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HPTLC Fingerprinting of Root Extracts of *Vitis vitigenia* L.

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Mariyan R. Patel[1]*, Jinal D. Solanki[1], Hiteksha S. Panchal[2]

[1] Indukaka Ipcowala pharmacy college, New V.V. Nagar, Anand, Gujarat, India.*

[1]Vidyabharati trust college of Pharmacy, Umrakh, Gujarat, India.

[2]Anand Pharmacy College, Anand, Gujarat, India.

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ABSTRACT

To establish the fingerprint profile of *Vitis vitigenia* using high-performance thin-layer chromatography (HPTLC) technique. HPTLC studies were carried out in three different solvent systems. HPTLC fingerprinting confirmed the presence of the tannins, triterpenoid saponins and flavonoids. Rf value of Gallic acid is 0.37, and both the extract also show the same Rf value from which we can say plants contains the tannins. It can be concluded that different Rf value of various phytochemicals provides valuable clue regarding their polarity and selection of solvents for separation of phytochemicals. The study will help in future for identifying this plant for further research.

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1. INTRODUCTION

The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. The present study intended to find out the active constituents. *Vitis vitigenia* plant is rarely mentioned in published books and there is no data of showing which constituents are present in this plant Qualitative chemical examination of various successive extracts of roots of *Vitis vitigenia* showed presence of triterpenes, carbohydrates, flavonoids and tannins were present. Aqueous and methanolic extracts were both showed presence of tannins, saponins and flavonoids so they can be confirmed by standard gallic acid and rutin by using different analysis techniques. 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. Major advantage of HPTLC is its ability to analyze several samples simultaneously using a small quantity of mobile phase.

2. MATERIALS AND METHODS

2.1. Collection, identification and preparation of plant materials

Plant of *Vitis vitigenia* L. collected from roadside between Umred and Bhiwapur (Maharashtra). The collection was done in the month of January and is authenticated by Dr. B.R. Patel, The Patidar Gin Science College, Bardoli.

2.2. Preparation of Extracts[1-4]

The coarsely powdered roots of *Vitis vitigenia* L. were extracted with 70% v/v alcohol hot percolation method, separately. Aqueous extracts were also prepared by using chloroform water I.P. by maceration process.

a.) Preparation of alcoholic extract

About 200 g of dried powder was extracted with 70% v/v alcohol in a soxhlet extractor. The extraction was continued until the solvent in the thimble became clear. After complete extraction, the extract was filtered and solvent was distilled off in a rotary flash evaporator at 50^0C. The extract was concentrated to dry residue, in a desiccator over anhydrous sodium sulfate.

b.) Preparation of aqueous extract

About 300 g of dried powder was subjected to cold maceration with chloroform water I. P. in a conical flask, for about 7 days at room temperature. The flask was securely plugged with absorbent cotton and was shaken periodically. Then the material was filtered through a muslin cloth and marc was pressed. The filtrate was refiltered through whatman filter paper to get the clear filtrate (free from suspended material). The filtrate was concentrated to dry residue, in a desiccator over anhydrous sodium sulfate.

2.3 HPTLC Profile (High-Performance Thin Layer Chromatography)[5,6]

HPTLC fingerprinting of ethanol extract and water extract of *Vitis vitigenia* L. roots and quantitative determination of Gallic acid, Ellagic acid, and Rutin.

2.3.1 SamplePreparation

Accurately weighed 20 mg of both extracts of *Vitis vitigenia* roots and standard were taken, dissolved in methanol and transferred to a 10 ml volumetric flask. The volume made up to the mark with methanol.

2.3.2 Developing Solvent System

A number of solvent systems were tried, for extract. The satisfactory resolution obtained for the phytochemical constituent tannins was in the solvent Toluene: Ethyl acetate: formic acid (5:5:1); for saponins was in the solvent Chloroform: Glacial acetic acid: Methanol: water (64:32:12:8) and for flavonoids was in the solvent Ethyl acetate: Formic acid: glacial acetic acid: water (100:11:11:26)

2.3.3 Chromatography

Sample application is the most critical step for obtaining good resolution for quantification in HPTLC. The automatic application devices are preferable. The most recent automatic device "CAMAG LINOMAT V" was used to apply 1 band of 6 mm width with different concentration of all the extracts and marker solution also.

2.3.4 DevelopmentofChromatogram

The plate was developed in CAMAG glass twin trough chamber (10-10 cm) previously saturated with the solvent for 60 min (temperature 25.2 ^{0}C, relative humidity 40%). The development distance was 8 cm. Subsequently, scanning was done. The mobile phase or solvent system for all the raw herbs, raw ingredients, and marker compound which is given in the Table

2.3.5 DetectionofSpots

The plate was scanned at UV 200 nm, 250 nm, 300 nm, 350 nm, 400 nm and 450nm using CAMAG TLC Scanner-3 and LINOMAT-V. R_f value of each compound which were separated on plate and data of peak area of each band was recorded.

