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RESEARCH ARTICLE

A HIGH PERFORMANCE THIN LAYER CHROMATOGRAPHIC (HPTLC) METHOD FOR SPECIES IDENTIFICATION OF GRASS LEAF STAINS

Priyanka and *Rajinder Singh

Department of Forensic Science, Punjabi University, Patiala, Punjab, India - 147002

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ABSTRACT

Grass stains are frequently encountered as evidentiary material particularly in outdoor criminal investigations. These stains, if analyzed properly and identified correctly can solve the mysteries of outdoor criminal cases. High Performance Thin Layer Chromatographic (HPTLC) being highly sensitive, reliable and reproducible technique is best suited for the analysis of grass stains. In the present study, we report a HPTLC method for the separation and subsequent analysis of various phytoconstituents of leaf stains of forty grass species commonly found in Punjab, a state in northwestern India. It has been found that the HPTLC profiles of leaf stains of selected grasses can differentiate them and can be used successfully as a characteristic tool for the species identification of grass leaf stains.

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INTRODUCTION

The botanical materials are encountered as evidence in outdoor crime cases in intact form, degraded, fragmented form or in the form of stains. They can be useful to provide links between a crime scene and suspect(s), testing *alibis*, and ascertains whether a crime scene is a primary or secondary, *etc*. Besides their importance as evidence, they are also used as weapon in criminal investigations (Chandra *et al*., 2014). Among various types of plants or their trace materials, grasses are presumably largest amongst the plant species stumble upon forensic evidence and have the tendency to offer links between individuals and the crime scene or give any vital information because of their ubiquitous distribution in almost every habitat available to flowering plant. So, this reveals that, the grasses are specific to each and every location and thus show large range of morphological variation (Ward *et al.,* 2005). This botanical trace evidence like pollens, vegetative or reproductive parts or stains can be easily transferred to the clothing's of the victim or suspect from the scene of crime in accordance with the Locard's exchange principle. Thus, the correct identification of the plant species from the parts recovered is mandatory. But, due to the lack of botanical knowledge regarding their identification, it remains underutilized evidence (Bock *et al*., 1997). The various types of plant or botanical material encountered as evidence include their vegetative or reproductive parts or their stains if they get rubbed against any external rough material. These stains usually have number of phytoconstituents (alkaloids, pigments, tannins, waxes etc.) of different polarity (Chen, 1987). This wide composition of phytoconstituents varies from plant species to species. Therefore, it is possible to link a particular plant stain to its species which then subsequently related to particular location for help in forensic investigations in various outdoor crime cases (Ward *et al*., 2005). The examination of plant stains usually involves analysis of their phytoconstituents like chlorophyll a and b, carotenoids (Chen *et al*., 1987, Hayashiba *et al*., 1989); flavonoids (Staaij *et al*., 2002), anthocyanins (Fossen *et al*., 2002), alkaloids *etc*. which can be brought about only by the chromatographic techniques as morphological identification is not possible. The first investigation on plant pigments was performed in 1906 by M. Tswett to analyze complex mixture of plant pigments using thin layer chromatography (Janero *et al*., 1981). After that, number of researches has been performed to separate and identify the phytoconstituents or pigments (carotenoids, chlorophyll, xanthophylls *etc.*) from plant parts or their stains using various instrumental techniques like Thin Layer Chromatography (TLC) (Sun et al., 2005; Li *et al*., 1999;

Corresponding author: Rajinder Singh,
Department of Forensic Science, Punjabi University, Patiala, Punjab, India – 147002.

Wagner *et al.,* 1984; Lade *et al*., 2014; Sethi, 1996; Banu *et al*., 2014); High Performance Liquid Chromatography (HPLC) (Sun *et al.,* 2005; Li *et* al., 1999; Kosa *et al.,* 2001) Reversed Phase High Performance Liquid Chromatography (RP-HPLC) (Pocock *et al*., 2004), High Performance Thin Layer Chromatography (HPTLC) (Ojha *et al.,* 2012; Sathiavelu *et al*., 2012; Devi *et al*., 2013; Banu *et al*., 2014, Seasotiya *et al*., 2014) *etc.*

From the available chromatographic techniques, HPTLC, the advanced version of TLC is quick, reliable automated technique to study the phytoconstituents of grass leaf stains. In this technique the sorbent has thin, small and uniform particle size and plates were developed for short distance (approximately 5 cm). This leads to less solvent system consumption, faster and better separation efficiency and lower detection limits. Moreover, this technique requires very less sample quantity and more number of samples can be applied per plate. The estimation of phytoconstituents can be done with reasonable accuracy in a shorter time in comparison to TLC.
HPTLC has been used firstly for fingerprint profiling of plant extracts by Wagner, *et al.,* 1984 and Sethi, 1996. Thereafter number of studies has been performed to study the phytoconstituents of bark extracts of *Ficus nervosa* (Devi *et al*., 2013), leaf extracts of *Wedelia chinensis* (Banu *et al*., 2014), leaf extract of *Cassia fistula* (Sathiavelu *et al*., 2012). Hayashiba *et al.* (1989) identified leaf stains of thirteen common grass weed species using High Pressure Liquid Chromatography. Geetha (2015) discussed a case study in which the phytochemical constituents of *Cynodon dactylon* were studied for medicinal purpose. It is lament that very limited work has been done on the analysis of leaf stains from forensic perspective. Keeping this significant aspect in view, the present study has been undertaken with an aim to study the number of phytoconsituents chiefly components of chlorophyll and carotenoids present in selected samples. The differentiation of leaf stains of different grasses on the basis of number of spots and Rf value has also been considered for their taxonomic significance in the field of forensics.

