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Full Length Original Research Paper

Phytochemical and Chromatographic Fingerprint Studies on Chloroformic Extracts of *Cassia tora* L.

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Abstract:

Objective: To lay down the chromatographic profile of chloroform extracts of *Cassia tora* L. by HPTLC technique. **Methods:** Chloroformic extracts prepared from leaf, flower and seed of *Cassia tora* L. using Soxhlet apparatus. Qualitative phytochemical screening was done and HPTLC analysis was carried out using CAMAG HPTLC system equipped with automatic TLC sampler IV, TLC scanner 3, REPROSTAR 3 with 12 bit CCD camera for photo documentation, winCATS Planer Chromatography software. **Results:** The results of qualitative phytochemical analysis confirmed the presence of alkaloids, tannins, flavonoids and terpenoids, but absence of saponins. HPTLC determination of leaf extract showed the presence of 10 components with Rf values ranging from 0.33 - 0.92; seed revealed the presence of 8 components with Rf values in the range of 0.35 – 0.91; flower extract displayed presence of 11 components with Rf values in the range of 0.33 – 0.94. **Conclusion:** The presence of phytochemicals like flavonoids, terpenoids, tannins and alkaloid in *Cassia tora* L. increase the probable role in biological activities and their use in medicine. The HPTLC finger printing of *Cassia tora* L. may be useful in differentiating the species and act as a biochemical marker for this plant.

Keywords: HPTLC, medicinal plant, phytochemicals, *Cassia tora*, biochemical marker

Introduction

Cassia tora is a tropical herb grows as waste land rainy season weed in India possess various biological and pharmacological activities including antihepatotoxic [1], antiallergic [2], antimutagenic [3], antioxidant [4], antibacterial [5] and antifungal [6]. The presence of different phytochemical substances in plant found to exhibit antioxidant/free radical scavenging properties [7-8]. Secondary metabolites provide defensive action to the plants against predation caused by many microorganisms, insects and herbivores [9-10]. Advancement in chromatographic and spectral fingerprints plays an important role in the quality control of complex herbal medicines [11]. Chemical finger prints obtained by chromatographic techniques are strongly recommended for the purpose of quality control of herbal medicines, since they represent appropriately the chemical integrities of the herbal medicines and its products and therefore used for authentication and identification of herbal plant [12]. HPTLC is more efficient, faster and the results are more reliable and reproducible. In combination with digital scanning profile, HPTLC also provides accurate and precise Rf values and quantitative analysis of sample by *in situ* scanning densitometry aided by formation of easily detected derivatives by post-chromatographic chemical reactions as required, as well as a record of the separation in the form of a

chromatogram with fractions represented as peaks with defined parameters including absorbance (intensity), Rf, height and area [13]. Furthermore, the pictorial fluorescence image of HPTLC coupled with a digital scanning profile is more and more attractive to herbal analysts for constructing herbal chromatographic fingerprint by means of HPTLC and provide adequate information and parameters for comprehensive identification, assessment and comparison of major active constituents fingerprint serve as a basis for their use in medicinal preparations [13-14]. Though different parts of *Cassia tora* L. possess significant medicinal properties, there is no published data available on phytochemical constituents and HPTLC profiling of *Cassia tora* L chloroformic extracts. In the previous study, HPTLC profile of *Cassia tora* L. in petroleum ether and methanol were standardized and developed [15-16]. Consequently, the main objective of present study was to develop the chemical fingerprinting of *Cassia tora* extracts prepared in non-polar solvent chloroform by HPTLC technique.

Materials and Methods

Collection and Identification

The *Cassia tora* plant parts viz. leaf, flower and seed were collected from the waste land and road side of Amravati city of Maharashtra, India. The plant materials were identified and authenticated (Auth. 13-023) by Scientist, Plant Drug Authentication Service Botany Group, Plant Sciences Division, Agharkar Research Institute, Pune (MS) India.