3. RESULTS

3.1HPTLC:

HPTLC study we can conclude that both the extract show the presence of tannins, saponins and flavonoids. R_f value of Gallic acid is 0.37, and both the extract also show the same R_f value from which we can say plants contains the tannins. However, further study is required to identify which tannins, saponins and flavonoids are present in it

3.1.1 HPTLC chromatogram of Standard, Ethanol and Aqueous extracts of *Vitis vitigenia* L. roots.

1) TANNINS

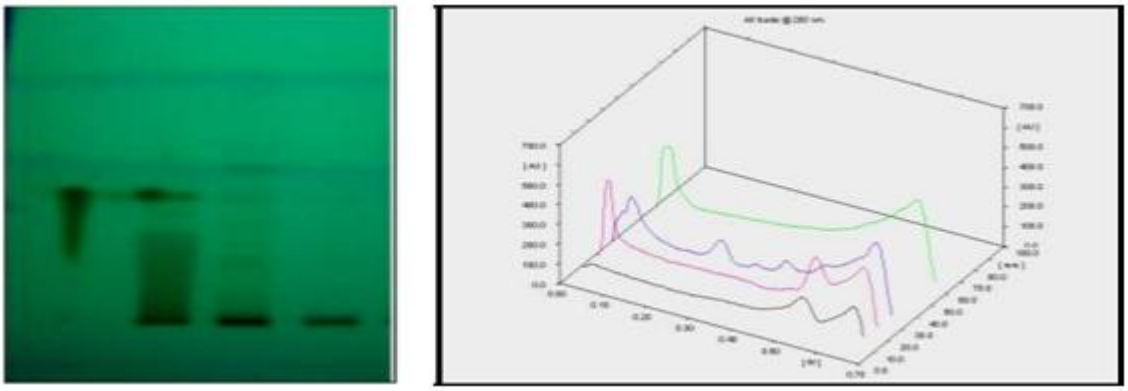

Figure 1: HPTLC and 3D Chromatogram of Tannins

[**Track 1**: 6 µl of Gallic acid, **Track 2** : 6 µl of Ellagic acid, **Track 3**: 6 µg/ml of Ethanol extract; **Track 4**: 8 µl of Aqueous extract]

Table 1: R_f value and area of Gallic acid, Ellagic acid, ethanol and aqueous extract of *Vitis vitigenia* L. Roots.

Peak	Gallic acid		Ellagic acid		Alcoholic Extract		Aqueous extract	
	R_f	AREA	R_f	AREA	R_f	AREA	R_f	AREA
1	0.37	231.8	0.34	1571.7	0.21	5844.4	0.17	2658.5
2	0.41	6676.7	0.42	12246.8	0.30	2816.2	0.37	37697.1
3	0.56	5947.4	0.55	13677.0	0.37	5211.2		
4					0.52	20406.9		

2) SAPONINS

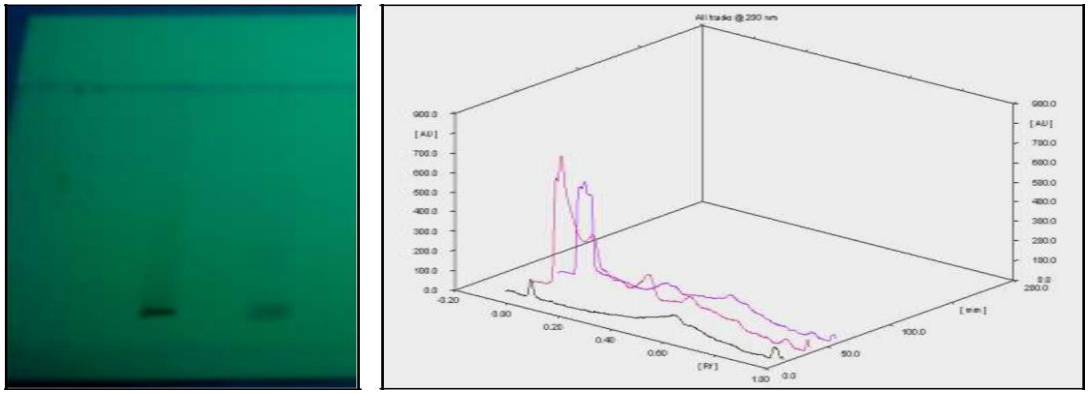

Figure 2: HPTLC and 3D Chromatogram of Saponin

[**Track 2**: 6 µg/ml of Ethanol extract; **Track 3**: 8 µl of aqueous extract]

Table 2: R_f value and area of ethanol and aqueous extract of *Vitis vitigenia* L. roots for saponins.