MATERIALS AND METHODS

Sampling

Five or more samples each of forty grass species belonging to six subfamilies were collected from various districts of Punjab state in Northwest India. All the grass species collected were stored separately by sandwitching in the newspapers. Species identification was done by morphological methods using the keys given by Sharma and Khosla (2001). The details of the information regarding their subfamily, location of collection and number of species collected has been given in Table 1.

Sample Preparation

Four stains of each selected grass species collected from different locality were prepared by gently rubbing the leaves of respective grass species on washed white cotton cloth pieces until visible green mark is obtained. The stain samples were then dried under shade, serially marked and stored in separate zip lock polythene to prevent cross contamination.

Sample Extraction

The stained part of the cloth pieces (1 x 1 cm) was cut for extraction and placed in 1 ml micro centrifugation tubes separately. 1 ml of methanol was added to the tubes separately and allows it undisturbed for overnight. The white cotton piece was treated in the same manner which serves as a negative control.

High Performance Thin Layer Chromatography

HPTLC (CAMAG Linomat 5) analysis was performed using 20 x 20 cm pre-coated silica gel aluminum plates 60 F254 with 0.2 mm layer thickness (Merck, Darmstadt, Germany, Cat. No.1.05548). The 10µl aliquot of respective samples and standards *i.e.* chlorophyll (Hubbard Scientific Chippowa Falle, WI, Lot no. 10217100) and carotenoid (Hubbard Scientific Chippowa Falle, WI, Lot. No. 1020945) were spotted in the form of bands (band width 6mm) at a distance of 1cm from the bottom of precoated Silica Gel plates using Automated TLC sampler Linomat V (Camag, Muttenz, Switzerland) which was controlled by Win CATS software 1.3.3 (Camag, Muttenz, Switzerland). 20 x 20 cm twin trough glass chamber (Camag, Muttenz, Switzerland) was saturated with selected mobile phase for 20 min at room temperature (25 C ± 2). The solvent front was allowed to migrate to a distance of 10cm above the origin. After the run has been completed the developed plates were air dried at room temperature. The separated spots were visualized, scanned and hRf values were calculated with CAMAG TLC UV scanner at various wavelengths.

RESULTS

In the present study, high performance thin layer chromatographic profiles (chromatogram) of methanolic extracts of forty grass leaf stains has been developed with an aim to differentiate the selected grass species based on the phytoconstituents. The present method is based on method of Chandel *et al.,* 2013 in which the solvent system (mobile phase) comprising Toluene, Ethyl acetate, Formic acid and Methanol (60:15:15:10) was used for the separation of various phytoconstituents of grass leaf stains using HPTLC. The developed HPTLC plates were scanned at various wavelengths to find out the suitable wavelength which can show more number of bands and peaks for the selected samples.

Selection of wavelength

The peaks of phytoconstituents of selected samples were scanned at various wavelengths between 200-380 nm. The best chromatogram for the selected samples has been observed at 366 nm. Thus, the latter was selected as a detection wavelength for the analysis of phytoconstituents of selected grass stains.

HPTLC profiles of selected samples

The chromatograms of methanol extract of forty selected samples and standard sample of chlorophyll and carotenoid pigment were developed under chamber saturation conditions using Toluene-Ethyl acetate-Formic acid-Methanol (60:15:15:10) as mobile phase or solvent system.

