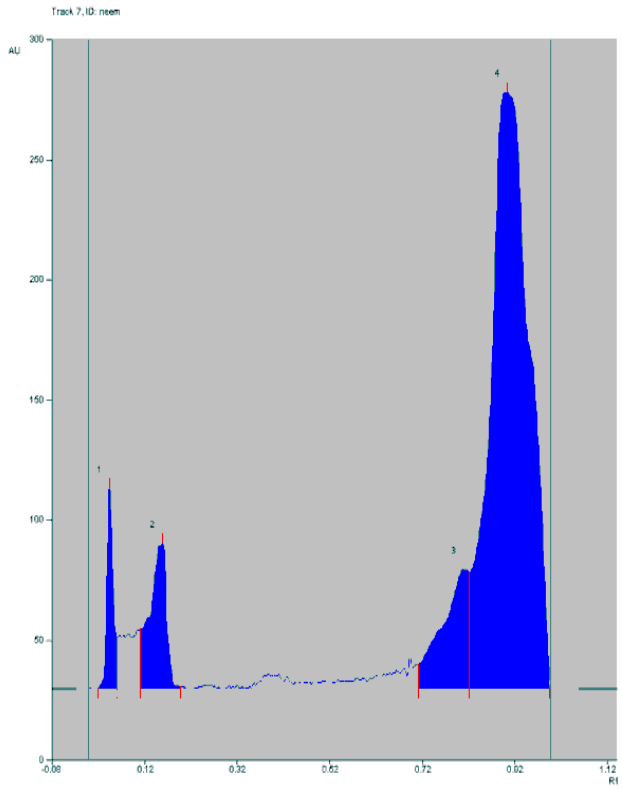

Figure 1j: Densitogram obtained from Neem.

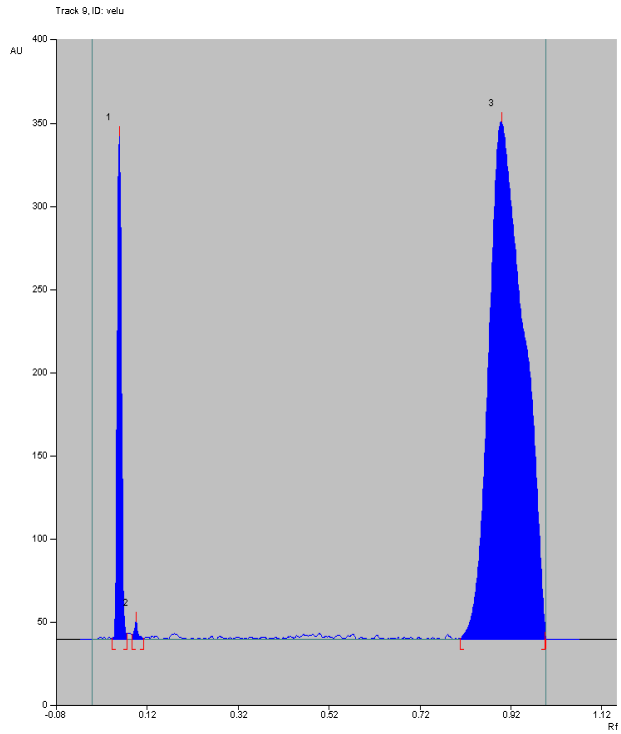

Figure 1l: Densitogram obtained from Velu.

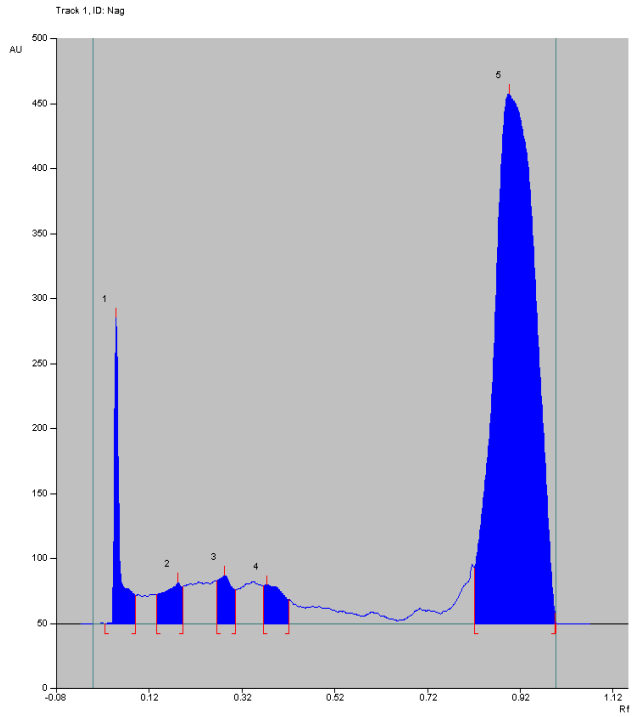

Figure 1k: Densitogram obtained from Nag.