Extraction of Plant Material

The shade dried plant parts were ground by mechanical grinder into coarse particles using the sieve number 2000µm. The ground material was extracted with 250ml of chloroform in the ratio of 1:6 w/v in a Soxhlet apparatus at 35-40°C until the extract was clear or colourless. Controlled conditions of temperatures were maintained to avoid loss of heat sensitive phytochemicals. Extracts were filtered through Whatman No.1 filter and clarified extracts were concentrated in a rotary evaporator under reduced pressure at 40°C. Dried extracts were weighed in an analytical balance. The extracted materials were stored at 4°C until use.

Phytochemical Screening

Preliminary phytochemical analysis was performed for establishing the phyto-constituents (alkaloids, tannins, saponins, flavonoids and terpenoids) present in the chloroform extract [17-19].

Test for Alkaloids

Solvent free extract (50 mg) was stirred with few ml of diluted HCl and filtered. The filtrate was tested carefully with alkaloid reagents as follows:

Mayer's test

To 1.2 ml of filtrate, 0.1 ml of Mayer's reagent was added by the side of the test tube. A white creamy precipitate indicated the presence of alkaloid.

Mayer's reagent:

Mercuric chloride, 1.358 g was dissolved in 60 ml of water and 5 g of potassium iodide was dissolved in 10 ml of water. The two solutions were mixed and made up to 100 ml with water.

Test for Tannins

About 0.1 g each portion was stirred with about 2 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. Occurrence of a blue-black, green or

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blue-green precipitate indicates the presence of tannins.

Test for Saponins

One hundred milligram of each plant extracts were boiled with 1 ml of distilled water, filtered. To the filtrate, about 0.5 ml of distilled water was further added and shaken vigorously for about 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins.

Test for Flavonoids

Alkaline reagent

One hundred milligram of each plant extracts were dissolved in 5 ml of water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of flavonoids.

Shinoda's test

In a test tube containing 0.5 ml of extract, 5-10 drops of diluted HCl and small piece of ZnCl or magnesium were added and the solution was boiled for few min. Appearance of reddish brown colour indicated the presence of flavonoids.

Test for Terpenoids

Three ml of each extract was mixed in 1 ml of chloroform, and concentrated H_2SO_4 (1 ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

HPTLC Fingerprinting

Chemical and Equipments: CAMAG HPTLC system equipped with automatic TLC sampler IV, TLC scanner 3, REPROSTAR 3 with 12 bit CCD camera for photo documentation, winCATS Planer Chromatography software. All the solvents used for HPTLC analysis were obtained from Merck, India.

HPTLC Method: HPTLC analysis was carried out using the method described by Misra *et al.*, 2008 with some modification [20]. The 20 mg of extract was dissolved in 1 ml methanol and centrifuged at 3000 rpm for 5 minutes and used for HPTLC analysis as test sample. The aliquot of 5 µl of the above samples and a mixture of standard solution containing gallic acid, quercetin and anthranol glycoside were loaded as 8 mm band length at a 10 mm application position in a 10 × 10 cm silica gel 60 F 254 TLC plate using a CAMAG automatic TLC sampler IV (CAMAG, Muttenz, Switzerland). The plates were prewashed with chloroform and dried in an oven at 60°C for 5 minutes. The samples loaded plates were kept in TLC Twin Trough Chamber (20x10cm) for saturation with the solvent vapours with respective mobile phase. The plates were developed in a linear ascending mode upto 80 mm.

The mobile phase n-Hexane: Ethyl acetate: Formic acid: Acetic acid (60:40:2.5:2.5) used. After the chromoplate development time of 20 min, the plate was air-dried for 15 min to evaporate solvents. These plates were kept in photo-documentation chamber (CAMAG REPROSTAR 3) observed under white light, UV at 254 and 366 nm and the images were documented. Separated bands were quantified by HPTLC densitometric scanning using Camag TLC Scanner III in the remission–absorption mode at 254 and 366 nm operated by winCATS planar chromatography software 4 to obtain the Rf value and peak areas of the chromatogram. The slit dimension of 4 ×

0.30 mm and the scanning speed of 20 mm/s were maintained to record the peak areas of the resolved bands. After derivatization, the plate was fixed and scanning was done at 298 nm by TLC Scanner 3. The peak table, display and densitogram were recorded.