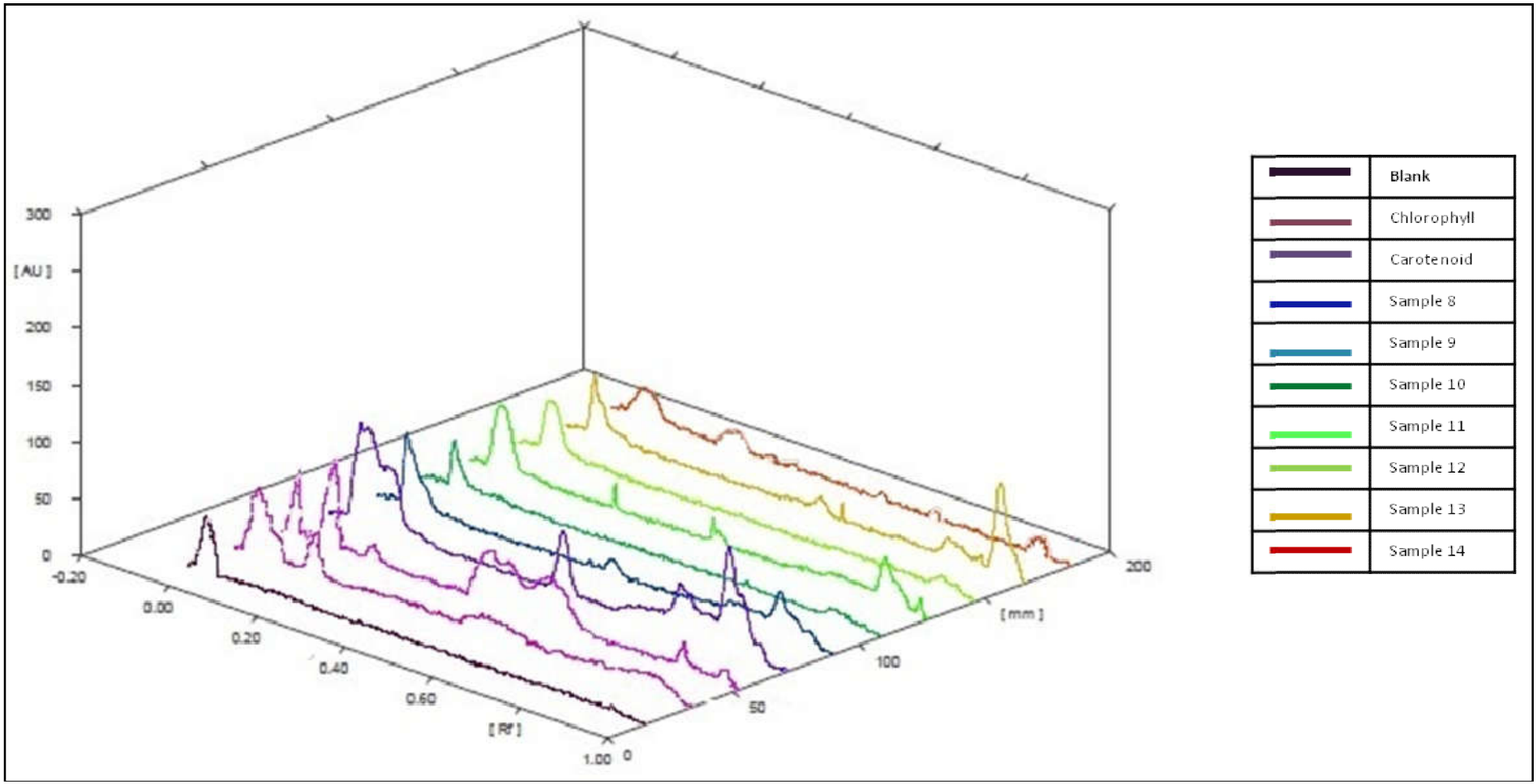

Fig.1. HPTLC chromatogram of selected standards and extracts of selected samples

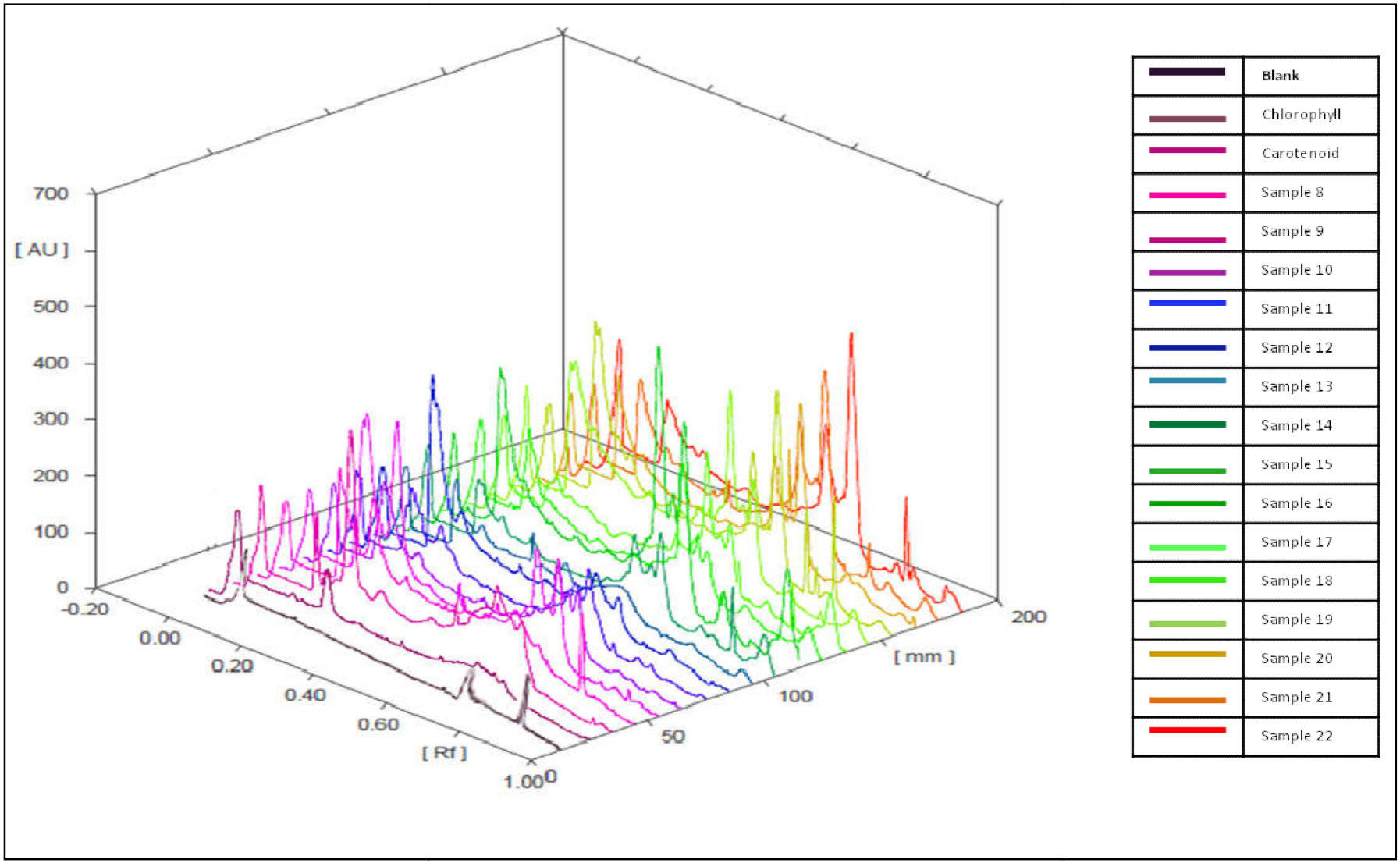

