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PHYTOCHEMICAL SCREENING AND HPTLC FINGER PRINT ANALYSIS OF AERIAL PARTS OF *URARIA PICTA* DESV. – A DASHMOOL SPECIES

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ABSTRACT

Objectives: *To screen phytochemicals and develop finger prints of medicinally important aerial parts of Uraria picta Desv.*
Methods: *The powdered plant material was extracted successively in different solvents of increasing polarity (petroleum ether, chloroform, ethyl acetate, ethanol and water) using soxhlet apparatus. Extracts were subjected to phytochemical screening following standard methods and HPTLC fingerprints were developed using mobile phase, Ethyl acetate: Formic acid: Acetic acid: Water (8: 0.3: 0.3: 0.2).*
Results: *The phytochemical screening revealed the presence of cardiac glycosides, steroids, tannins, terpenoids in petroleum ether extract, cardiac glycosides, flavonoids, saponins, steroids, terpenoids in chloroform extract, cardiac glycosides, flavonoids, saponins, steroids, terpenoids in ethyl acetate extract, alkaloids, cardiac glycosides, flavonoids, saponins, steroids, tannins, terpenoids in ethanol extract and flavonoids, saponins, steroids, terpenoids in water extract. HPTLC fingerprinting of different extracts has shown several peaks with different Rf values and peak areas.*
Conclusion: *The phytochemical screening of different extracts showed the presence of important active phytoconstituents which have been described to have tremendous medicinal values in literature. HPTLC finger prints would be helpful in identification and authentication of aerial parts of this prestigious species.*

Key words*: Uraria picta, Aerial parts, Phytochemical screening, Chemical fingerprinting, HPTLC.*

INTRODUCTION

Since ancient era, nature functions as a complete store house of remedies to cure all aliments of mankind [1] and provides us drugs in the form of herbs, plants and algaes to cure diseases without any toxic effect [2]. In present time also, more than 80% of world population are still relying on traditional system of medicines to cure their diseases [3, 4]. Due to being safe and effective, the world market for herbal medicines is growing at the rate of 7-15% annually [5, 6]. India being one of the richest biodiversity countries may capture the opportunity of come out as a leader in the trade and commerce of pharmaceuticals, phytochemicals, neutraceuticals, cosmetics and other herbal products [5]. But, due to lack of scientific validation and quality standardization, Indian herbal drugs fetch typical bias in western countries [7]. Hence, the standardization of the herbal raw materials is the need of the hour to make the Indian branded drugs most reliable. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical characters. Hence the modern methods describing the identification and quantification of active chemical constituents in the plant material may be helpful for proper standardization of herbs and their formulations [8 - 10]. World Health Organization (WHO) has also emphasized on the quality assurance of medicinal plants using modern sophisticated techniques and applying suitable standards [11, 12].

High Pressure Thin Layer Chromatography (HPTLC) has emerged as a simple, versatile, accurate, cost effective, rapid and reliable tool for identification, quantification and standardization of herbal materials [8, 13]. Chromatographic fingerprints generated through HPTLC can be visualized and stored as electronic images [14].

Uraria picta Desv. (Syn. *Doodia picta* Roxb., *Hedysarum pictum* Jacq.) is commonly known as Prishnaparni or Pithvan and belongs to family Leguminosae: Papilionoidae (fig. 1). It is an erect, little branched, perennial herb, 90 – 180 cm tall, stems with short, rough hairs, leaves imparipinnate with 5-9 leaflets (lowermost leaves often 1-3-foliolate); leaflets narrowly lanceolate, 7-25 cm long (lowermost smaller), often variegated, shiny and hairless above, rough hairy below; margins entire, inflorescence a long terminal densely many-flowered spike-like raceme, up to 55 cm long, covered in long whitish hairs, flowers pink, bluish or reddish, fruit 5-9 mm long, folded into 3-6 segments, brown to black, turning greyish-white when old. It is widely distributed throughout India, Bangladesh, Sri Lanka, Tropical Africa, Malay Islands, Philippines, Australia, Africa and almost all parts of Asia [15 - 19].