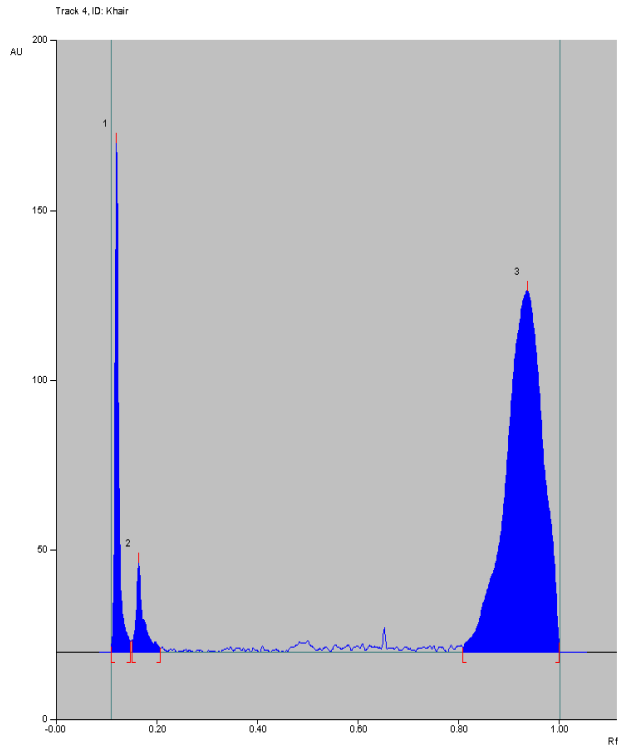

Figure 1m: Densitogram obtained from Khair.

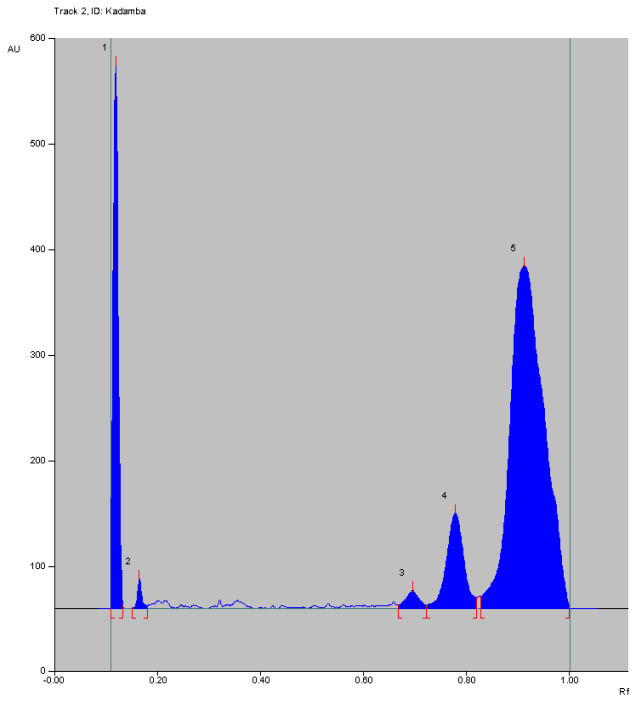

Figure 1n: Densitogram obtained from Kadamba.

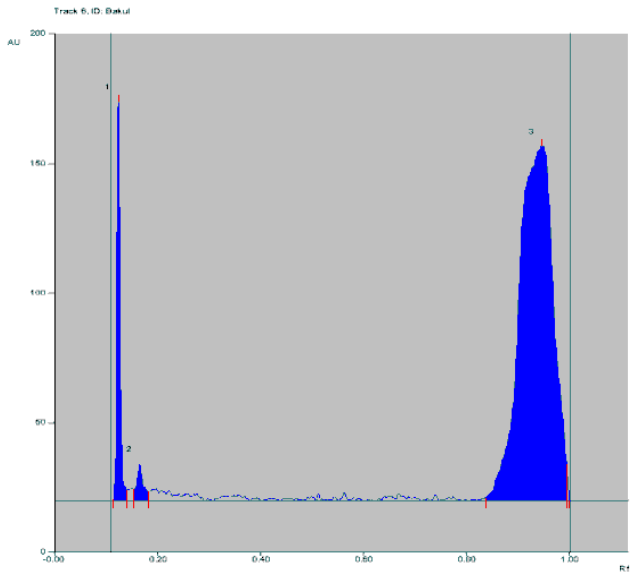

Figure 1o: Densitogram obtained from Bakul.