Fig.2. HPTLC chromatogram of selected standards and extracts of selected samples

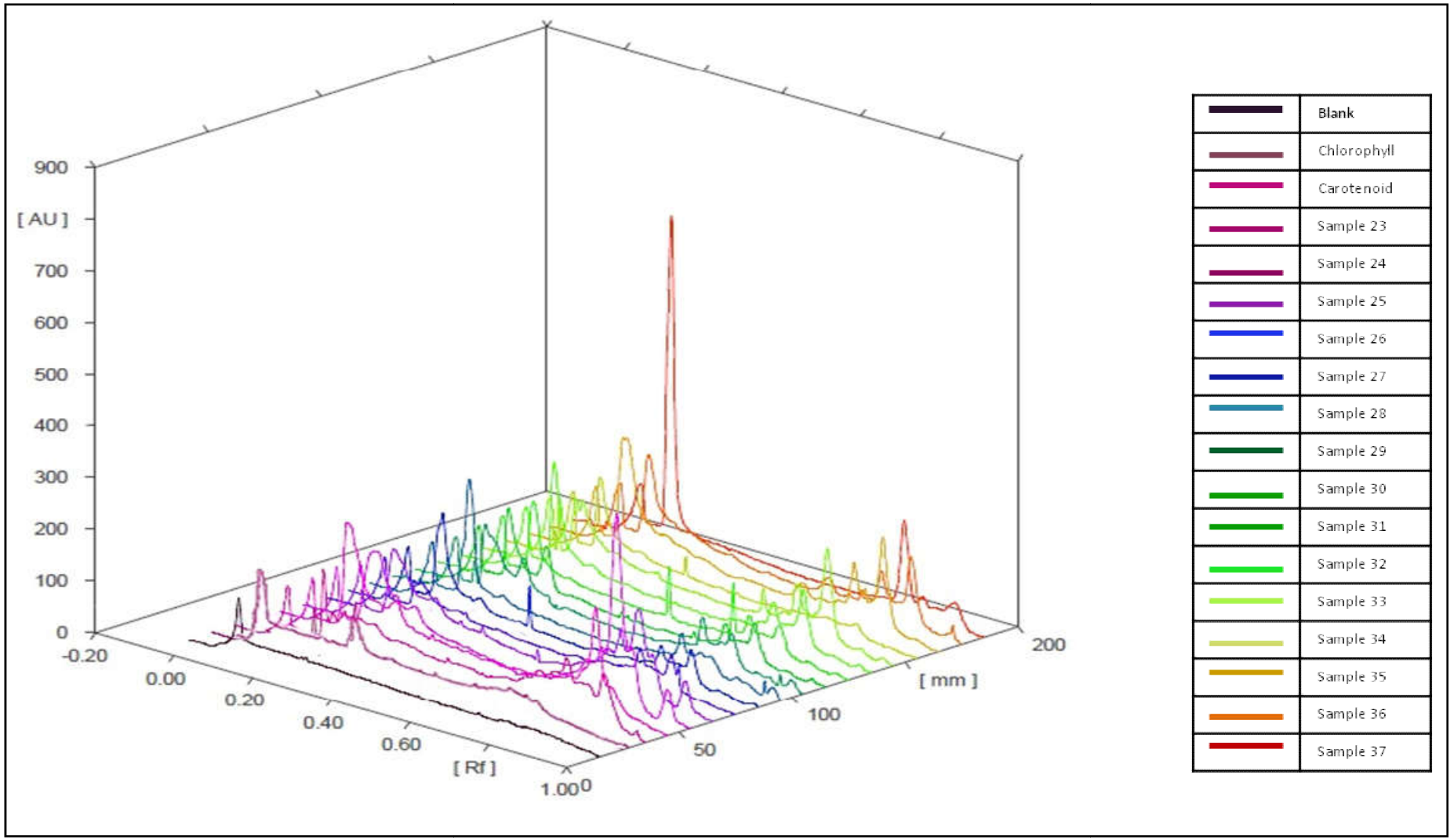

Fig.3. HPTLC chromatogram of selected standards and extracts of selected samples