It is one of the ten plant formulation called "Dashmoola", a well established Ayurvedic drug of the Indian System of Medicines used for treating general fatigue, oral sores and several gynaecological disorders [16, 17, 20]. A flavonoid rhoifolin (Apigenin-7-o-neohesperidoside, fig. 2) has been isolated from aerial parts of this plant which exhibited a partial vasorelaxing effect [21, 22] and found effective against hypodynamic [23] and pulmonary hypertensive cases [24]. Based on effectiveness, it has been established as marker compound for quality standardization of aerial parts of this species [21].

This study has been planned to identify the phytochemicals and to develop chemical finger prints using HPTLC technique which may serve as a basis for quality standardization of the plant material.

Fig. 1: *Uraria picta* in Wild

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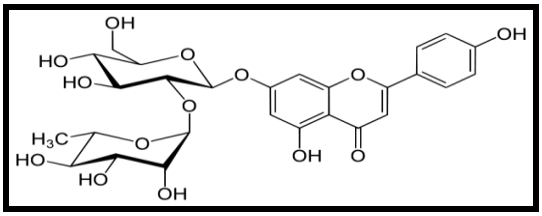


Fig. 2: Chemical structure of Rhoifolin

MATERIAL AND METHODS

Plant Material:

The aerial parts of *Uraria picta* were collected in November – December from Chhindwara district of Madhya Pradesh. Taxonomic identification of the plant specimen was authenticated by Biodiversity and Sustainable Management Division of Tropical Forest Research Institute (TFRI), Jabalpur (Identification no. 1763). The herbarium of plant specimen was prepared and deposited in the same division of TFRI.

Chemicals:

Rhoifolin standard was purchased from Sigma Aldrich, India. All chemicals and solvents used were of AR grade.

Processing and extraction of plant material:

Fresh aerial parts were washed in running water to remove unwanted foreign particles and dried in shade. The shade dried material was grinded to make powder and stored in air –tight polythene bags for further analysis. The powdered sample was subjected to successive solvent extraction with different solvents (petroleum ether, chloroform, ethyl acetate, ethanol and water) in increasing order of polarity using soxhlet apparatus. A total of 20 g of dried plant powder was extracted in 25 ml of various solvents for 8 hours. Extracts were evaporated to dryness to yield the respected extracts which were used for phytochemical screening and development of chemical fingerprints.

Phytochemical screening of plant extracts:

The preliminary phytochemical screening of crude extracts of aerial parts of *U.picta* was carried out according to the method described by various workers [25 – 28].

Development of chemical finger prints using HPTLC:

Sample preparation:

Dried extracts were dissolved in 10 ml of respective AR grade solvents which were used for development of HPTLC fingerprints.

Preparation of standard solution:

A solution of 0.1 mg/ml of rhoifolin in methanol was prepared to compare the presence of rhoifolin compound in different crude extracts.

Standardization of mobile phase:

Ethyl acetate: Formic acid: Acetic acid: Water (8: 0.3: 0.3: 0.2) solvent system was found appropriate for better resolution of peaks.

HPTLC fingerprint analysis:

3 µL of each test solution and 3 µL of standard solution were applied in the form of bands of width 8 mm using a 100 µL CAMAG syringe on 10 x 10 cm aluminum packed TLC plate prewashed with methanol and coated with 0.2 mm layer of silica gel 60F 254 (E. Merck Ltd., Darmstadt, Germany) with the help of a 100 µL Hamilton syringe and LinomatV applicator attached to CAMAG HPTLC system, which was programmed through Win CATS software. Samples loaded TLC plate was developed by the ascending technique using 10 ml of mobile phase [Ethyl acetate: Formic acid: Acetic acid: Water (8: 0.03: 0.03: 0.2)] in a CAMAG twin-through glass chamber (10 cm x 10 cm) saturated with mobile phase and covered with a stainless steel lid. Developed TLC plate was dried by hot air to evaporate solvents from the plate. The image of plate was taken at day (visible) light. The plate was kept in photo-documentation chamber and images were captured at 254 nm and 366 nm. Densitometric scanning was then performed with a CAMAG TLC Scanner 4 equipped with WinCATS software at λ_{max} = 254 and 366 nm using deuterium and mercury light sources respectively. Rf values, peak tables and HPTLC chromatograms were recorded.