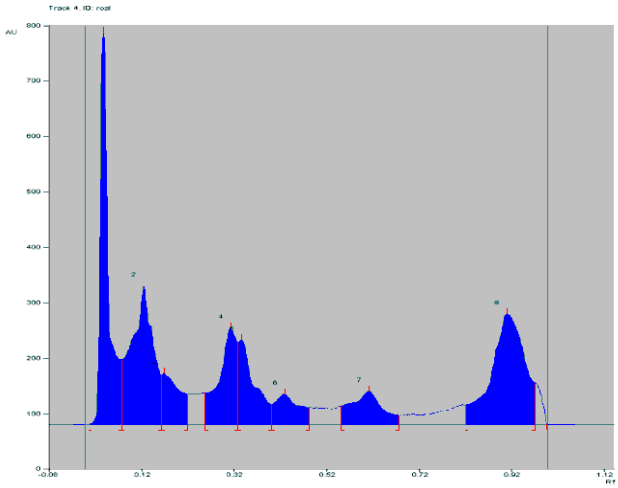

Figure 1p: Densitogram obtained from Roal

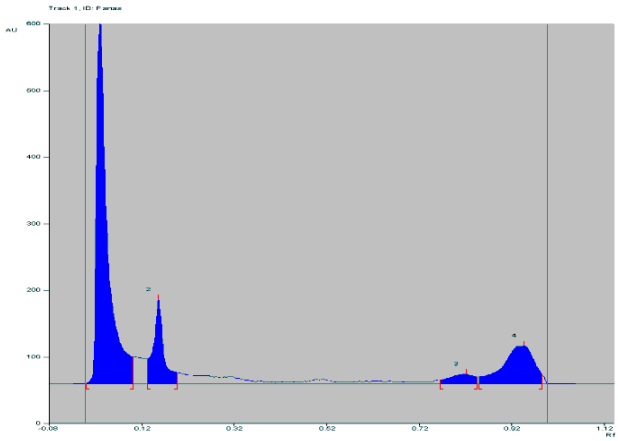

Figure 1q: Densitogram obtained from Panas.

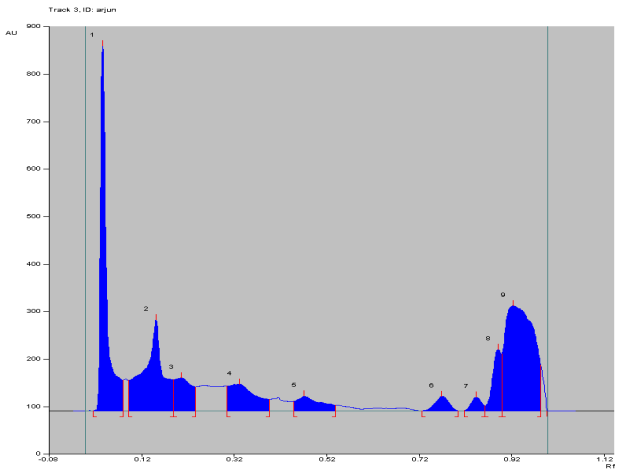

Figure 1r: Densitogram obtained from Arjun.

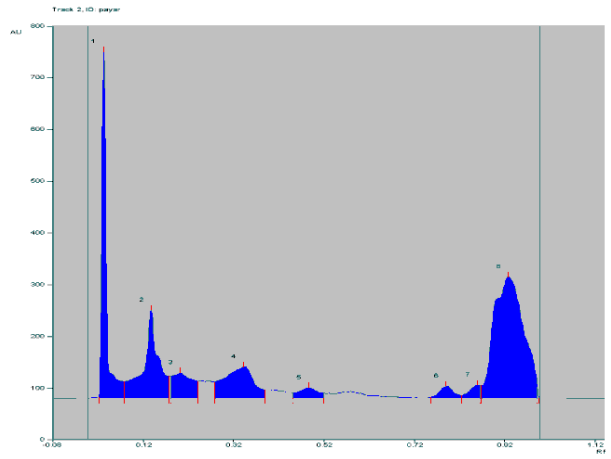

Figure 1s: Densitogram obtained from Payar.