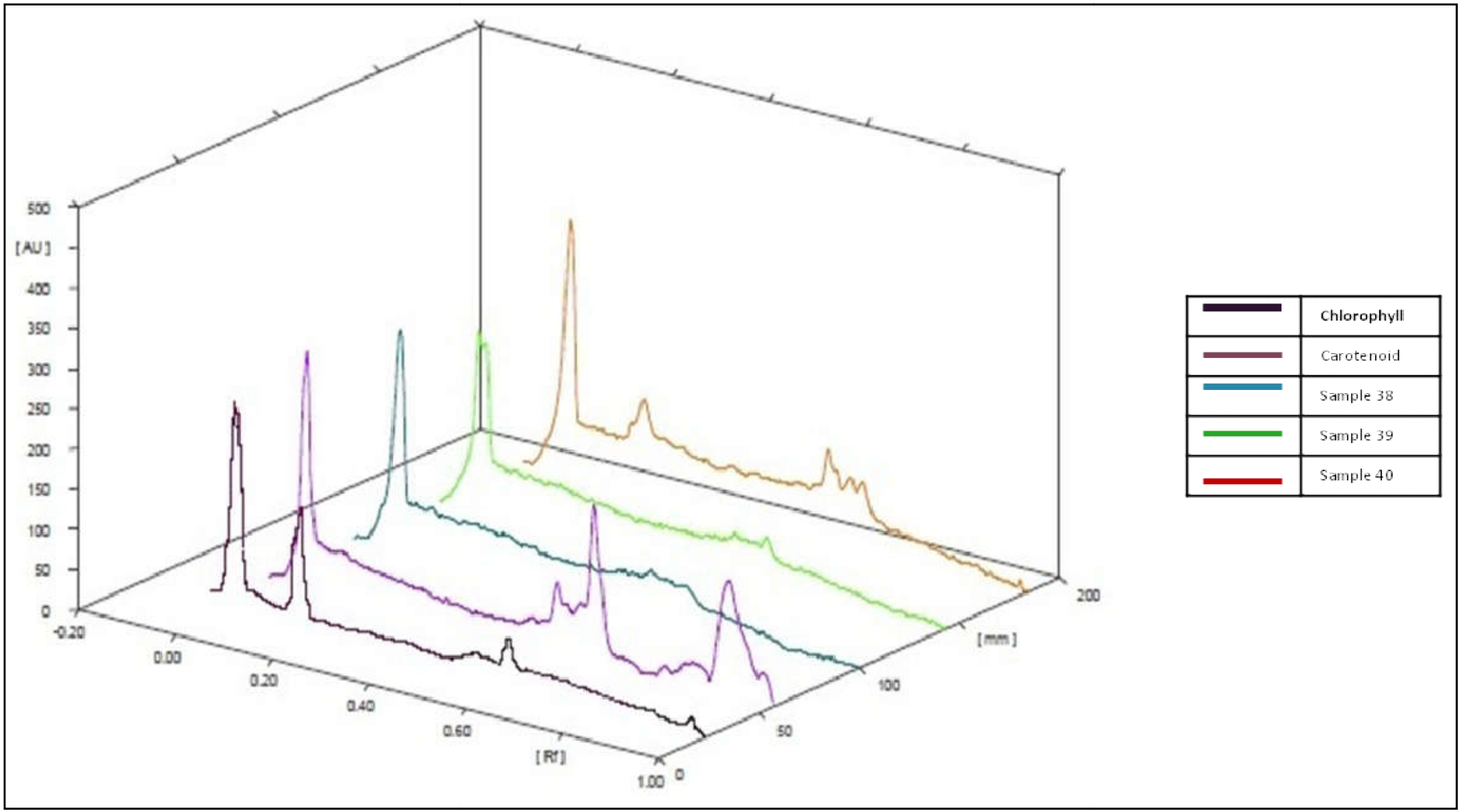

Fig.4. HPTLC chromatogram of selected standards and extracts of selected samples

Table 1. Detailed information of selected grass species collected for the present study

