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SEPARATION OF PHYTOCHEMICALS FROM *ABRUS PRECATORIUS* USING TLC AND HPTLC TECHNIQUES

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ABSTRACT

In the present investigations, the methanol extracts of *Abrus precatorius* in five different accessions (Hosur-Ap_1, Karaiyar-Ap_2, Nilgiri-Ap_3, Thenmala-Ap_4 and Varushanad-Ap_5) were screened and their phytochemical compounds were separated using the advanced chromatographic techniques TLC and HPTLC. For TLC analysis the chromatogram was observed under UV (365nm), UV (265nm), visible light and Iodine chamber to observe the variously colored bands. Maximum numbers of bands (twelve) were observed in leaves extracts of Ap_1 accession. In HPTLC analysis 13 peaks were eluted in Ap_1 accession, of these the 1st peak had the maximum height (160.7 AU) with Rf value 0.01 and percentage of area (13.05%), the 2nd peak had the minimum height (15.5 AU) with Rf value 0.12 and percentage of area (2.42 %). Hence the present study clearly shows that *A. precatorious* in Hosur accession can be used as potential medicinal plant for having more phytochemicals among the five populations.

INTRODUCTION

Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots that work with nutrients and fibers to act as an defense system against disease or more accurately, to protect against disease [2]. The chromatographic fingerprint technique was introduced as a tool to evaluate the quality of herbal samples or their derived products [3,4,5]. TLC is a rapidly developing technique that offers great potential for monitoring the quality of herbal materials, particularly for identifying a particular herb and distinguishing it from closely related species [6,7]. High Performance Thin Layer Chromatography has emerged as one of the most efficient tools in the last two decades for the separation and quantification of secondary metabolites especially for the evaluation of botanical materials [8].

Abrus precatorius L. commonly known as Rosary Pea. It belongs to the family Fabaceae. The seeds used as abortifacient [9] and treat diabetes and chronic nephritis [10]. The paste of roots, seeds and leaves cure abdominal pains, tumors, to treat tetanus, to prevent rabies and used as oral contraceptives [11]. Abrin is the major active principle of the plant. It is a highly toxic, galactose-binding lectin [12]. Abrin has the antitumor activity in mice [13] and in humans [14]. Hence the present study was designed to analyses the Abrus plant for the presence of phytochemicals which cause antitumor and other medicinal activity.

MATERIALS AND METHODS

Sample preparation

The wild fully grown *Abrus precatorius* were randomly collected from five different locations of Southern Western Ghats. The leaves were shade dried for a week and powdered using mixer grinder. 25 gms of the leaf powder from each location was continuously extracted with 250 ml of methanol for 18 hrs at 60°C using Soxhlet apparatus. The extracts were filtered through Whatman No.1 filter paper. The filtered extract was evaporated by a rotatory vaccum evaporator. The evaporated residues with constant weight were stored prior to analyses in dark at 4°C.

Thin Layer Chromatography (TLC) 10 ml methanol extract of each sample were taken and evaporated, the paste of the evaporated extracts was used for TLC. A combination of Petroleum

ether, Methanol and Benzene in the ratio 8:1:1.5 was used as solvent mixture. The TLC plates were coated with silica gel prepared using solvent mixture. The extracts to be analyzed were spotted on the plate. The plates were placed in TLC chamber and the chromatogram was developed with the solvent mixture. The TLC plates were taken out and visualized in visible light, UV light (254 nm & 366 nm) and iodine chamber and spots were marked. The migration pattern was recorded and the Rf value of each spot was calculated using the formula and tabulated.

Data analysis

The Rf values of different spots were calculated using the formula

$$\text{Rf value} = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

High Performance Thin Layer Chromatography (HPTLC)

Sample preparation

The methanol extract of each sample was used for HPTLC analysis. The extracts were filtered through a Whatman no.1 filter paper. 25 ml filtered extract was evaporated by a rotatory vaccum evaporator. 10 ml evaporated extracts were used for analysis.

Procedure

HPTLC analysis was performed on a CAMAG semi automated HPTLC system equipped with an automatic TLC sampler (ATS4), TLC scanner 3 integrated with documentation device Reprostar 3 with win CATS version 1.4.4 planar chromatography manager software. The plates were developed to a height of about 8 cm from the base in Toluene: Ethylacetate (5:2). To check the identity of the bands, UV absorption spectrum of each standard was overlaid with the corresponding band in the track [15,16].

Data analysis

The Rf value, area of peak, percentage of area were calculated from the calibration graph.

