**RESEARCH ARTICLE**

# HPTLC Fingerprint Profile of Petroleum Ether Extracts from Wasteland Herb *Cassia tora* L.

**Patil SD *, Wankhade SJ and Maknikar PP**

*Department of Microbiology, Shri Shivaji Science College, Amravati - 444603 (MS) India.*

***Correspondence Author:** Dr. Sahadeo D. Patil, Associate Professor and Head, Department of Microbiology, Shri Shivaji Science College, Amravati - 444603 Maharashtra, India. Email ID: sdpatil20957@yahoo.co.in*

**Manuscript details:**

Received : 28 December, 2013
Finally accepted : 15 February, 2014

Date of publication (online):
30 March, 2014

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

**Editor: Dr. Arvind Chavhan**

**Citation:** Patil SD, Wankhade SJ and Maknikar PP (2014) HPTLC Fingerprint Profile of Petroleum Ether Extracts from Wasteland Herb *Cassia tora* L., *International Journal of Life Sciences,* 2 (1): 23-29.



## ABSTRACT

*Cassia tora* L. (Family: Caesalpiniaceae) is a wild herb generally distributed in tropical countries. In India, it grows as a wasteland rainy season weed. Traditionally, *Cassia tora* L. is used for treating various ailments like leprosy, ringworm infection, ulcers and many more. The aim of the study is to develop a HPTLC fingerprint profile of petroleum ether extracts from aerial parts of *Cassia tora*. The HPTLC method was developed for the separation of phytoconstituents present in petroleum ether extracts using mobile phase of n-Hexane: Ethyl acetate: formic acid: Acetic acid (60:40:2.5:2.5) and scanned under UV at 254 nm, 366nm and under white light. HPTLC fingerprinting of leaf extract displayed 10 constituents, seed (7 constituents) and flower (11 constituents). The HPTLC fingerprinting profile for petroleum ether extract may provide appropriate identification and quantification of non polar marker compounds and in the authentication of this plant.

**Key Words:** HPTLC, *Cassia tora* L., Medicinal plant, Standardization.

## INTRODUCTION

*Cassia tora* has known to possess various biological and pharmacological activities including antihepatotoxic (Wong *et al.,* 1989), antiallergic (Zhang and Yu, 2003), antimutagenic (Choi *et al.,* 1997), antioxidant (Zhenbao *et al.,* 2007), antibacterial (Sharma *et al.,* 2010) and antifungal (Mukherjee *et al.,* 1996). Advancement in chromatographic and spectral fingerprints plays an important role in the quality control of complex herbal medicines (Gong *et al.,* 2005). 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 (Liang *et al.,* 2004). 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 (Moffat, 2001). 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 (Moffat, 2001; Rajani *et al.,* 2001). The present study sought to investigate HPTLC profile of *Cassia tora* for analyzing marker chemical constituents.

## MATERIALS AND METHODS

### Collection and Identification

The *Cassia tora* plant parts viz. leaves, 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 solvent petroleum ether 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.

### 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. 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 methanol 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 for petroleum ether extracts. 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 petroleum ether extracts of *Cassia tora* L. were analyzed by HPTLC using specific solvent system n-Hexane: Ethyl acetate: Formic acid: Acetic acid (60:40:2.5:2.5) and detected under UV at 254 nm, 366 nm and also using white light. In the present study HPTLC finger printing of petroleum ether extracts of *Cassia tora* revealed several peaks along with the mixture of three standards (Quercetin, Anthranol glycoside and Gallic acid). HPTLC profile under 254 nm, 366 nm and white light was recorded along with their corresponding HPTLC chromatograms. Selected solvent system was found to be suitable for quantitative analysis. The HPTLC images; shown in Figure 1 indicate that all the sample constituents were clearly separated without any tailing and diffuseness.