S.No.	Name of grass species	Subfamily	Place of collection (Districts)	No. of samples collected (n=)
1.	*Cynodon dactylon*	Chloridoideae	Patiala, Sangrur, Bathinda, Jalandhar, SAS Nagar	11
2.	*Dactyloctenium aegyptium*	Chloridoideae	Patiala, Sangrur, Bathinda, Faridkot, Kapurthala, SAS Nagar, Jalandhar	12
3.	*Cenchrus biflorus*	Panicoideae	Patiala, Sangrur, SAS Nagar, Ludhiana	8
4.	*Denebra retroflexa*	Chloridoideae	Patiala, Ludhiana	5
5.	*Brachiaria ramose*	Panicoideae	Patiala, Sangrur, Faridkot, Ludhiana	6
6.	*Echinochloa colonum*	Panicoideae	Patiala, SAS Nagar, Ludhiana	6
7.	*Panicum maximum*	Panicoideae	Patiala, Bathinda	8
8.	*Digitaria abludens*	Panicoideae	Patiala, Sangrur	6
9.	*Arundo donax*	Arundinoideae	Patiala, SAS Nagar, Sangrur	5
10.	*Arundinella nepalensis*	Arundinoideae	Patiala, Bathinda	5
11.	*Cenchrus setigerus*	Panicoideae	Patiala, Sangrur	6
12.	*Poa annua*	Pooideae	Patiala, SAS Nagar	9
13.	*Triticum aestivum*	Festucoideae	Sangrur, Patiala	12
14.	*Sporobolus diander*	Chloridoideae	Patiala, SAS Nagar, Sangrur	6
15.	*Bothriochloa pertusa*	Panicoideae	Patiala, Sangrur, Ropar	7
16.	*Leptochloa panacea*	Chloridoideae	Patiala, Faridkot	6
17.	*Dichanthium annulatum*	Panicoideae	Patiala, Sangrur, Bathinda, SAS Nagar	10
18.	*Polypogon monspeliensis*	Pooideae	Patiala, Sangrur, SAS Nagar	10
19.	*Paspalidium flavidum*	Panicoideae	Patiala, SAS Nagar	12
20.	*Digiteria ciliaris*	Panicoideae	Patiala, Sangrur, SAS Nagar	10
21.	*Leptochloa chinensis*	Chloridoideae	Sangrur, Patiala	5
22.	*Eragrostis poaeoides*	Chloridoideae	Patiala, Ludhiana	7
23.	*Eleusine indica*	Chloridoideae	Sangrur, Patiala, SAS Nagar	9
24.	*Cenchrus pennisetiformis*	Panicoideae	Patiala, Sangrur	10
25.	*Cenchrus ciliaris*	Panicoideae	Patiala, Sangrur, Jalandhar	8
26.	*Setaria glauca*	Panicoideae	Patiala, Sangrur, SAS Nagar,	11
27.	*Pancium antidotale*	Panicoideae	Sangrur, Jalandhar, Bathinda	10
28.	*Vetiveria zizanioides*	Panicoideae	Patiala, Sangrur	8
29.	*Oriza sativa*	Oryzodeae	Sangrur, Patiala	8
30.	*Avena sativa*	Pooideae	Patiala, Ludhiana	7
31.	*Acrachne racemosa*	Chloridoideae	Patiala, Sangrur, SAS Nagar, Ludhiana	5
32.	*Urochloa panicoides*	Panicoideae	Patiala, SAS Nagar, Sangrur	9
33.	*Sorghum halepense*	Panicoideae	Patiala	5
34.	*Setaria verticillata*	Panicoideae	Patiala, Sangrur, SAS Nagar, Kapurthala	9
35.	*Imperata cylindrical*	Panicoideae	Sangrur, Jalandhar, Patiala	12
36.	*Setaria intermedia*	Panicoideae	Patiala, Sangrur, SAS Nagar,	8
37.	*Paspalum scrobiculatum*	Panicoideae	Jalandhar, Faridkot	9
38.	*Echinochloa crusgalli*	Panicoideae	Kapurthala, Sangrur, Ludhiana	6
39.	*Panicum trypheron*	Panicoideae	Sangrur, Bathinda	8
40.	*Eragrostis diplachnoides*	Chloridoideae	Patiala, Sangrur	7

Table 2. HPTLC profilies of selected grass leaf stains using solvent system Toluene: Ethyl Acetate: Formic acid: Methanol (60:15:15:10 v/v/v/v) under UV light at 366nm

S. No.	Rf → / Standard/Grass species ↓	0.03	0.07	0.18	0.21	0.28	0.32	0.36	0.40	0.45	0.49	0.52	0.57	0.62	0.66	0.71	0.75	0.81	0.85	0.90	0.95
S1	Chlorophyll	*				*				*					*	*	*				
S2.	Carotenoid		*	*	*	*		*					*		*		*		*		*
1.	*Cynodon dactylon*	*		*		*		*				*	*	*	*		*		*	*	
2.	*Dactyloctenium aegyptium*	*		*		*		*				*		*	*	*			*	*	*
3.	*Cenchrus biflorus*	*		*		*				*			*	*	*	*			*		
4.	*Dinebra retroflexa*	*	*	*		*		*						*	*		*	*	*		
5.	*Bracheria ramose*	*		*		*				*	*		*	*	*				*		
6.	*Echnochloa colonum*		*	*		*		*			*			*			*	*			*
7.	*Panicum maximum*	*		*		*	*				*	*		*	*	*		*	*	*	*
8.	*Digitaria abludens*		*		*		*		*		*		*	*	*	*		*	*		
9.	*Arundo donax*		*	*				*						*	*		*	*			*
10.	*Arundinella nepalensis*	*			*	*			*			*		*	*	*	*		*		*

Table 3. HPTLC profiles of selected grass leaf stains using solvent system Toluene: Ethyl Acetate: Formic acid: Methanol (60:15:15:10 v/v/v/v) under UV light at 366nm

S. No.	Rf → / Standard/Grass species ↓	0.03	0.07	0.18	0.21	0.28	0.32	0.36	0.40	0.45	0.49	0.52	0.57	0.62	0.66	0.71	0.75	0.81	0.85	0.90	0.95
S1	*Chlorophyll*	*				*				*					*	*	*				
S2.	*Carotenoid*		*	*	*	*		*					*		*		*		*		*
11.	*Cenchrus setigerus*		*	*	*				*		*	*	*	*	*		*		*		*
12.	*Poa annua*		*	*	*	*			*			*		*	*	*		*	*		*
13.	*Triticum aestivum*		*	*		*		*			*		*	*	*			*	*		*
14.	*Sporobolus diander*	*		*		*			*			*		*		*		*	*		*
15.	*Bothriochloa pertusa*																				
16.	*Leptochloa panacea*		*	*		*		*	*				*			*	*	*	*		*
17.	*Dichanthum annulatum*		*	*	*		*								*		*		*		
18.	*Polypogon monospeliensis*		*	*		*		*	*							*	*	*	*	*	
19.	*Paspalidium flavidum*		*	*	*		*			*			*				*		*		
20.	*Digitaria ciliaris*		*		*	*				*							*		*	*	