Results and Discussion

The preliminary phytochemical studies revealed the presence of flavonoids, tannins and terpenoids in the leaf extract, while alkaloids and saponins were found to be absent. Flavonoids and terpenoids were present but no alkaloids, tannins and saponins in flower extract. In the seed extract; alkaloids, flavonoids and terpenoids were found to be present, whereas saponins and tannins were absent. HPTLC profile of chloroform extracts was studied by using solvent system n-Hexane: Ethyl acetate: Formic acid: Acetic acid (60:40:2.5:2.5), detected under UV 366, 254 nm and white light is shown in the Fig. 1.

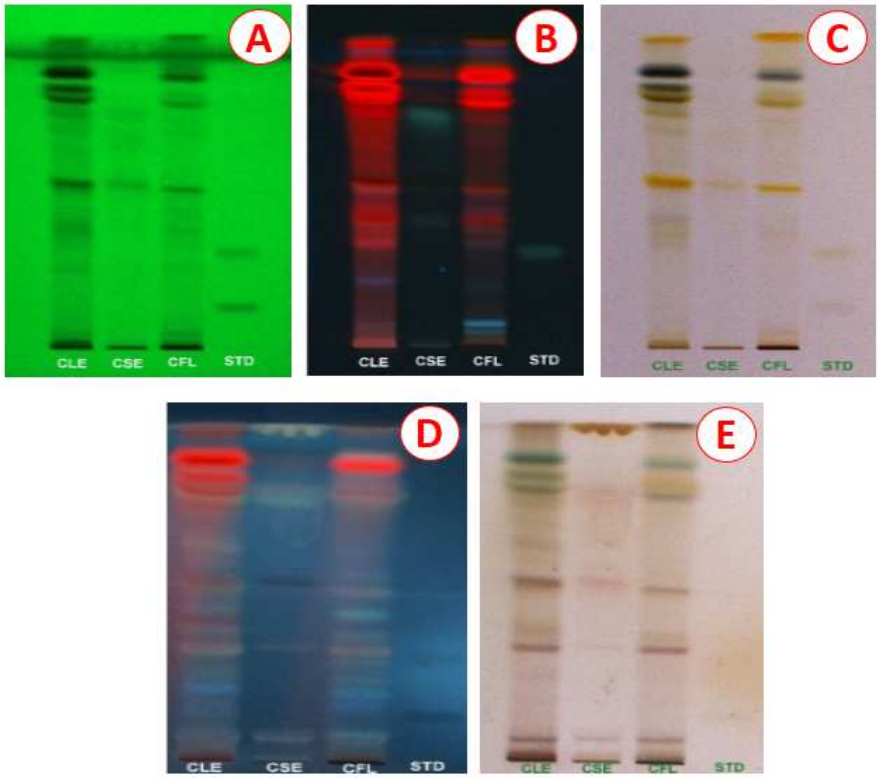

Fig. 1 HPTLC profile of chloroform extracts of *Cassia tora* under 254 nm, UV 366 nm and white light. A) 254nm B) 366 nm C) White light D) After derivatization - 366 nm E) After derivatization - white light (Extracts: CLE: Chloroform leaf, CFL: Chloroform flower, CSE: Chloroform seed, STD: Standard).

The HPTLC densitogram of chloroform extract of *Cassia tora* leaf, seed and flower revealed several peaks which are presented in Fig. 2. Standards including quercetin, gallic acid and anthranol glycoside also clearly revealed three peaks with Rf values 0.14, 0.30 and 0.91 respectively. The corresponding HPTLC chromatograms are presented in Fig. 3-6. The leaf extract revealed 10 spots with Rf values in the range 0.33-0.92 (Fig. 3 and Table 1). Out of these 10 spots, 5 spots with the Rf value 0.51, 0.71, 0.75, 0.79 and 0.83 were recorded as principal components with the percentage area of 13.93, 11.04, 12.02, 11.05 and 21.57 respectively. The chloroformic extract of seed exhibited 8 peaks with Rf values in the range of 0.35-0.91, but the

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spots with Rf value 0.50, 0.62, 0.71 and 0.88 possesses maximum percentage area of 30.64, 21.74, 12.71 and 13.37 respectively. While other exhibited comparatively lower percentage area and reported below 10% in the range of 3.13-9.21 (Fig. 4 and Table 2). These 4 components with high percentage area may be responsible for biological activities of *C. tora* seed either individual or combined state.