RESULTS

The successive extraction of aerial parts of *Uraria picta* provided the petroleum ether, chloroform, ethyl acetate, ethanol and water extracts in 5.731%, 5.631%, 6.726%, 10.238% and 16.727% yields respectively. The phytochemical characters of different extracts of aerial parts of *U. picta* are summarized in Table 1. The study revealed the presence of cardiac glycosides in ethanol, ethyl acetate, chloroform and petroleum ether extracts, steroids and terpenoids in all extracts, tannins in petroleum ether and ethanol extracts, flavonoids and saponins in chloroform, ethyl acetate, ethanol and water extracts and alkaloids only in ethanol extract. Flavonoids and saponins were not detected in petroleum ether extract, alkaloids in all extracts except ethanol and cardiac glycosides in water extract. Tannins were found absent in chloroform, ethyl acetate and water extracts. Anthraquinones, anthocyanides, coumarins, phlobatannins and phenols were not detected in all extracts.

HPTLC fingerprint profiles of *Uraria picta* under visible light, UV 254 nm and 366 nm were recorded (fig. 3). HPTLC chromatograms of different tracks resulted from densitometric scanning of TLC plate at 254 and 366 nm were documented (fig. 4 and fig. 5). Desitometric scanning at 254 nm revealed 12 peaks of water extract with maximum Rf values in the range of 0.05 to 1, 10 peaks of ethanol extract with maximum Rf values in the range 0.05 to 0.97, 1 peak of standard solution with maximum Rf 0.09, 8 peaks of ethyl acetate extract with maximum Rf values in the range 0.04 to 0.97, 7 peaks of chloroform extract with maximum Rf values in the range 0.02 to 0.97 and 5 peaks of petroleum ether extract with maximum Rf values in the range 0.02 to 0.96 (fig. 3 and Table 2). Similarly, desitometric scanning at 366 nm revealed 4 peaks of water extract with maximum Rf values in the range of 0.06 to 0.70, 5 peaks of ethanol extract with maximum Rf values in the range 0.05 to 0.71, 1 peak of standard solution with maximum Rf 0.09, 8 peaks of ethyl acetate extract with maximum Rf values in the range 0.04 to 0.89, 5 peaks of chloroform extract with maximum Rf values in the range 0.03 to 0.85 and 3 peaks of petroleum ether extract with maximum Rf values in the range 0.02 to 0.85 (fig. 4 and Table 3).

Table No. 1: Phytochemical screening of aerial parts of *Uraria picta*

Phytochemicals	Extracts of aerial parts of *Uraria picta*				
	Petroleum Ether	Chloroform	Ethyl acetate	Ethanol	Water
Alkaloids	-	-	--	+	-
Anthraqunones	-	-	-	-	-
Anthocyanides	-	-	-	-	-
Cardiac glycosides	+	++	+	+	-
Coumarins	-	-	-	-	-
Flavonoids	-	+	+	+	++
Phenols	-	-	-	-	-
Phlobatannins	-	-	-	-	-
Saponins	-	++	-	+	+
Steroids	+	+	+	+	+
Tannins	+	-	+	+	-
Terpenoids	+	+	+	+	+