Table 4. HPTLC profiles of selected grass leaf stains using solvent system Toluene: Ethyl Acetate: Formic acid: Methanol (60:15:15:10 v/v/v/v) under UV light at 366nm

S. No.	Rf → / Standard/Grass species ↓	0.03	0.07	0.18	0.21	0.28	0.32	0.36	0.40	0.45	0.49	0.52	0.57	0.62	0.66	0.71	0.75	0.81	0.85	0.90	0.95
S1	Chlorophyll	*				*				*					*	*	*				
S2.	Carotenoid		*	*	*	*		*					*		*		*		*		*
21.	*Leptochloa chinensis*		*		*	*								*			*	*	*	*	*
22.	*Eragrostis poaeoides*	*	*	*		*		*									*	*		*	*
23.	*Eleucine indica*		*	*		*		*			*				*		*	*			
24.	*Cenchrus pennisetiformis*			*	*	*						*				*	*	*		*	
25.	*Cenchrus ciliaris*		*	*		*			*					*			*		*	*	
26.	*Seteria glauca*	*	*	*	*		*		*						*	*	*		*	*	
27.	*Panicum antidotale*			*	*		*		*	*					*		*		*		
28.	*Vetiveria zizinoides*	*		*	*		*		*						*		*	*	*		*
29.	*Oriza sativa*		*		*		*		*						*		*	*	*		*
30.	*Avena sativa*	*	*	*	*					*				*				*	*	*	

Table 5. HPTLC profiles of selected grass leaf stains using solvent system Toluene: Ethyl Acetate: Formic acid: Methanol (60:15:15:10 v/v/v/v) under UV light at 366nm

S. No.	Rf → / Standard/Grass species ↓	0.03	0.07	0.18	0.21	0.28	0.32	0.36	0.40	0.45	0.49	0.52	0.57	0.62	0.66	0.71	0.75	0.81	0.85	0.90	0.95
S1	Chlorophyll	*				*				*					*	*	*				
S2.	Carotenoid		*	*	*	*		*					*		*		*		*		*
31.	*Arachne racemosa*	*								*	*						*			*	*
32.	*Urochloa panicoides*	*	*								*						*		*	*	*
33.	*Sorghum halepense*		*	*		*		*	*				*			*	*	*	*		*
34.	*Seteria verticilata*		*	*	*		*										*		*		
35.	*Imperata cylindrica*		*	*		*		*	*				*			*	*	*	*	*	
36.	*Seteria intermedia*		*	*	*		*			*							*		*		
37.	*Paspalum scorbiculatum*		*		*	*				*							*		*	*	
38.	*Echnochloa crusgalli*		*		*	*											*	*	*	*	*
39.	*Panicum trypheron*	*	*	*		*		*									*	*		*	*
40.	*Eragrostis diplachnoides*		*	*		*		*			*				*		*	*			

The methanolic extracts of respective samples on development with selected solvent system showed number of distinctive peaks of different Rf values with different area percentage at 366 nm, thus showing qualitative variations of the phytoconstituents in the selected extracts. The chromatograms of all the selected samples and pigment standards at 366 nm revealed that all sample constituents were clearly separated without any tailing and diffuseness. The results of chromatogram for methanolic extract of selected samples with their Rf values has been given in Table 2-5. The chromatograms of the selected samples have been recorded and are depicted in Fig. 1-4. The similarity in the Rf values of the peaks indicated the presence of specific compounds in the extracts of respective samples. The difference in Rf values in peaks reflected qualitative variation in the phytoconstituents of selected grass species. From the analysis of chromatogram of selected standards, the chlorophyll standard showed total of six peaks with Rf values 0.03, 0.28, 0.45, 0.66, 0.71 and 0.75 as given in Table 2-5 and Fig 1-4. This indicate the occurrence of six different components i.e. chlorophyll a, b, c_1, c_2, d and f with different polarity. The components chlorophyll c_1 and c_2 are more polar as compared to other components. In general, more the number of separated components of samples, higher is degree of diversity. Maximum number of five peaks of chlorophyll has been observed in samples 3 and 9. The peak with Rf value 0.03 of chlorophyll component has been found in sample 1-5, 7, 10, 14, 22, 26, 28, 30-32 and 39. The peak with Rf value 0.28 has been found in sample no. 1-7, 10, 12-14, 16, 18, 20-25, 31, 34, 35 and 37-40. The peak of component with Rf value 0.45 has been shown by very less number of samples i.e. 3, 5, 19, 20, 27, 30, 31, 36 and 37. The samples 1-5, 7-13,17, 23, 26-29, 32 and 40 possess peak of chlorophyll with Rf value 0.66. Peak of carotenoid with Rf value 0.71 has been shown by samples 2, 3, 7, 8, 10, 12, 14, 16, 18, 24, 33 and 35. All the samples except 2, 3, 5, 7, 8, 12-15 and 30 showed peaks of chlorophyll component with Rf value 0.75.