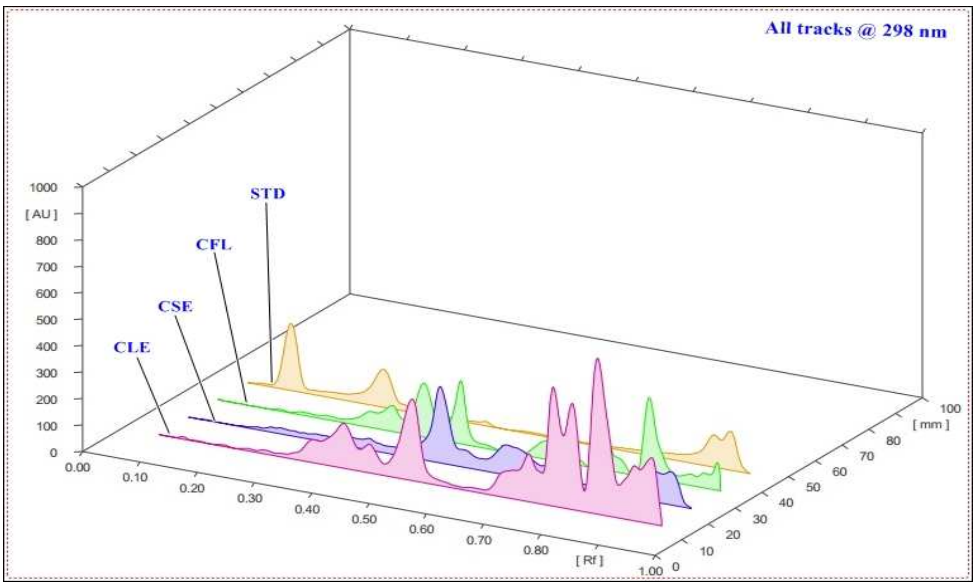

Fig. 2 HPTLC densitogram of chloroform extracts of *Cassia tora* L.

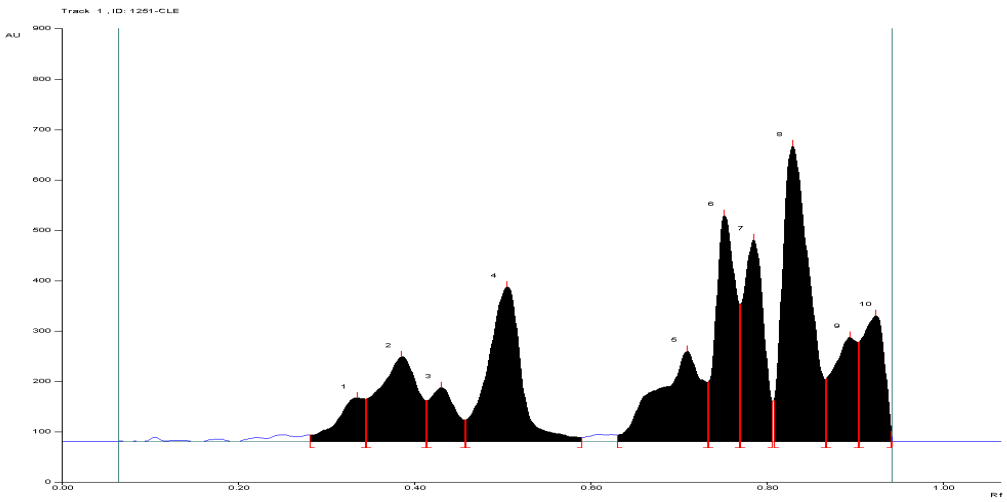

Fig. 3 HPTLC chromatogram of chloroform leaf extracts showing different peaks of phytoconstituents.