(+) = detected, (++) = detected in more amount and (-) = not detected

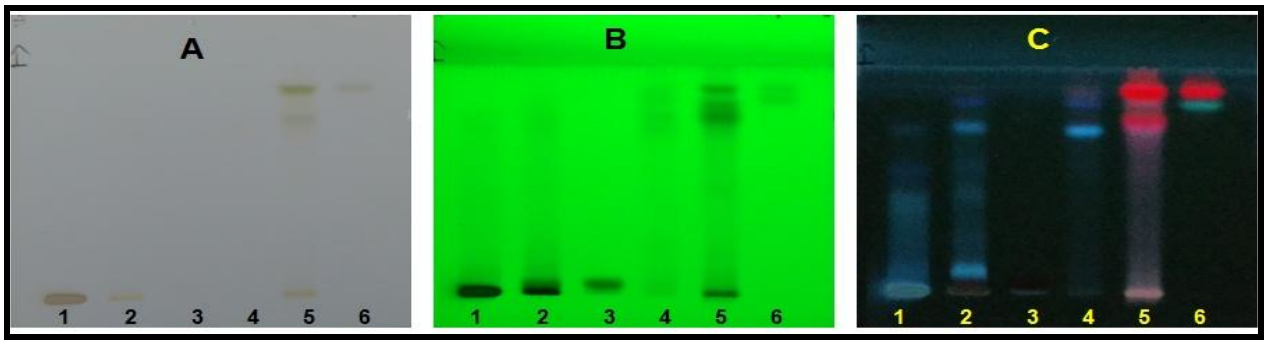
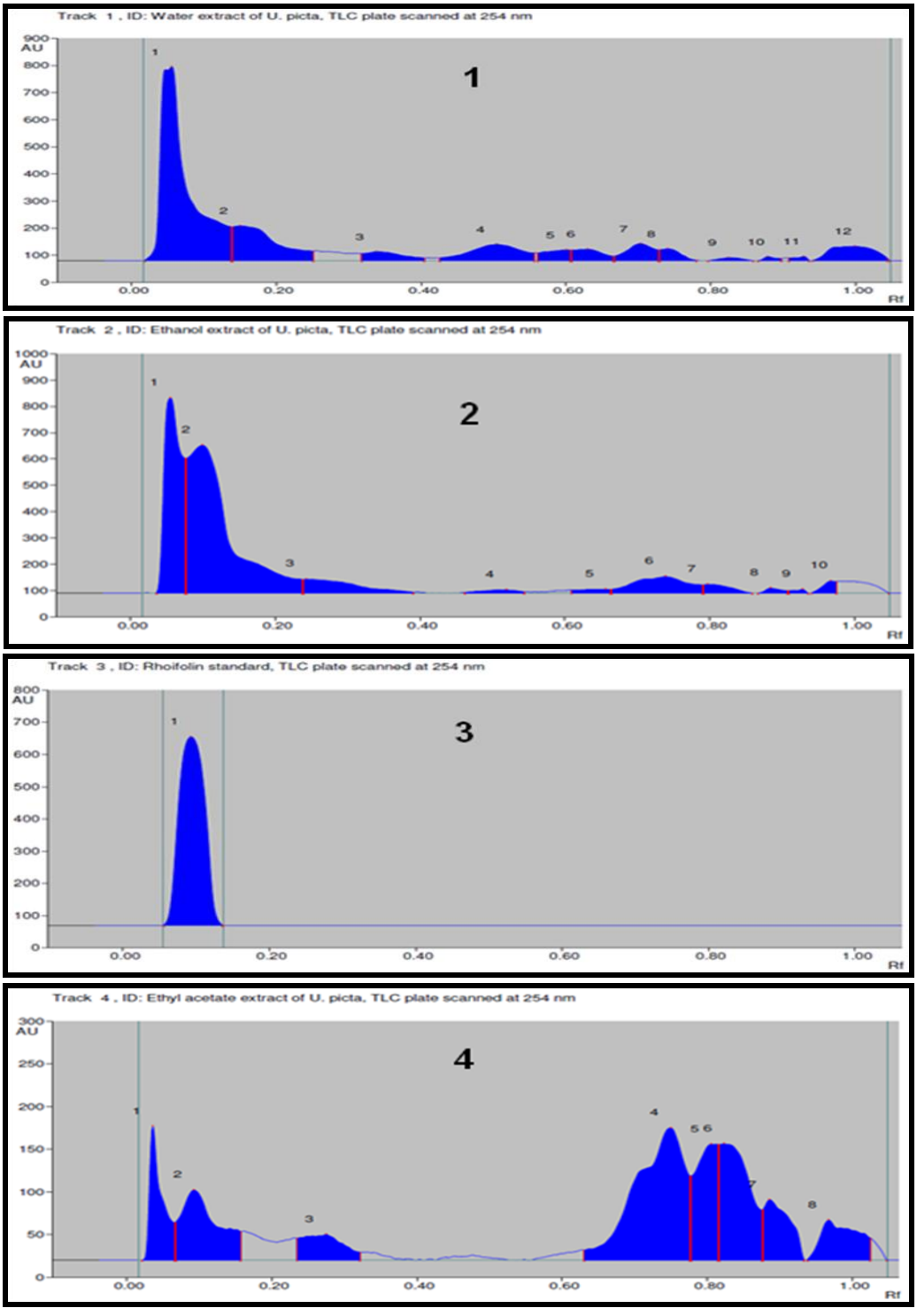