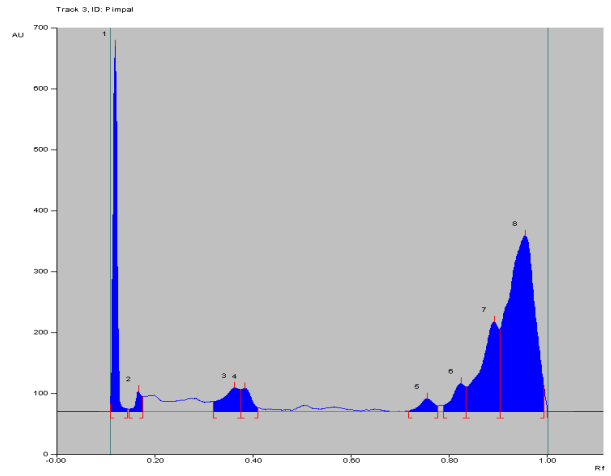

Figure 1t: Densitogram obtained from Pimpal.

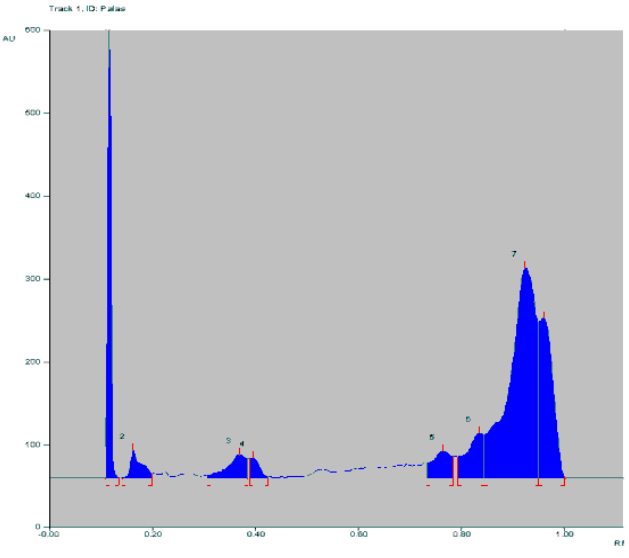

Figure 1u: Densitogram obtained from Palas.

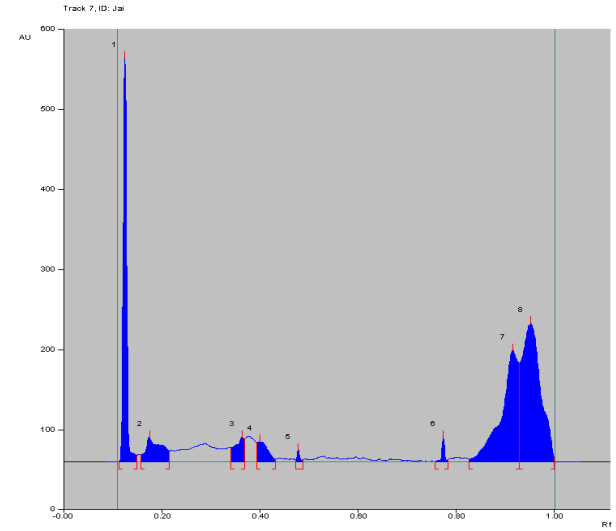

Figure 1v: Densitogram obtained from Jai.

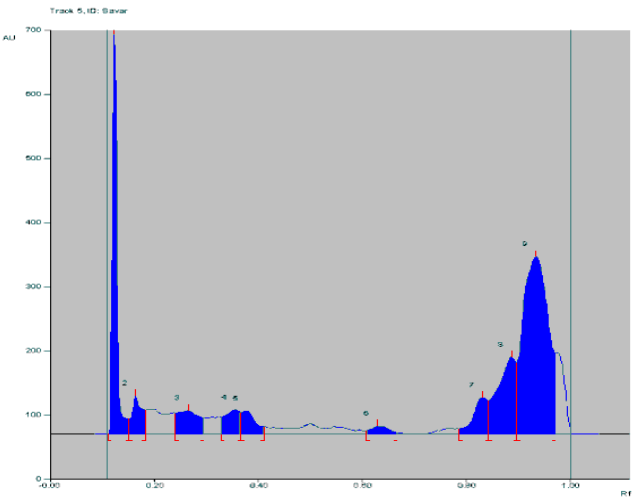

Figure 1w: Densitogram obtained from Savar.