Table 1: Peak list and Rf value of the chromatogram of 5µl of chloroform extract of leaf

Track	Peak	Max Rf	Max Height	Max %	Area	Area %
1	1	0.33	86.9	3.17	2330.4	3.28
1	2	0.39	168.6	6.15	6333.3	8.91
1	3	0.43	107.2	3.91	2666.1	3.75
1	**4**	**0.51**	**306.8**	**11.19**	**9896.4**	**13.93**
1	**5**	**0.71**	**179.7**	**6.55**	**7843.3**	**11.04**
1	**6**	**0.75**	**448.9**	**16.37**	**8542.8**	**12.02**
1	**7**	**0.79**	**400.1**	**14.59**	**7848.4**	**11.05**
1	**8**	**0.83**	**586.6**	**21.39**	**15324.2**	**21.57**
1	9	0.89	207.2	7.56	4881.4	6.87
1	10	0.92	249.8	9.11	5379.2	7.57

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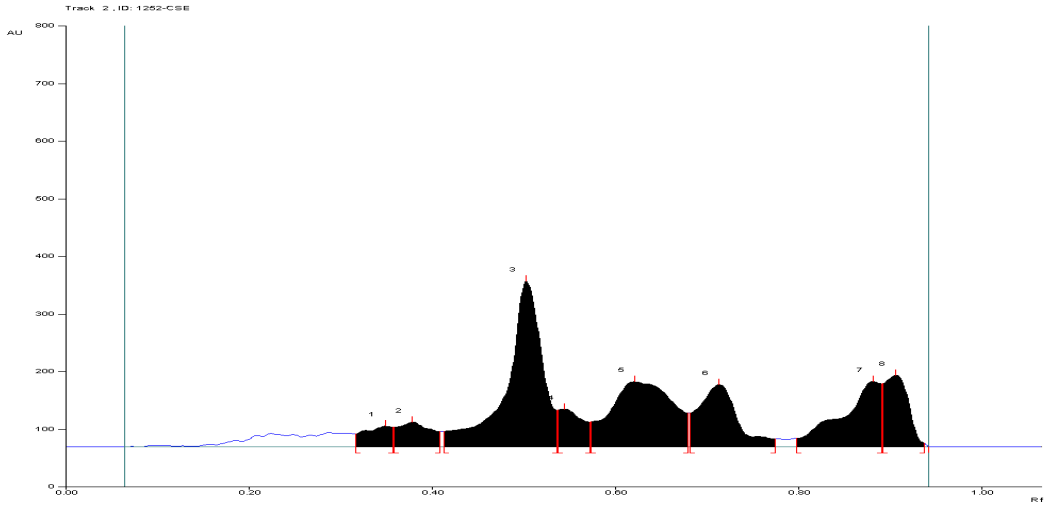

Fig. 4 HPTLC chromatogram of chloroform seed extracts showing different peaks of phytoconstituents.

Table 2 Peak list and Rf value of the chromatogram of 5µl of chloroform extract of seed

Track	Peak	Max Rf	Max Height	Max %	Area	Area %
2	1	0.35	36.0	4.04	969.4	3.13
2	2	0.38	43.1	4.83	1336.9	4.32
2	**3**	**0.50**	**287.5**	**32.28**	**9489.4**	**30.64**
2	4	0.54	65.6	7.37	1511.0	4.88
2	**5**	**0.62**	**113.0**	**12.68**	**6731.3**	**21.74**
2	**6**	**0.71**	**108.0**	**12.13**	**3935.1**	**12.71**
2	**7**	**0.88**	**113.4**	**12.73**	**4140.8**	**13.37**
2	8	0.91	124.2	13.94	2852.4	9.21