The HPTLC densitogram (Figure 2) was developed for qualitative/quantitative analysis of various P.E. extracts of *C. tora* using HPTLC coupled with detector at 298 nm. The Rf values of the corresponding components were obtained through the software system attached with the instrument. Area corresponding to each peak for the corresponding spot or component determines the concentration of the component in the solution. It is evident from the Table 1 that in 5µl of P.E. extract of leaf; there are 10 spots at the Rf values 0.45, 0.51, 0.55, 0.66, 0.71, 0.77, 0.79, 0.87, 0.91 and 0.93 as shown in

Figure 3, indicating the occurrence of atleast 10 different components. Out of 10 components, the components with Rf values 0.71, 0.87, 0.91, and 0.93 at 298 nm were found to be more predominant as the percentage area is more with 15.13, 21.28, 15.15 and 11.04% respectively, while remaining components were found to be negligible in quantity as the percentage area for all the spots was less than 10%. Table 2 shows that in 5µL of P.E. extract of *Cassia tora* flower there are 11 spots as shown in Figure 4 indicating the occurrence of at least 11 different components in P.E. extract of flower. Out of 11 components, the component with Rf values 0.55, 0.70, 0.86 and 0.91 were found to be more predominant as the percentage area was more with 10.57, 10.08, 14.42 and 22.73 respectively. The Rf values of remaining components were found to be below 10 percentage area.

Table 3 shows that in 5µL of P.E. extract of *Cassia tora* seed there are 7 spots as shown in Figure 5; indicating the occurrence of at least 7 different components in P.E. extract. Out of these, the component with Rf values 0.86 and 0.91 were found to be major ones as the percentage area was more with 37.19 and 37.47 respectively. The mixture of three standards (gallic acid, quercetin and anthranol glycoside) revealed the presence of three major peaks (Figure 6 and Table 4) at Rf values 0.21, 0.32 and 0.91 were recorded predominantly with the percentage area of 42.61, 31.08 and 11.37 respectively.