RESULTS AND DISCUSSION

Thin Layer Chromatography (TLC)

TLC chromatogram of *A. precatorius* under visible light revealed 10 bands for Ap_1, 8 bands in Ap_2, 9 bands in A_3 and Ap_4 and 8 bands for Ap_5 accessions. The highest Rf values were 0.97 in Ap_1 and Ap_2, 0.96 in Ap_4, 0.94 in Ap_3 and 0.92 in Ap_5 with yellow colored bands. The least Rf

values were 0.12 in Ap_3, 0.13 in Ap_5 and 0.15 in Ap_1 and Ap_4 accessions with green colored bands. In Ap_2 accession, the least Rf values was 0.24 with brown colored band (Table 1).

The chromatogram under UV light (254 nm) revealed 11 bands in Ap_1, and Ap_2 accessions and 8 bands in Ap_3 accession and 12 bands in Ap_4 and Ap_5 accessions. The highest Rf values were 0.86 in Ap_5, 0.85 in Ap_1, 0.82 in Ap_2 accessions. In Ap_3 and Ap_4 accessions the highest Rf values were 0.81 and 0.80 with yellow colored bands. The least Rf value was 0.14 in Ap_1 and Ap_4, 0.15 in Ap_2 and Ap_5, 0.20 in Ap_3 accessions with green colored bands (Table 2).

Under UV light (366 nm), the chromatogram revealed 10 bands in Ap_1, Ap_3 and Ap_4 accessions and 11 bands in Ap_2, 9 bands in and Ap_5 accessions. The highest Rf value was 0.82 with dark yellow colored bands in Ap_1 and Ap_4 accessions. In Ap_2 and Ap_3 accessions the highest Rf value was 0.80 with dark yellow colored bands. In Ap_5 accession the highest Rf value was 0.81. The least Rf values were 0.14 in Ap_2, Ap_3 and Ap_4 accessions, 0.13 in Ap_1 and accessions with fluorescent white colored bands and 0.28 in Pop $_5$ accessions with brown colored band. A fluorescent green colored band was observed in all the accessions except Ap_1 accession with Rf values 0.43 (Table 3).

When the chromatograms were exposed to iodine vapour, it revealed 11 bands in Ap_1, Ap_2, Ap_4 and Ap_5 accessions, 9 bands in Ap_3 accession. The highest Rf value was 0.87 in Ap_5 and 0.85 in Ap_1 and Ap_3 accessions with brown colored bands. In Ap_2 and Ap_4 accessions the highest Rf value was 0.81 and 0.82 respectively. The least Rf values were 0.21 in Ap_2 and Ap_4 accessions with brown colored bands. In Ap_1 and Ap_5 accessions least Rf values was 0.22. In Ap_3 accession least Rf values was 0.26 (Table 4).

High Performance of Thin Layer Chromatography (HPTLC)

In *A.precatorius* 13 peaks were eluted in Ap_1 accession, of these the 1st peak had the maximum height (160.7 AU) with Rf value 0.01 and percentage of area (13.05%), the 2nd peak had the minimum height (15.5 AU) with Rf value 0.12 and percentage of area (2.42 %) (Fig.1). In Ap_2 accession 10 peaks were eluted, of these the 5th peak had the maximum height (313.9 AU) with Rf value 0.29 and maximum percentage of area (40.58 %). The 4th peak had the minimum height (16.6 AU) with Rf value 0.26 and minimum percentage of area (0.83 %) (Fig. 2).

9 peaks were eluted in Ap_3 accession, of these the 5th peak had the maximum height (213.7 AU) with Rf value 0.28 and percentage of area (33.44%), the 4th peak had the minimum height (19.0

AU) with Rf value 0.25 and percentage of area (1.19 %) (Fig. 3). In Ap_4 accession 11 peaks were eluted, of these the 8th peak had the maximum height (136.1 AU) with Rf value 0.49 and percentage of area (24.22 %). The 7th peak had the minimum height (12.9 AU) with Rf value 0.44 and percentage of area (1.20 %) (Fig. 4). 14 peaks were eluted in Ap_5 accession, of these the 4th peak had the maximum height (124.0 AU) with Rf value 0.31 and a percentage of area (15.58 %), the 2nd peak had the minimum height (18.7 AU) with Rf value 0.12 and minimum percentage of area (1.80 %) (Fig. 5). Of the five accessions the Ap_1 accessions had more number of peaks (13), (i.e.) chemical compounds with a maximum height 160.7 AU.