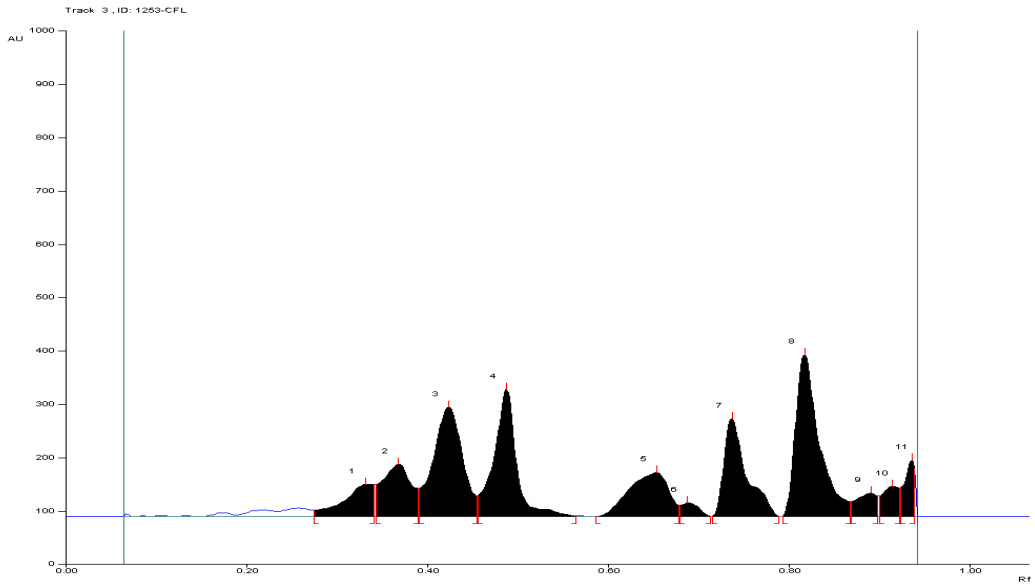

Fig. 5 HPTLC chromatogram of chloroform flower extracts showing different peaks of phytoconstituents.

Table 3: Peak list and Rf value of the chromatogram of 5µl of chloroform extract of flower

Track	Peak	Max Rf	Max Height	Max %	Area	Area %
3	1	0.33	60.8	4.33	1748.2	5.40
3	2	0.37	98.1	7.00	2706.2	8.36
3	**3**	**0.42**	**204.1**	**14.56**	**5719.7**	**17.68**
3	**4**	**0.49**	**238.6**	**17.01**	**5088.9**	**15.73**
3	5	0.65	82.3	5.87	3130.5	9.68
3	6	0.69	25.1	1.79	424.0	1.31
3	**7**	**0.74**	**183.6**	**13.09**	**3934.4**	**12.16**
3	**8**	**0.82**	**303.8**	**21.67**	**6870.6**	**21.24**
3	9	0.89	43.4	3.10	861.2	2.66
3	10	0.91	56.7	4.04	907.3	2.80
3	11	0.94	105.7	7.54	961.4	2.97

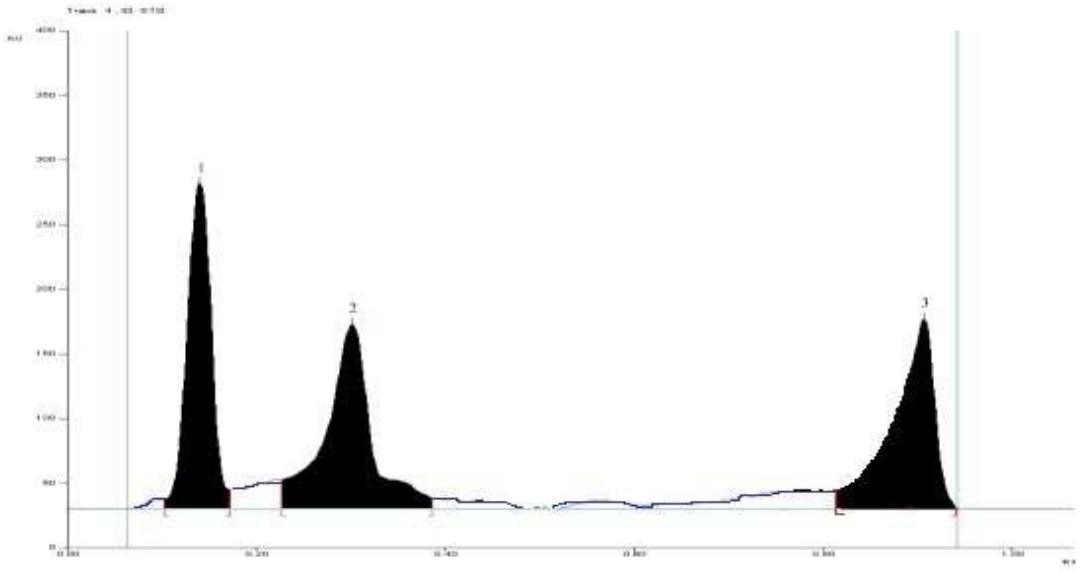

Fig. 6: HPTLC chromatogram of mixture of standards (Quercetin, gallic acid and anthranol glycoside) showing different peaks.