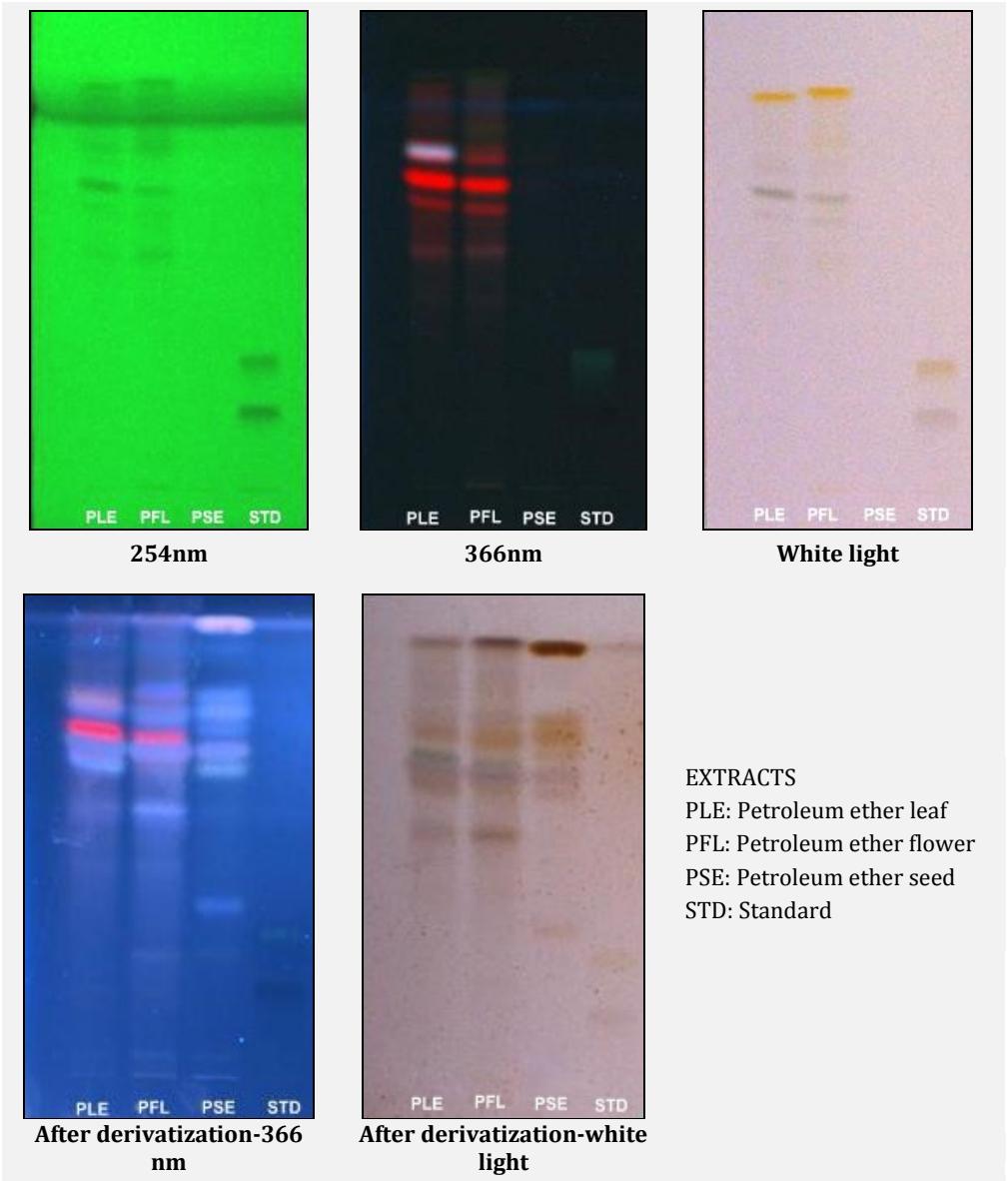


**Figure 1:** HPTLC profile of the P.E. extract of *Cassia tora* under UV at 254 nm, 366 nm and white light.

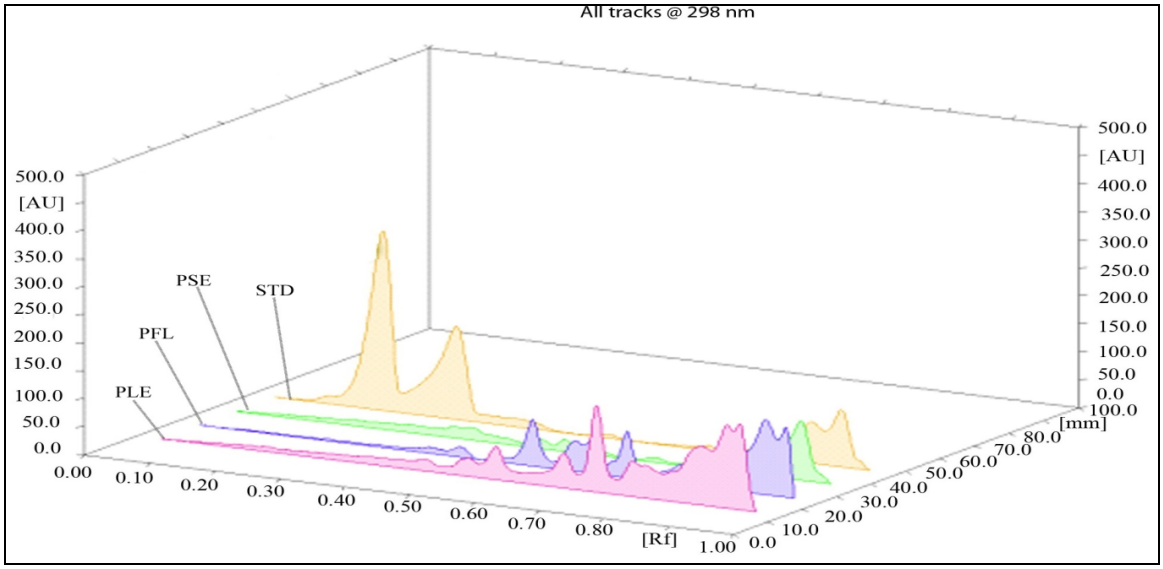


**Figure 2:** HPTLC densitogram of petroleum ether extracts of *Cassia tora* L.

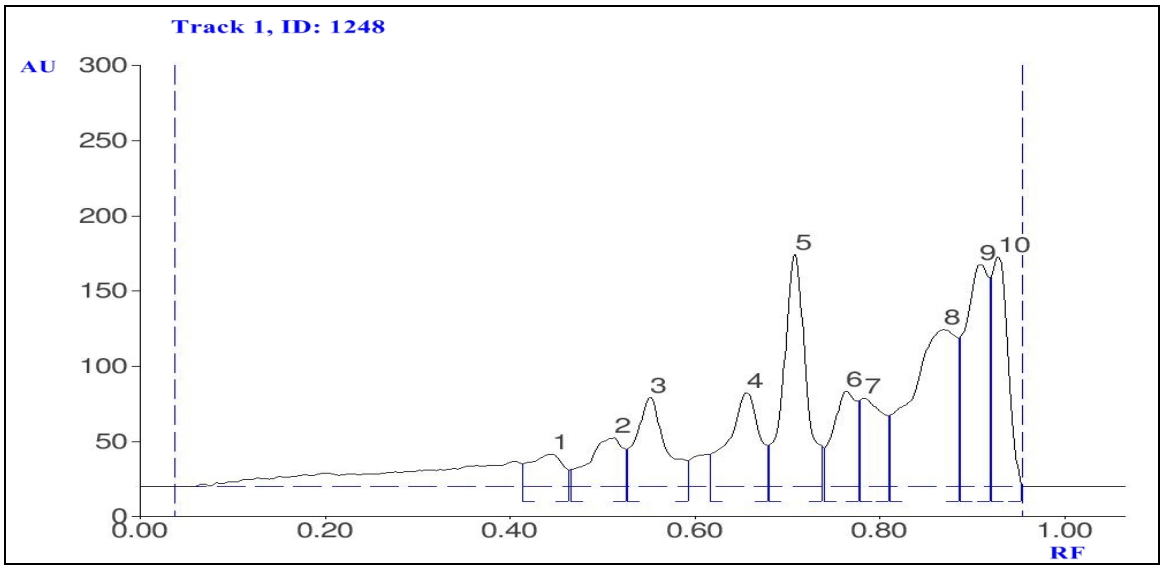


**Figure 3:** HPTLC chromatogram of P.E. leaf extracts showing different peaks of phytoconstituents.

**Table 1:** Peak list and Rf value of the chromatogram of 5µl of P.E. extract of *Cassia tora* L. leaf.

| Track | Peak | Max Rf | Max Height | Max % | Area | Area % |
|---|---|---|---|---|---|---|
| **1** | 1 | 0.45 | 21.8 | 2.54 | 685.0 | 3.22 |
| **1** | 2 | 0.51 | 32.4 | 3.78 | 1034.3 | 4.86 |
| **1** | 3 | 0.55 | 59.4 | 6.92 | 1687.3 | 7.93 |
| **1** | 4 | 0.66 | 62.5 | 7.28 | 1801.6 | 8.47 |
| **1** | **5** | **0.71** | **154.4** | **17.99** | **3219.1** | **15.13** |
| **1** | 6 | 0.77 | 63.3 | 7.37 | 1411.4 | 6.63 |
| **1** | 7 | 0.79 | 58.8 | 6.85 | 1335.6 | 6.28 |
| **1** | **8** | **0.87** | **104.6** | **12.19** | **4527.5** | **21.28** |
| **1** | **9** | **0.91** | **147.9** | **17.23** | **3223.9** | **15.15** |
| **1** | **10** | **0.93** | **153.1** | **17.84** | **2348.1** | **11.04** |