Fig. 2: HPTLC fingerprint profiles of aerial parts of *Uraria picta*
A. Visible light, B. 254 nm, C. 366 nm
(Track 1: Water extract, Track 2: Ethanol extract, Track 3: Rhofolin standard, Track 4: Ethyl acetate extract, Track 5: Chloroform extract, Track 6: Petroleum ether extract)



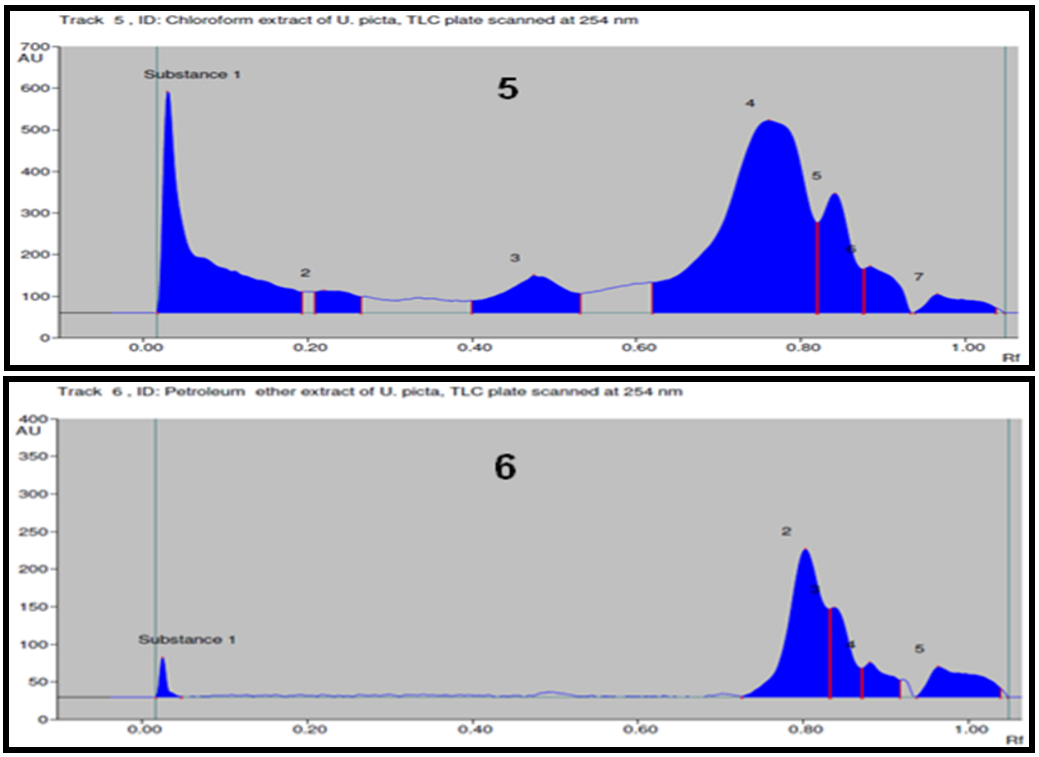
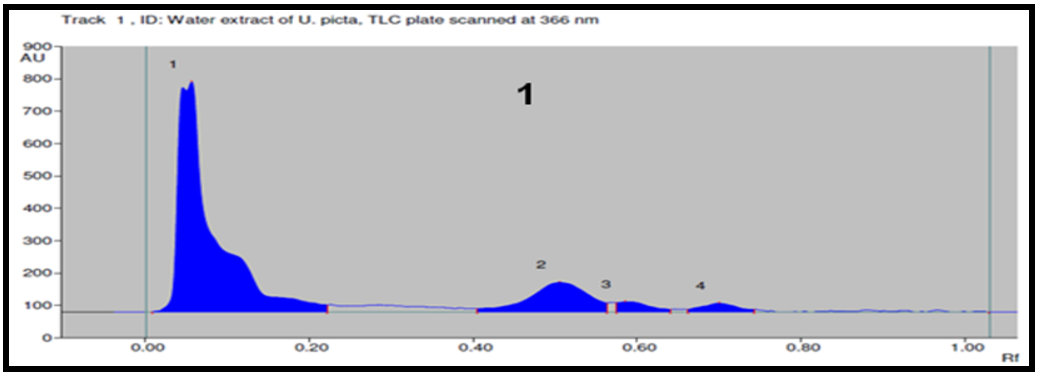