A simple, robust and reproducible TLC method for the separation of phytochemicals were reported in *Vitex trifolia* by [17], in *Radix Polygoni* by [18] and in *Mucuna pruriens* by [19]. [20] suggested that the colours of the separated spots in TLC and their position relative to standard substances are important characteristics for the plant extract identification. [21] investigated the comparative quantification of phyllanthin through High Performance Thin Layer Chromatography (HPTLC). [22] evaluated the pharmacognostical characters of *Nothapodytes nimmoniana* leaf including anatomy, physicochemical characters and phytochemical analysis with HPTLC fingerprinting of extracts revealed the presence of camptothecin (Rf value 4.7). [23] employed a sensitive, simple, and accurate High-Performance Thin-Layer Chromatographic (HPTLC) method for quantitation of β-sitosterol from seed powder of *Caesalpinia bonduc* (Linn.) collected from different regions of India.

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Table: 1 TLC fingerprint profile of *Abrus precatorius* under visible light

S.No	Colour of the bands	Rf values				
		Ap_1	Ap_2	Ap_3	Ap_4	Ap_5
1.	Yellow	0.97	0.97	0.94	0.96	0.92
2.	Pale green	0.95	0.94	0.92	0.95	-
3.	Green	0.85	0.85	-	0.84	-
4.	Pale green	0.81	0.81	0.83	0.81	0.81
5.	Yellow	0.76	0.74	0.76	0.75	0.76
6.	Brown	0.64	0.63	0.64	0.64	0.62
7.	Pale green	0.52	-	0.53	0.52	0.54
8.	Pale green	0.32	0.31	0.31	-	0.31
9.	Brown	0.24	0.24	0.24	0.24	0.24
10.	Green	0.15	-	0.12	0.15	0.13

Table: 3 TLC fingerprint profile of *Abrus precatorius* under UV light (366 nm)

S.No	Colour of the bands	Rf values				
		Ap_1	Ap_2	Ap_3	Ap_4	Ap_5
1.	Dark Yellow	0.82	0.80	0.80	0.82	0.81
2.	Green	0.79	0.78	0.79	0.81	0.78
3.	Dark green	0.72	0.74	0.72	0.74	0.72
4.	Brown	0.62	0.68	0.62	0.62	0.62
5.	Yellowish green	0.59	0.62	-	0.60	0.58
6.	Dark Brown	0.52	0.52	0.52	0.52	0.52
7.	Fluorescent green	-	0.43	0.43	0.43	0.43
8.	Green	0.38	0.38	0.38	0.38	-
9.	Light Brown	0.30	0.31	0.30	-	0.31
10.	Brown	0.27	0.29	0.29	0.27	0.28
11.	Fluorescent white	0.13	0.14	0.14	0.14	-

Table: 4 TLC fingerprint profile of *Abrus precatorius* under iodine vapour

S.No	Colour of the bands	Rf values				
		Ap_1	Ap_2	Ap_3	Ap_4	Ap_5
1.	Brown	0.85	0.81	0.85	0.82	0.87
2.	Dark brown	0.80	0.80	0.79	0.81	0.79
3.	Brown	0.78	-	0.76	0.77	0.76
4.	Pale brown	0.72	0.71	0.70	0.72	0.70
5.	Pale green	0.68	0.69	0.64	-	0.69
6.	Green	0.62	0.64	-	0.62	0.64
7.	Brown	-	0.55	0.55	0.56	0.58
8.	Dark green	0.50	0.51	0.49	0.51	0.51
9.	Green	0.42	0.43	0.42	0.43	-
10.	Brown	0.33	0.32	-	0.32	0.33
11.	Brown	0.26	0.28	0.26	0.28	0.26
12.	Brown	0.22	0.21	-	0.21	0.22

Table: 2 TLC fingerprint profile of *Abrus precatorius* under UV light (254 nm)

S.No.	Colour of the bands	Rf values				
		Ap_1	Ap_2	Ap_3	Ap_4	Ap_5
1.	Yellow	0.85	0.82	0.81	0.80	0.86
2.	Pale green	0.82	0.83	-	0.82	0.85
3.	Green	0.76	0.73	0.76	0.72	0.75
4.	Pale green	0.72	-	0.72	0.72	0.73
5.	Yellow	0.68	0.66	0.69	0.71	0.67
6.	Brown	0.62	0.62	0.63	0.65	0.63
7.	Pale green	0.49	0.49	0.51	0.49	0.52
8.	Pale green	0.42	0.43	-	0.44	0.43
9.	Brown	0.35	0.36	0.34	0.39	0.39
10.	Green	-	0.31	-	0.29	0.31
11.	Dark brown	0.20	0.21	0.20	0.19	0.21
12.	Green	0.14	0.15	-	0.14	0.15