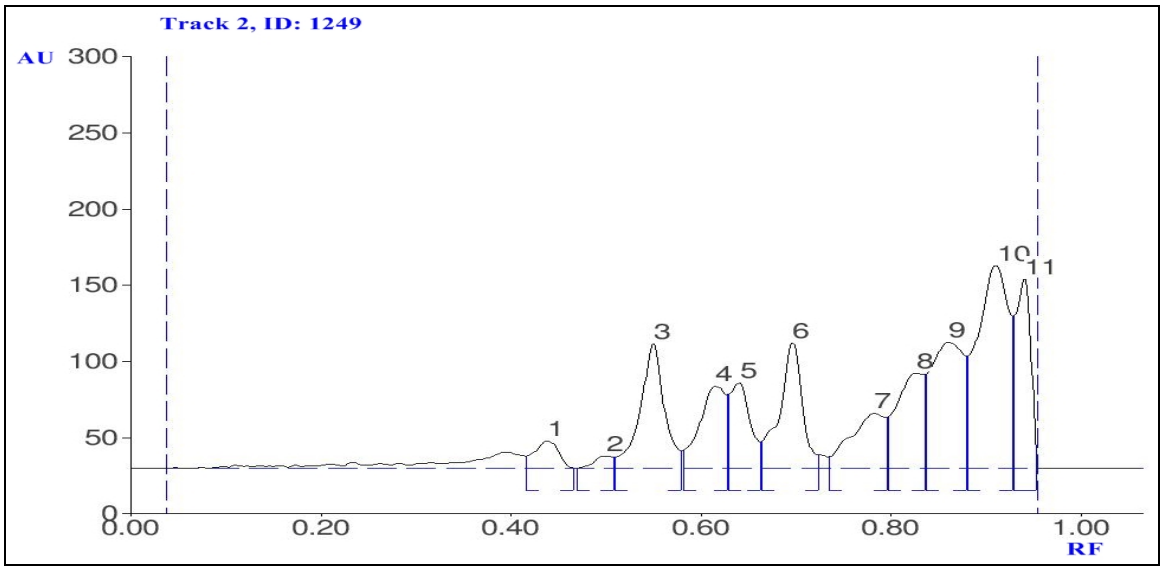


**Figure 4:** HPTLC chromatogram of P.E. flower extracts showing different peaks of phytoconstituents.

**Table 2:** Peak list and Rf value of the chromatogram of 5µl of P.E. extract of *Cassia tora* L. flower.

| Track | Peak | Max Rf | Max Height | Max % | Area | Area % |
|---|---|---|---|---|---|---|
| **2** | 1 | 0.44 | 17.9 | 2.43 | 400.9 | 2.33 |
| **2** | 2 | 0.50 | 8.4 | 1.13 | 135.3 | 0.79 |
| **2** | **3** | **0.55** | **81.9** | **11.08** | **1821.1** | **10.57** |
| **2** | 4 | 0.62 | 53.9 | 7.29 | 1286.2 | 7.46 |
| **2** | 5 | 0.64 | 56.0 | 7.58 | 1055.8 | 6.13 |
| **2** | **6** | **0.70** | **82.1** | **11.11** | **1737.7** | **10.08** |
| **2** | 7 | 0.78 | 36.1 | 4.89 | 1133.9 | 6.58 |
| **2** | 8 | 0.83 | 62.6 | 8.47 | 1549.2 | 8.99 |
| **2** | **9** | **0.86** | **82.8** | **11.20** | **2484.6** | **14.42** |
| **2** | **10** | **0.91** | **133.1** | **18.01** | **3918.2** | **22.73** |
| **2** | 11 | 0.94 | 124.3 | 16.81 | 1713.1 | 9.94 |