The carotenoid standard exhibited ten peaks with Rf values 0.07, 0.18, 0.21, 0.28, 0.36, 0.57, 0.66, 0.75, 0.85 and 0.95 indicates the occurrence of ten different components i.e. α-carotene, β-carotene, γ-carotene, xanthophylls (lutein, zeaxanthin, neoxanthin, violaxanthin, flavoxanthin, α-cryptoxanthin, β-cryptoxanthin). The carotenoid standard showed the presence of ten different types of carotenoids with different Rf values as shown in Table 2-5 and Fig. 1- 4. Maximum number of six carotenoid components peaks has been observed in samples 11, 13, 16, 18, 33 and 35. The peak with Rf value 0.07 of component of carotenoid has been found in sample 4, 6, 8, 11-13, 16-23, 25, 26, 29, 30, 32-40. The peak with Rf value 0.21 of carotenoid component has been found in sample no. 8, 10-12, 17, 19-21, 24, 26-30, 34, 36-38. The peak of component with Rf value 0.36 has been found in samples 1, 2, 4, 6, 9, 13,16,18, 22, 23, 33, 35, 39 and 40. The samples 1, 3, 5, 8, 11, 13, 16, 19, 33 and 35 possess peak of component of carotenoid with Rf value 0.57. All the samples except 6, 9, 15, 22, 23, 24, 31, 39 and 40 showed peaks of carotenoid component with Rf value 0.80. The peak of carotenoids with Rf value 0.90 and 0.95 has been shown by samples 1, 2, 7,18, 20, 21, 22, 24, 25, 26, 29, 30, 31, 35, 37, 38, 39 and 2, 6, 7, 9, 10, 11, 12, 13, 14,16, 21, 22, 28, 29, 31, 32, 33, 38, 39 respectively. This difference in Rf values of the appeared peaks reflected the qualitative variation in the phytoconstituents of the methanolic extracts of the selected samples. No spot was observed for negative controlled extract. In respect to the discrimination between leaf stain of two grasses of same species collected from different locality, no difference in their chromatogram was observed. The aliquot of leaf stains of grass samples were analyzed five times under same set of experimental conditions to check the reproducibility of results and they were in concordance with each other. Thus, the results obtained showed that HPTLC with appropriate solvent system as mobile phase permits better separation of the constituents of leaf stained samples of selected grass species as compared to TLC.

DISCUSSION

The identification and differentiation of extracts of selected samples at generic and species level is very decisive. Various morphological, anatomical, cytological or molecular based methods are used to identify and differentiate them. But the latter are costly. So, the chromatographic based techniques can serve the purpose. The chromatographic based techniques analyze the different phytoconstituents present in the samples.
The HPTLC is the fastest and automated technique with high sensitivity, accuracy and precision in comparison to TLC. The spots/bands are better resolved than TLC. In the present study, the results of HPTLC profiling were scanned at wavelength 366 nm for the methanolic extracts of standards chlorophyll and carotenoid and different extracts of leaf of selected samples. The Rf values for standards chlorophyll and carotenoid and different extracts of selected samples were given in Table: 2-5 respectively and the chromatograms were given in Fig: 1-4. All the phytocontituents of standards and samples were separated without any tailing and shadowing. The results obtained with HPTLC analysis were better as compared to TLC because with former more number of bands and peaks has been observed for the standards and extracts of each selected sample. The analysis of chromatogram showed that the each sample showed different chromatographic profile i.e. each sample contain different group of combination of phytoconstituents. The difference in number of peaks and Rf values is the evidence of qualitative variations of the phytoconstituents in the different extracted samples studied. Very fewer studies have been conducted on the HPTLC profiling of grass species, their parts or stains. As per our knowledge and literature reviewed, very less work has been conducted on HPTLC profiling of grass species from their parts or stains. The total of eight phytochemical constituents with Rf value 0.09, 0.10, 0.18, 0.26, 0.34, 0.48, 0.58 and 0.86 had been extracted from plant *Cynodon dactylon* using solvent system Ethyl acetate : n hexane (20:80) (Geetha, 2015). In the present study, total of eleven phytochemical constituents have been observed with Rf values 0.03, 0.18. 0.28, 0.36, 0.57, 0.66, 0.75, 0.85 and 0.95 using HPTLC of *C. dactylon*. In the previous study, the TLC analysis of methanolic extract of stains the twenty grass species belonging to subfamily Panicoideae was recorded using the above discussed solvent system. The standard carotenoid showed total of five spots *i.e.* five components with TLC (Chandel *et al*., 2013) but with HPTLC, the selected standard resolved into ten different

components with different Rf values. The same findings were observed for the samples analyzed. This study could serve as an appropriate approach for analyzing the phytoconstituents mainly chlorophyll and carotenoid components in the extracts of the selected samples studied herein. The HPTLC technique has been proved to be a liner, precise, accurate method for differentiating the selected species.

Conclusion

The results indicated that extracts of selected grass stains is composed of number of different phytoconstituents which cause variation among the samples. Hence it is very important to obtain reliable chromatographic profile with chromatographic techniques. Among sophisticated techniques used for phytochemical profiling, TLC serves as a preliminary method in providing the chromatographic profiles but the HPTLC profiling can be used a diagnostic tool for the identification and differentiation of selected species. From the HPTLC studies, the results reveled the qualitative evaluation of HPTLC finger print profiles which could be used in differentiating the selected species. The presence or absence of any phytochemical constituent or the combination of phytochemical constituents present can help in differentiating the samples. Though, further work is required to characterize the other unknown phytoconstituents with marker compounds so that the findings can be used for proper species identification for the use in criminal justice system and other biological field.

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