Fig. 1 HPTLC densidogram of *A.precatorius* from Hosur

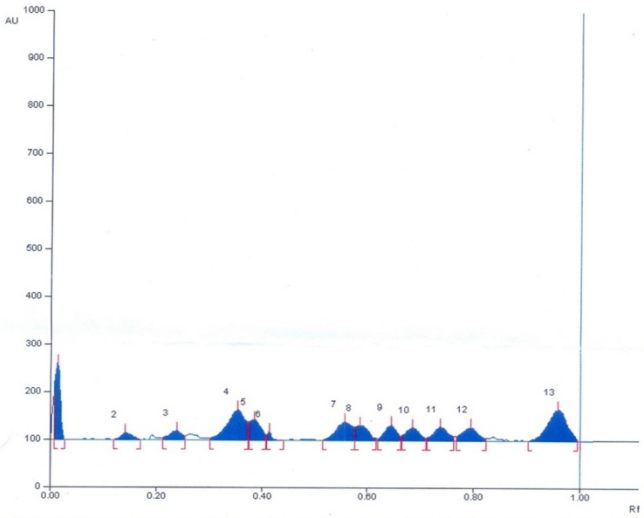

Fig. 2 HPTLC densidogram of *A.precatorius* from Karaiyar

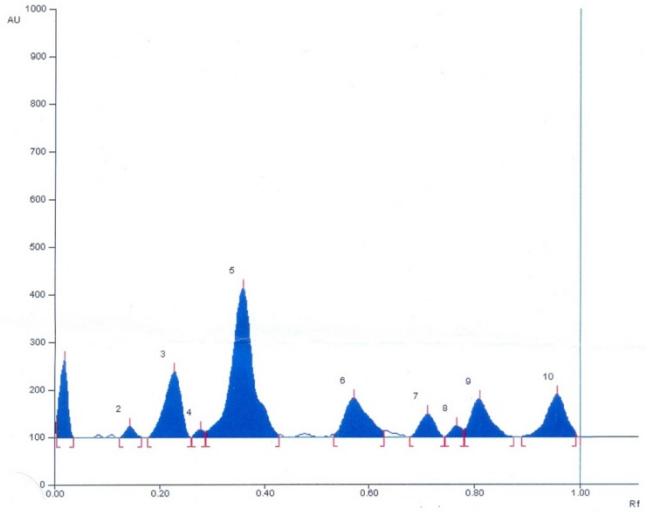

Fig. 3 HPTLC densidogram of *A.precatorius* from Nilgiri hills

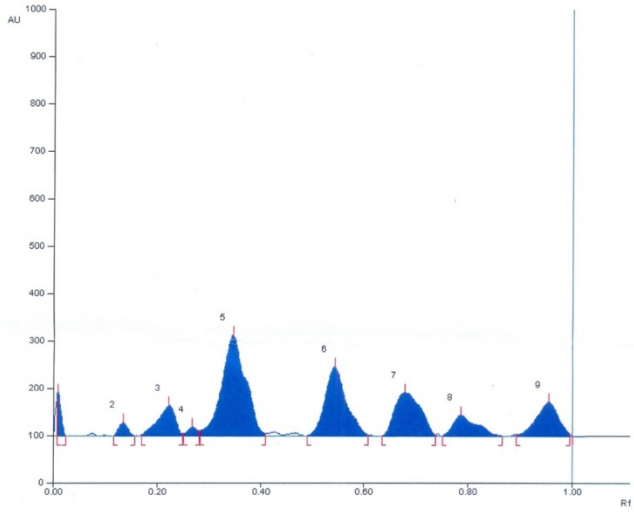

Fig. 4 HPTLC densidogram of *A.precatorius* from Thenmala

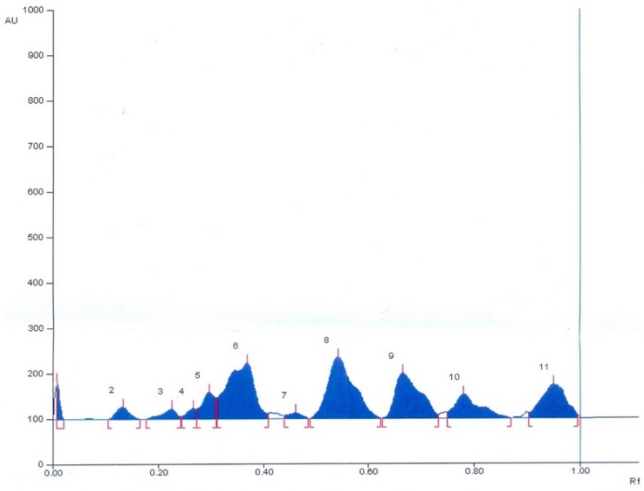

Fig. 5 HPTLC densidogram of *A.precatorius* from Varushanad hills