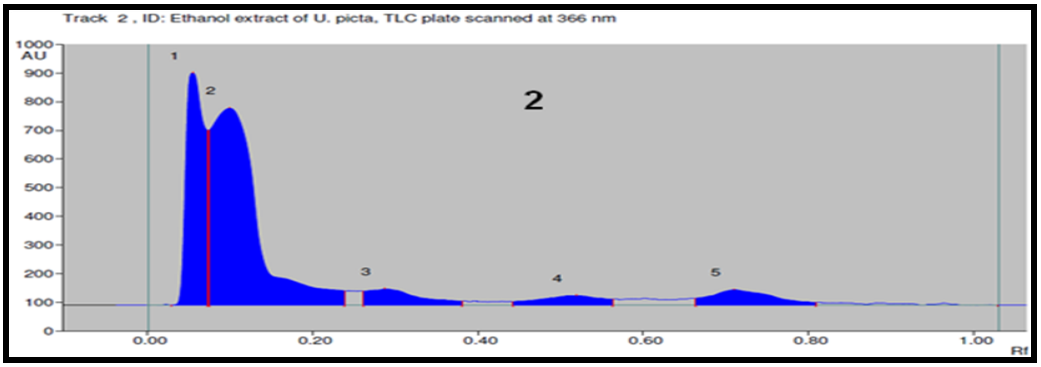
Fig. 4: HPTLC chromatograms of different extracts of aerial parts of *Uraria picta* showing phytochemicals at 254 nm

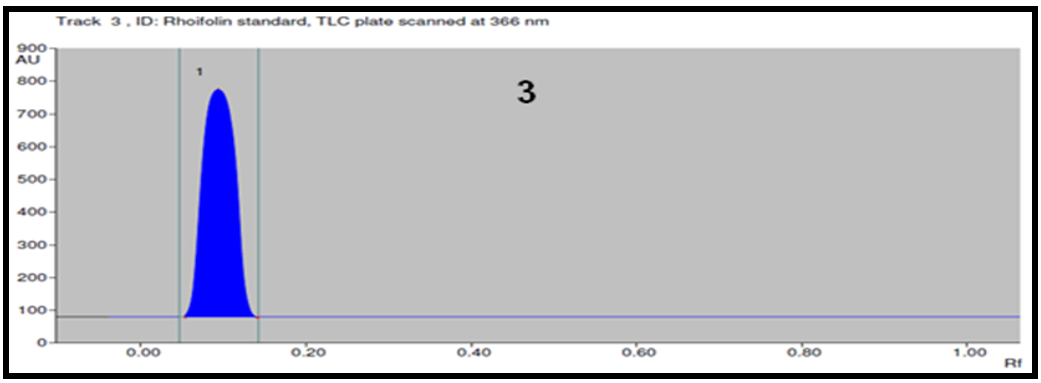
Table No. 2: Peak list and Rf values of phytchemicals in HPTLC chromatograms of different extracts of aerial parts of *U. picta* at 254 nm