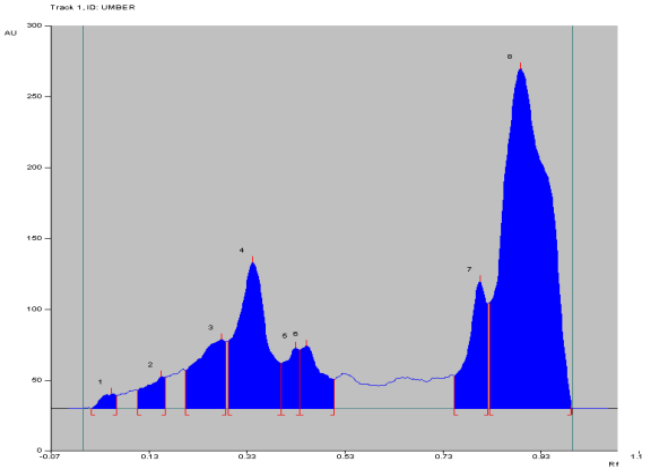

Figure 1x: Densitogram obtained from Umber.

Plant name and Wave length (nm)	Peak	Start R_f	End R_f	Area	Area%
Mango at 254					
	1	0.01	0.11	6882.2	14.74
	2	0.30	0.38	710.6	1.57
	3	0.60	0.62	163.4	0.36
	4	0.67	0.74	510.4	1.13
	5	0.78	0.82	437.0	0.96
	6	0.82	0.99	36820.6	81.24
Vet at 254					
	1	0.12	0.20	806.9	2.63
	2	0.24	0.30	1214.8	3.97
	3	0.31	0.36	1929.3	6.30
	4	0.36	0.42	1927.1	6.29
	5	0.44	0.48	748.5	2.44
	6	0.51	0.58	770.3	2.52
	7	0.68	0.76	1408.6	4.60
	8	0.76	0.92	12886.3	42.08
	9	0.92	1.00	8933.1	29.17
Moha at 304					
	1	0.28	0.34	528.8	3.41
	2	0.34	0.41	978.8	6.32
	3	0.75	0.79	239.5	1.55
	4	0.79	0.99	13743.4	88.72
Amla at 304					
	1	0.04	012	3439.6	11.74
	2	0.57	0.64	424.8	1.45
	3	0.76	0.80	258.7	0.86
	4	0.81	0.93	17205.8	58.70
	5	0.93	1.00	7987.3	27.25
Chandan at 304					
	1	0.74	0.85	3281.1	14.39
	2	0.85	0.92	8392.2	36.81
	3	0.92	1.00	11126.8	48.80
Beal at 304					
	1	0.00	0.07	7279.0	27.71
	2	0.09	0.18	3205.2	12.20
	3	0.81	0.94	9046.8	34.44
	4	0.94	1.00	6740.2	25.66
Vad at 304					
	1	0.03	0.1	2938.5	9.87
	2	0.35	0.39	350.9	1.18
	3	0.77	0.99	26475.9	88.96
Shami at 304					
	1	0.05	0.07	300.9	1.1
	2	0.82	0.99	27171.9	98.9
Rui at 304					
	1	0.01	0.10	4517.4	12.05
	2	0.79	0.90	11842.2	31.58
	3	0.90	1.00	21144.5	56.38
Neem at 304					
	1	0.02	0.06	1095.2	4.22
	2	0.11	0.20	2095.5	8.08
	3	0.72	0.83	2696.8	10.40
	4	0.83	1.00	20037.9	77.29
Naagkeshar at 304					
	1	0.03	0.09	2508.9	6.3
	2	0.04	0.19	12209.3	3.09
	3	0.27	0.81	1084.8	2.72
	4	0.37	0.42	1184.4	2.96
	5	0.82	1.0	33841.2	84.93
Jamun at 304					
	1	0.03	0.11	4142.5	18.24
	2	0.12	0.18	1195.9	5.26
	3	0.76	0.83	1008.6	4.44
	4	0.83	1.00	16369.2	72.06
Velu at 304					
	1	0.01	0.11	5367.3	16.01
	2	0.83	1.00	28153.5	83.99
Khair at 304					
	1	0.4	0.15	1163.4	12.00
	2	0.15	0.21	402.0	4.25
	3	0.81	1.00	8128.4	83.85
Kadam at 304					
	1	0.11	0.15	3222.0	12.62
	2	0.15	0.18	624.5	2.95
	3	0.18	0.23	502.0	1.97
	4	0.74	0.80	1210.9	4.74
	5	0.81	1.00	19973.6	78.23
Bakul at 304					
	1	0.4	0.14	980.5	9.23
	2	0.15	0.18	183.8	1.73
	3	0.84	0.99	9456.5	89.04
Roal at 354					
	1	0.01	0.08	11996.4	20.56
	2	0.08	0.17	11250.7	19.28
	3	0.17	0.22	3478.5	5.96
	4	0.26	00.33	6403.1	10.97
	5	0.33	0.40	5095.6	8.73
	6	0.40	0.49	2722.6	4.77
	7	0.55	0.68	3837.1	6.58
	8	0.82	0.97	13503.8	23.14
Phanas at 404					
	1	0.00	0.11	12403.3	63.76
	2	0.14	0.20	2641.6	13.62
	3	0.77	0.85	748.8	3.85
	4	0.85	0.99	3551.9	18.77
Arjun at 404					
	1	0.12	0.08	9903.3	23.94
	2	0.09	0.19	7348.1	17.77
	3	0.19	0.24	2550.3	6.17
	4	0.31	0.40	3184.3	7.70
	5	0.45	0.54	1555.4	3.76
	6	0.73	0.81	865.2	2.09
	7	0.82	0.87	612.7	1.48
	8	0.87	0.90	2334.0	5.06
	9	0.90	0.94	13001.7	31.44
Payar at 404					
	1	0.02	0.08	6647.1	19.41
	2	0.08	0.18	5184.4	15.54
	3	0.18	0.24	2082.1	6.08
	4	0.28	0.39	3725.5	10.88
	5	0.45	0.52	821.2	2.40
	6	0.76	0.83	669.2	1.95
	7	0.83	0.87	580.9	1.70
	8	0.87	1.00	14535.8	42.45
Pimpal at 404					
	1	0.11	0.14	4870.7	15.57
	2	0.15	0.17	430.7	1.38
	3	0.32	0.37	1446.3	4.62
	4	0.37	0.41	833.5	2.66
	5	0.72	0.78	621.1	1.99