Table 4 Peak list and Rf value of the chromatogram of mixture of standards

Track	Peak	Max Rf	Max Height	Max %	Area	Area %
4	1	0.14	252.6	37.11	5577.7	28.76
4	2	0.30	143.0	21.00	5955.1	30.71
4	5	0.91	146.8	21.56	3002.1	15.48

The flower of *Cassia tora* revealed the presence of 11 constituents in the chloroform extract. The number of constituent in the extract and their Rf values are summarized in Fig. 5 and Table 3. It highlighted that there are 11 spots at the following Rf 0.33, 0.37, 0.42, 0.49, 0.65, 0.69, 0.74, 0.82, 0.89, 0.91 and 0.94; thereby indicating the occurrence of atleast 11 different components in chloroform flower extract. Out of 11 components, the components with Rf values 0.42, 0.49, 0.74 and 0.82 were found to be predominant; with 17.68, 15.73, 12.16 and 21.24 percentage area values respectively. The remaining components were found to be very less in quantity as the

percentage area for all the spots were less than 9.68%. The mixture of standards (gallic acid, quercetin and anthranol glycoside) demonstrated the presence of three peaks with high percentage area as 28.76, 30.71 and 15.48 at Rf values 0.14, 0.30 and 0.91 respectively (Table 4).

Discussion:

Authentication of medicinal plants at genetic and chemical level is a critical step in the use of botanical materials for both research purpose and commercial preparations. For any living organism, identity is very important in order to distinguish itself from other organisms within the population and other populations. In plant taxonomy, during this molecular era, the morphological characters also play a vital role in plant systematic study and are used as a tool for the classification of a taxon. In recent times, in addition to morphological markers, anatomical, cytological, biochemical and molecular markers are also being used to classify the organisms. The presence or absence of chemical constituent has been found useful in the placement of the plant in taxonomic categories. HPTLC profile differentiation is such an important and powerful procedure which has often been employed for this purpose. HPTLC fingerprinting is proved to be a liner, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant. The developed HPTLC fingerprints help the manufacturer for quality control and standardization of herbal formulations. Such finger printing is useful in differentiating the species from the adulterant and act as a biochemical marker for medicinally important plant in the pharmaceutical industry and plant systematic studies [21]. HPTLC is an invaluable quality assessment tool for the evaluation of botanical materials, and it allows for the analysis of a broad number of compounds both efficiently and cost effectively. HPTLC studies have shown that it is more versatile than ordinary TLC methods, as the spots are well resolved. Further work is needed to characterize the chemical constituents and quantitative estimation with marker compounds is necessary for fixing standards to *Cassia tora* L.

HPTLC finger printing profile is useful as phytochemical marker and also a good estimation of genetic variability in plant populations. It is also a valuable tool for reliable identification and provides chromatographic finger prints that can be visualized and stored as electronic images which can be used several times without any errors and change [21].

To our knowledge HPTLC profile of *Cassia tora* L. chloroform extracts were generated for the first time to ascertain the total number of chemical moieties which will help in designing the method of isolation and characterization of bioactive compounds. The solvent system n-Hexane: Ethyl acetate: Formic acid: Acetic acid (60:40:2.5:2.5) standardized in the present work for *Cassia tora* L. chloroform extracts will provide basic information useful for the isolation, purification and characterization of marker chemical compounds.

Conclusion

The total number of chemical moieties present in chloroform extracts of *Cassia tora* L. will help in designing the method of isolation and characterization of bioactive compounds. The

developed chromatogram of *Cassia tora* will be specific with the selected solvent system, n-Hexane: Ethyl acetate: Formic acid: Acetic acid (60:40:2.5:2.5) for chloroform extracts. Further there is a need to characterize the major chemical constituents and their quantitative estimation with marker compounds.

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