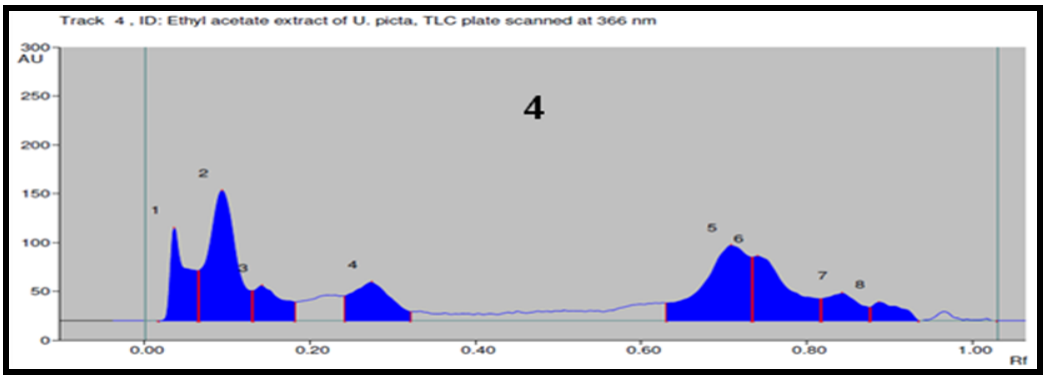
Track	Peak	Max Rf	Max Height (AU)	Area (%)
1	1	0.05	714.7	52.53
1	2	0.15	128.5	14.89
1	3	0.34	32.9	3.42
1	4	0.51	59.9	8.20
1	5	0.6	39.1	2.69
1	6	0.63	42.4	3.32
1	7	0.7	62.9	4.51
1	8	0.74	43.8	2.33
1	9	0.83	11.1	0.64
1	10	0.88	14.4	0.48
1	11	0.93	15.4	0.52
1	12	1	53	6.47
2	1	0.05	741.6	27.94
2	2	0.1	561.8	51.96
2	3	0.24	53.1	6.15
2	4	0.52	12.9	1.02
2	5	0.66	15.1	1.04
2	6	0.74	62.9	7.49
2	7	0.8	33.5	1.97
2	8	0.89	18.7	0.67
2	9	0.93	14.2	0.39
2	10	0.97	46.2	1.37
3	1	0.09	606.3	84.50
4	1	0.04	156.6	7.94
4	2	0.09	82.4	12.43
4	3	0.27	30.2	5.30
4	4	0.75	155	30.86
4	5	0.81	136	12.27
4	6	0.82	136.4	16.64
4	7	0.89	70.9	7.45
4	8	0.97	46.9	7.10
5	1	0.03	530.6	22.21
5	2	0.22	53.4	2.69
5	3	0.48	89.4	7.32
5	4	0.76	461.9	49.78
5	5	0.84	286.6	11.02
5	6	0.89	110.9	4.40
5	7	0.97	43.7	2.60
6	1	0.02	51.8	2.69

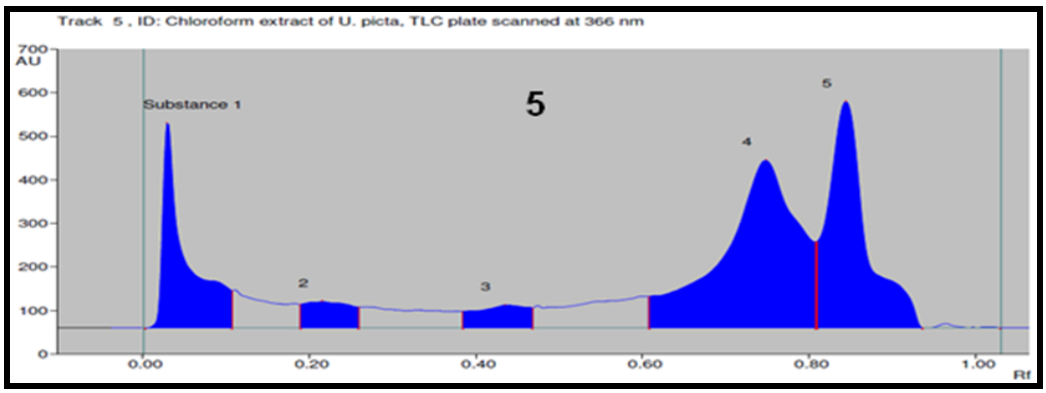
6	2	0.8	196.4	53.39
6	3	0.84	118.8	18.84
6	4	0.88	45.3	9.24
6	5	0.96	39.8	15.84











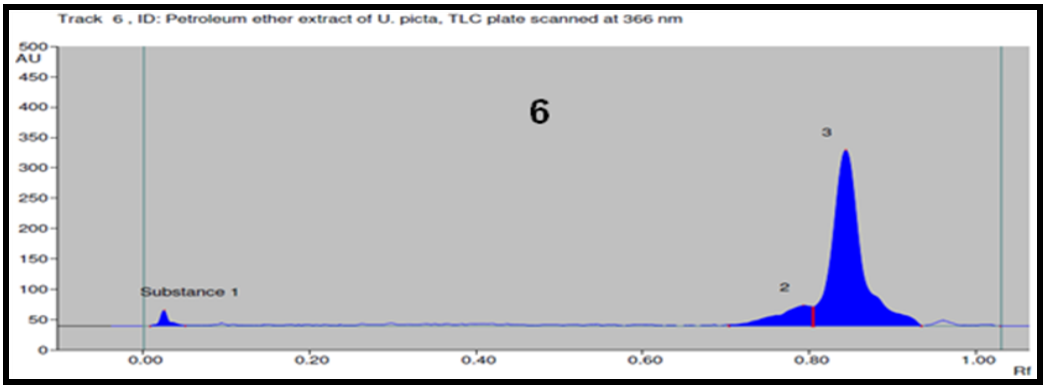


Fig. 5: HPTLC chromatograms of different extracts of aerial parts of *Uraria picta* showing phytochemicals at 366 nm