	6	0.79	0.83	1280.4	4.09
	7	0.83	0.90	5845.4	18.04
	8	0.90	0.99	16159.3	51.65
Palas at 404					
	1	0.11	0.13	3674.1	13.10
	2	0.14	0.20	719.9	2.57
	3	0.30	0.38	1088.8	3.88
	4	0.39	0.42	486.6	1.72
	5	0.73	0.78	1207.6	4.31
	6	0.79	0.84	1866.4	6.65
	7	0.84	0.95	1345.3	48.55
	8	0.95	1.00	5389.8	19.22
Jai at 404					
	1	0.11	0.15	354.7	19.70
	2	0.16	0.21	1030.0	5.73
	3	0.34	0.37	605.7	3.73
	4	0.39	0.43	573.7	3.14
	5	0.47	0.49	89.8	0.50
	6	0.76	0.78	184.9	1.03
	7	0.83	0.93	5068.2	28.20
	8	0.93	1.00	6876.9	38.27
Savar at 404					
	1	0.11	0.15	5594.0	17.96
	2	0.15	0.18	1166.4	3.75
	3	0.24	0.29	1574.3	5.06
	4	0.33	0.36	1190.6	5.82
	5	0.36	0.41	1100.4	3.71
	6	0.61	0.67	444.1	1.43
	7	0.79	0.84	16667.8	5.36
	8	0.84	0.90	4413.9	14.17
	9	0.90	0.97	14089.7	45.24
Umber at 404					
	1	0.02	0.07	261.9	0.85
	2	0.11	0.17	709.6	2.30
	3	0.21	0.29	2300.4	7.47
	4	0.30	0.41	4898.2	15.91
	5	0.41	0.44	1008.2	3.27
	6	0.44	0.51	1594.0	5.18
	7	0.76	0.83	2666.2	8.66
	8	0.83	1.00	17350.2	56.35

Table 3: HPTLC profile of the Nakshatra plants for glycosides.

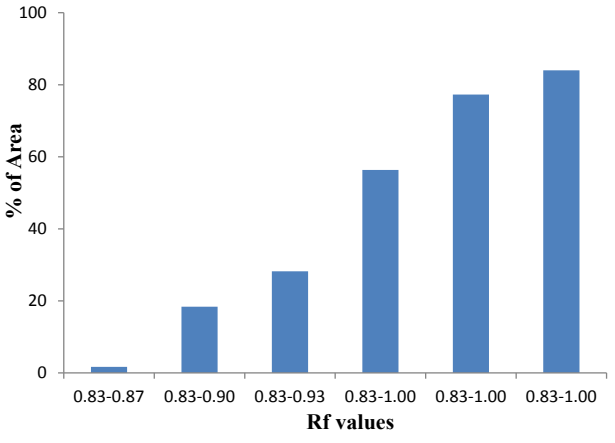

Figure 2: Percentage of Common glycosides present in Payar, Pimpal, Jai, Umber, Neem, Velu plant extracts.