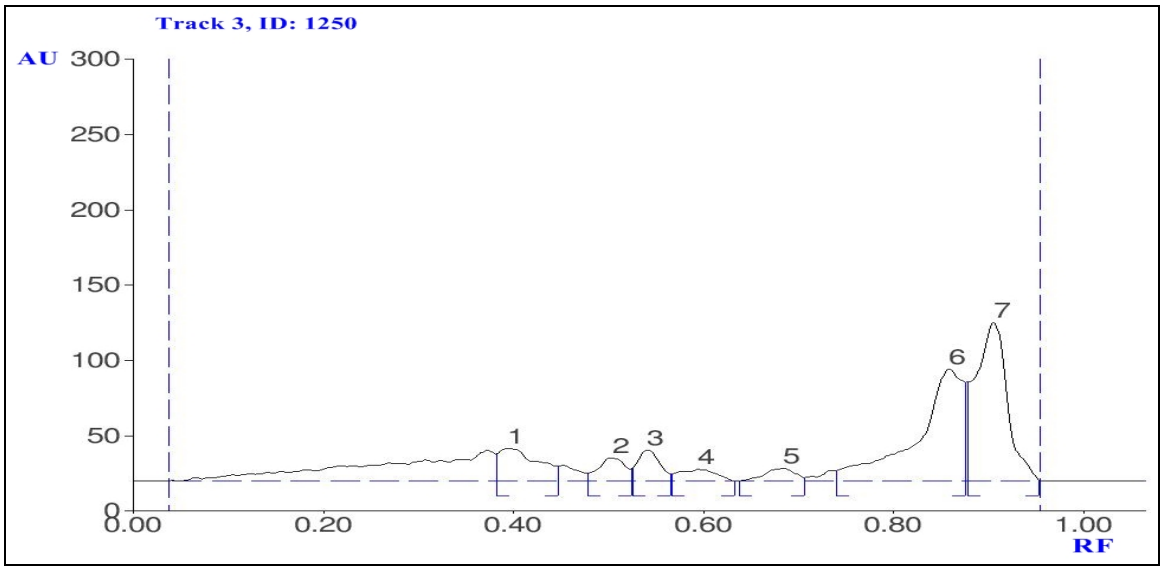


**Figure 5:** HPTLC chromatogram of P.E. seed extracts showing different peaks of phytoconstituents.

**Table 3:** Peak list and Rf value of the chromatogram of 5µl of P.E. extract of *Cassia tora* L. seed.

| Track | Peak | Max Rf | Max Height | Max % | Area | Area % |
|---|---|---|---|---|---|---|
| **3** | 1 | 0.39 | 21.8 | 8.56 | 782.5 | 9.48 |
| **3** | 2 | 0.51 | 15.6 | 6.14 | 379.0 | 4.59 |
| **3** | 3 | 0.54 | 20.36 | 8.11 | 421.2 | 5.11 |
| **3** | 4 | 0.60 | 7.9 | 3.12 | 268.2 | 3.25 |
| **3** | 5 | 0.69 | 8.6 | 3.38 | 239.9 | 2.91 |
| **3** | **6** | **0.86** | **74.5** | **29.31** | **3068.8** | **37.19** |
| **3** | **7** | **0.91** | **105.2** | **41.38** | **3091.3** | **37.47** |

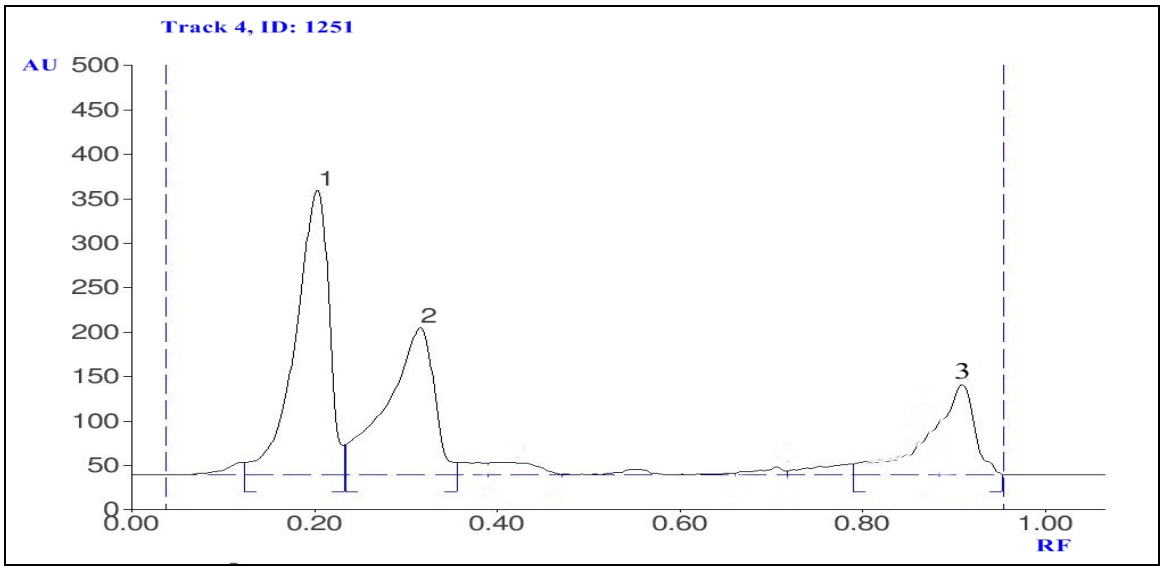


**Figure 6:** HPTLC chromatogram of mixture of standards (Quercetin, gallic acid and anthranol glycoside) showing different peaks.