Table No. 3: Peak list and Rf values of phytchemicals in HPTLC chromatograms of different extracts of aerial parts of *U. picta* at 366 nm

Track	Peak	Max Position	Max Height (AU)	Area (%)
1	1	0.06	710.3	77.49
1	2	0.51	91.0	16.40
1	3	0.59	30.9	3.11
1	4	0.70	26.5	2.99
2	1	0.05	809.7	27.14
2	2	0.1	686.9	57.45
2	3	0.29	55.9	5.45
2	4	0.52	33.4	3.81
2	5	0.71	53.5	6.15
3	1	0.09	729.4	95.62
4	1	0.04	95.0	10.40
4	2	0.09	133.2	24.07
4	3	0.14	35.9	6.55
4	4	0.27	39.4	10.29
4	5	0.71	77.2	23.16
4	6	0.74	66.1	15.75
4	7	0.84	28.2	6.14
4	8	0.89	18.9	3.63
5	1	0.03	469.1	16.66
5	2	0.22	60.4	4.49
5	3	0.44	51.9	4.30
5	4	0.75	384.3	43.95
5	5	0.85	519.2	30.60
6	1	0.02	24.6	2.08
6	2	0.79	32.8	12.28
6	3	0.85	288.7	85.65

DISCUSSION

Preliminary phytochemical screening actually helps in isolating and characterizing the chemical constituents present in the plant extracts and knowledge of chemical constituents of plants is necessary to understand herbal drugs and their preparations and finally in discovering the actual value of folkloric remedies [29]. Phytochemicals such as alkaloids, flavonoids, steroids, terpenoids, cardiac glycosides, saponins and tannins present in different extracts exhibit a number of biological activities and protect from most of the chronic diseases [30, 31].

Morphological characters play a crucial role in plant taxonomy. In recent times, anatomical, cytological, biochemical and molecular markers also play an important role in classifying species in addition to morphological markers. In the last two decades, chemical fingerprinting emerged as an effective tool to resolve problems in standardization of herbal drugs. Using chemical fingerprinting, plants can be demarcated on the basis of their species, strain and geographical origin [32]. The presence or absence of chemical constituents plays an important role in placing the plant in taxonomic categories. HPTLC fingerprinting is proved to be a better, linear, precise and accurate method for herbal identification, authentication, quality standardization and characterization of medicinal plants [13]. HPTLC profiles of plant extracts in solvents of different polarities were generated in order to find out total number of chemical moieties which will help in devising the methods of isolation and structure elucidation of active compounds [33] along with quality control and standardization of herbal drugs. The developed chromatograms will be specific with standardized solvent system Ethyl acetate: Formic acid: Acetic acid: Water (8: 0.3: 0.3: 0.2 v/v) and Rf value. Rhoifoin, a marker compound in aerial parts of *U.picta*, was run on TLC plate (Track 3) and compared for its presence in tracks of sample extracts. Chromatograms revealed its presence in the ethanol and ethyl acetate extracts at both wavelengths i.e. 254 and 366 nm (Rf values, Track 2 - 0.1, Track 3 – 0.09 and Track 4 - 0.09). The present investigation will provide enough information about the therapeutic potential of the plant and also help in quality control of herbal formulations.

CONCLUSION

The study revealed the presence of important phytoconstituents in extracts of aerial parts of *U.picta* prepared in solvents of increasing polarities. These phytoconstituents have been reported to confer with huge therapeutic potential. HPTLC finger print images would be helpful in identification, authentication and quality control of aerial parts of this prestigious species. These fingerprints will also serve as biochemical markers to distinguish between authentic drug and adulterants even in processed samples, thus will be of utmost importance for pharmaceutical industries.

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