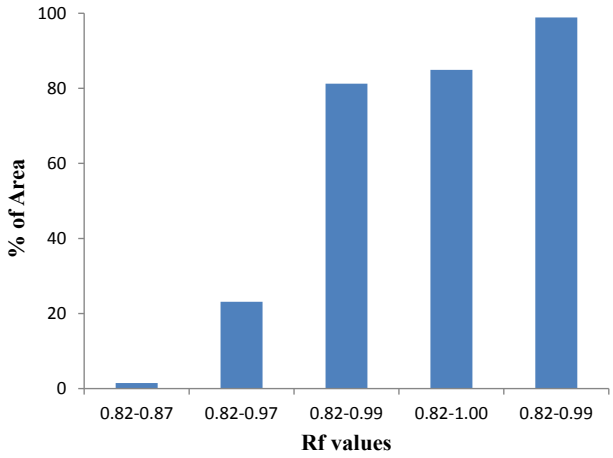

Figure 3: Percentage of Common glycosides present in Arjun, Roal, Mango, Naagakesher, Shami plant extracts.

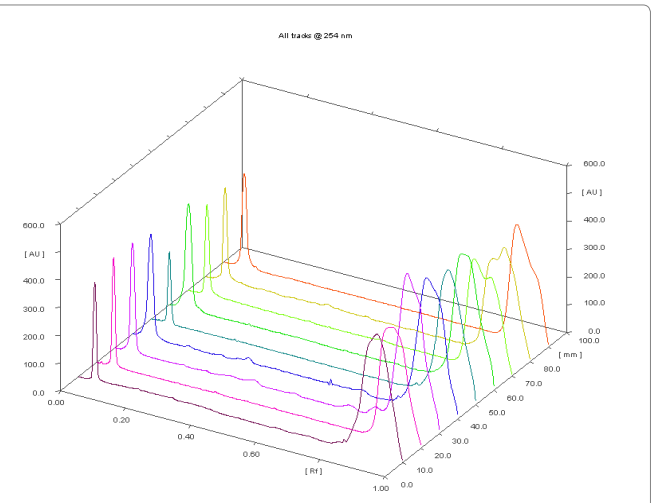

Figure 3a: HPTLC Spectrum of glycosides from Nagkesher, Vad, Jamun, Mango, Shami, Adulsa, Amla, Rui, Velu overlaid with the corresponding peaks at 254 nm.

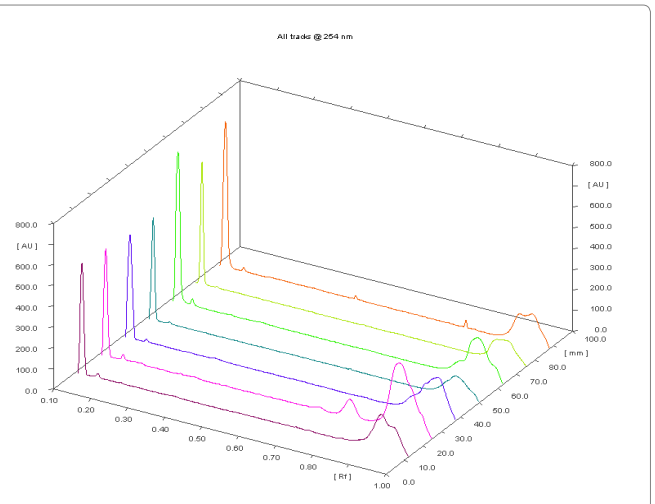

Figure 3b: HPTLC Spectrum of glycosides from Palas, Kadamba, Pimpal, Kair, Savar, Bakul, Jai overlaid with the corresponding peaks at 254 nm.

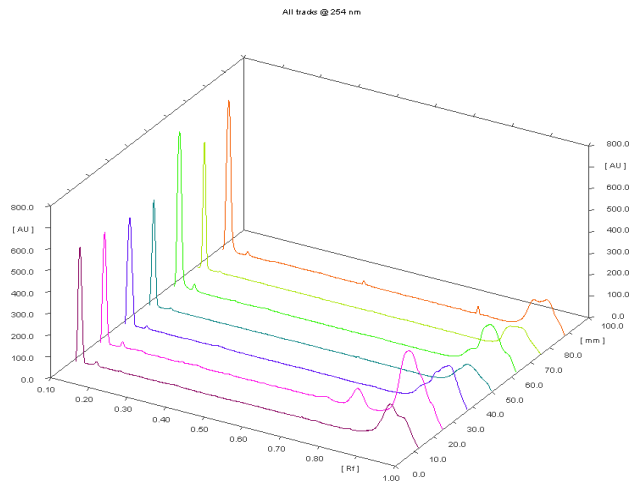

Figure 3c: HPTLC Spectrum of glycosides from Phanas, Payar, Arjun, Roal, Beal, Kuchala, Neem, Jambul overlaid with the corresponding peaks at 254 nm.