**Table 4:** Peak list and Rf value of the chromatogram of 5µl mixture of standards

| Track | Peak | Max Rf | Max Height | Max % | Area | Area % |
|---|---|---|---|---|---|---|
| 4 | 1 | 0.21 | 320.1 | 46.78 | 10228.2 | 42.61 |
| 4 | 2 | 0.32 | 165.7 | 24.21 | 7459.9 | 31.08 |
| 4 | 6 | 0.91 | 101.2 | 14.78 | 272 9.3 | 11.37 |

Authentication of medicinal plants at genetic and chemical level is a critical step in the use of botanical materials for both research purposes 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. HPTLC is useful as a phytochemical marker and also a good estimator of genetic variability in plant populations. 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 (Johnson *et al.,* 2011). 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.

In the present study *Cassia tora* L. extracts possess several chemical compounds. The isolation and identification of these compounds can be used to formulate new drugs to treat various diseases and disorders. 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 (Johnson *et al.,* 2011).

## CONCLUSION

The total number of chemical moieties present in *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 petroleum ether extracts. HPTLC fingerprinting of this plant species will also provide basic information useful for the isolation, purification and characterization of marker chemical compounds.

## ACKNOWLEDGEMENT

S. J. Wankhade is thankful to Rajiv Gandhi National Fellowship Scheme, UGC, New Delhi for providing fund.

## REFERENCES

Choi JS, Lee HJ, Park KY, Ha JO and Kang SS (1997) *In vitro* antimutagenic effects of anthraquinone aglycones and naphthpyrone glycoside from *Cassia tora*. *Planta Med*., 63(1): 11-14.

Gong F, Wang BT, Chau FT and Liang YZ (2005) Data preprocessing for chromatographic fingerprint of herbal medicine with chemometric approaches. *Anal. Lett.*, 38(14): 2475-2492.

Johnson M, Mariswamy Y and Gnaraj WE (2011) Chromatographic fingerprint analysis of steroids in *Aerva lanata* L. by HPTLC technique. *Asian Pac. J. Trop. Biomed*., 428- 433.

Liang YZ, Xie P and Chan K (2004) Quality control of herbal medicines. *J. Chromatogr. B*., 812: 53-70.

Misra H, Mehta BK and Jain DC (2008) Optimization of extraction conditions and HPTLC – UV method for determination of Quinine in different extracts of *Cinchona* species bark. *Rec. Nat. Prod*., 2 (4): 107-115.

Moffat CA (2001) Clarke's analysis of drugs and poisons. London: *Pharmaceutical Press*, pp. 392.

Mukherjee PK, Saha K, Saha BP and Pal M and Das J (1996) Antifungal activities of the leaf extract of *Cassia tora* Linn. (Fam. Leguminosae). *Phytotherapy Research*, 10: 521-522.

Rajani M, Ravishankara MN, Shrivastava N and Padh H (2001) HPTLC-aided phytochemical fingerprinting analysis as a tool for evaluation of herbal drugs. A case of Ushaq (*Ammoniacum gum*). *J. Planer. Chromatogr*., 14: 34-41.

Sharma S, Dangi MS, Wadhwa S, Daniel V and Tiwari A (2010) Antibacterial activity of *Cassia tora* leaves. *Int. J. Pharmaceutical and Biological Archives*., 1(1): 84-86.

Wong SM, Wong MM Seligmann O and Wagner H (1989) New antihepatotoxic naphtha-pyrone glycosides from the seeds of *Cassia tora*. *Planta Med*., 55(3): 276-280.

Zhang Z and Yu B (2003) Total synthesis of the antiallergic naphtha-α-pyrone tetraglucoside, cassiaside $C_2$, isolated from *Cassia* seeds. *J. Org. Chem*., 68 (16): 6309-6313.

Zhenbao J, Fei T, Ling G, Guanjun T and Xiaolin D (2007) Antioxidant properties of extracts from juemingzi (*Cassia tora* L.) evaluated *in vitro*. *LWT – Food science and technology.,* 40 (6): 1072 – 1077.