Peak	Alcoholic extract		Aqueous extract	
	Rf	AREA	Rf	AREA
1	0.13	15528.8	0.17	917.8
2	0.24	4248.8	0.25	3870.9
3	0.43	2620.8	0.36	1881.5
4	0.50	5732.7	0.41	3063.3
5	0.58	4065.3	0.53	5922.7
6	0.69	2576.6	0.63	3991.6
7	0.77	629.4	0.79	262.5
8	0.82	300.5	0.82	138.0
9	0.87	816.2	0.87	471.2
10	0.97	406.3	0.97	381.4

3) FLAVONOIDS

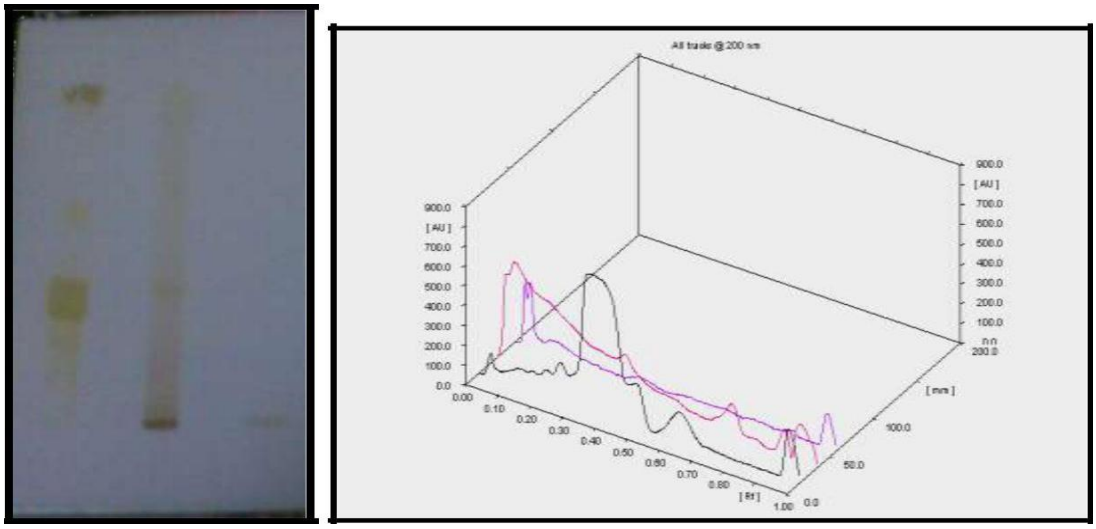

Figure 3 HPTLC and 3D Chromatogram of Flavonoids

[**Track 1**: 6 µl of Rutin extract; **Track 2**: 6 µg/ml of Ethanol extract; **Track 3**: 8 µl of aqueous extract]

Table 3: R_f value and area of Rutin, ethanol and aqueous extract of *Vitis vitigenia* L. roots for flavonoids.

Peak		Alcoholic extract		Aqueous extract		
	R_f	AREA	R_f	AREA	R_f	AREA
1	0.01	2231.6	0.37	15224.3	0.01	2900.8
2	0.07	4429.9	0.52	5191.7	0.04	4798.7
3	0.18	3762.5	0.65	8311.2	0.09	4182.5
4	0.23	6077.3	0.79	885.5	0.25	281.8
5	0.28	62162.2	0.85	2883.6	0.34	1526.2
6	0.47	7114.3	0.92	6795.6	0.50	231.0
7	0.55	8088.2			0.68	142.9
8	0.94	6217.7			0.72	386.3
9					0.76	282.5
10					0.84	577.3
11					0.95	3652.5

DISCUSSION

Both the extracts show the presence of tannins, saponins and flavonoids. And both the extracts were subjected to the HPTLC fingerprinting study which verifies the presence of the tannins, triterpenoid saponins and flavonoids. R_f value of Gallic acid is 0.37, and both the extract also show the same R_f value from which we can say plants contains the tannins. However, further study is required to identify which tannins, saponins and flavonoids are present in it.

CONCLUSION

Sophisticated modern techniques of standardization such as TLC and HPTLC provide quantitative and semi quantitative information about the main active constituents or marker compounds present in the crude drug or herbal products. TLC serves as one of the many methods in providing a chromatographic plant extract fingerprint. HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. Though further work to characterize the other chemical constituents and perform quantitative estimation with marker compounds is also necessary these data can also be considered along with the other values for fixings standards to this plant

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