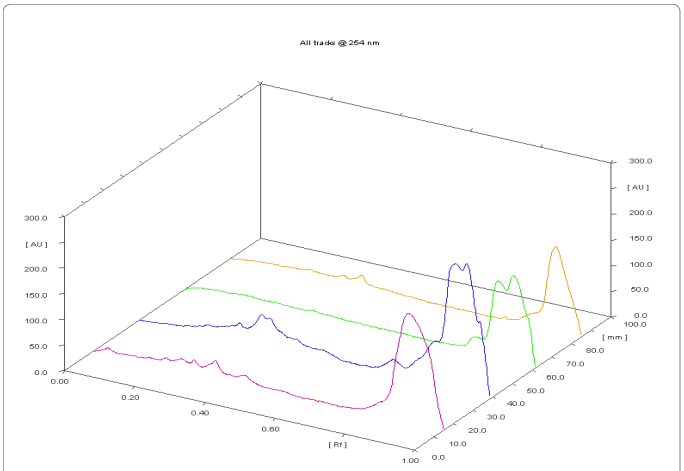

Figure 3e: HPTLC Spectrum of glycosides from Umber, Vet, Chandan, Moha overlaid with the corresponding peaks at 254 nm.

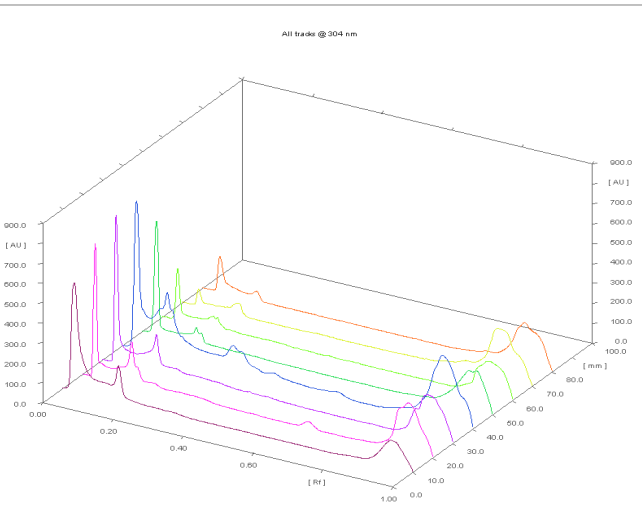

Figure 3d: HPTLC Spectrum of glycosides from Phanas, Payar, Arjun, Roal, Beal, Kuchala, Neem, Jambul overlaid with the corresponding peaks at 304 nm.

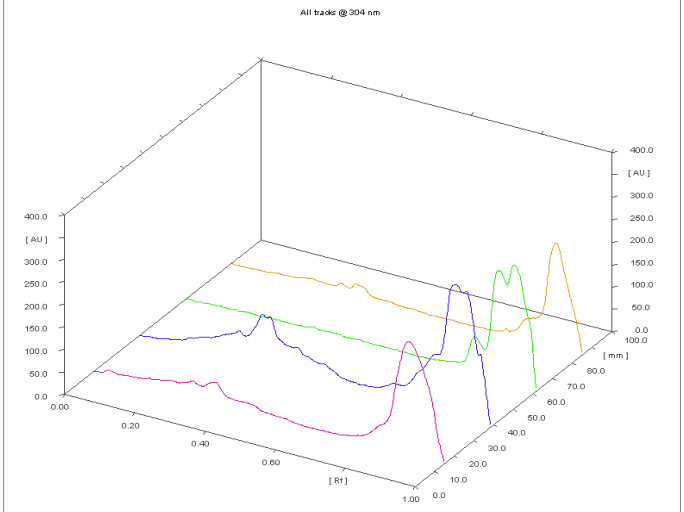

Figure 3f: HPTLC Spectrum of glycosides from Umber, Vet, Chandan, Moha Overlaid with the corresponding peaks at 304 nm.

sample. It is concluded that some glycosides in Nakshatra plants varied in their concentration when compared with other medicinal plants. A comparative HPTLC analysis of nakshtra plant extracts provides a comparative account of the amount of glycosides present in the particular plants. For further study, pure common glycoside compound should be isolated and identified on the basis of reference standards from leaves of Nakshtra plants for assessing its potent pharmacological properties.

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