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HPTLC profiling and antibacterial studies of *Cassia tora L* against some pus forming bacteria

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Abstract
Cassia tora L is a seasonal weed of the Leguminosae family, commonly reported to have medicinal properties, such as laxative, anti-helminthic, and is effective against leprosy, ringworm, cough, bronchitis, dizziness. of the heart, etc. In the Ayurvedic medicine system, the Cassia tora has a good reputation for all types of skin diseases. In the present study, we attempted to study the HPTLC profile and antibacterial activity of ethanolic extract from the root, stem, leaves, and seeds of the Cassia tora. Chromatographic Analysis (HPTLC) has shown the existence of several polyvalent phytochemical computers with variable Rf values and concentrations. Antimicrobial extract studies have shown significant growth inhibition against *E. coli, S. aureus, S. epidermidis S. pyogenes, S. pneumoniae,* and *P. aeruginosa*. A mixture of existing extruded polyvalent compounds may be responsible for antimicrobial activity against bacteria producing redness. The results obtained support the use of *C. tora* in a few traditional ethnomedicinal systems. In addition, advanced HPTLC fingerprints can detect their effectiveness in accurate C detection. tora and in finding adultery in herbal medicine and in the preparation of herbs.

Keywords: *Cassia tora* L, leguminaceae, phytochemical compounds, HPTLC

Introduction

Many plants used in India's traditional health care system. They have been promoted for their exciting work at many levels. Among the medicinal plants used in Ayurvedic preparations, some investigated for their medicinal action and others are yet to be tested (Paolo and Esther 2000). These plants are said to be the backbone of traditional remedies. The traditional medicines related to the treatment of both human and animal diseases with plant-derived preparations are providing valuable knowledge for treatment (Nwosu and Okfar, 1995). Cassia belong to Caesalpiniaceae family. The flower consists five identical sepals and petals. *Cassia* species have always been of great interest in phytochemical and pharmacological research because of their excellent therapeutic value (Suchita, 2018). *C. tora* L. is found in a variety of places including a height of 1,400 meters in Himachal Pradesh and especially in vacant areas, roads, field boundaries, etc. It grows best during the rainy season, on dry land in all tropical climates. Flowering and flowering occur in the months of August to October. The plant is usually propagated by seeds. It shows a wide variety of genes depending on plant size, morphology, fruit size, and fruit production. However, the type and number of chemical elements also varies from plant-to-plant Durgesh *et al*. (2013). Different parts of *C. tora* have various medicinal functions such as hepatoprotective, anti-inflammatory, immunostimulatory, antibacterial activity (Sonia and Manisa, 2014). The red bacteria are also called pyogenic bacteria that cause sepsis in various types of wounds. In the present study, we will attempt to study the effect of hydroalcoholic extract on some of these bacteria. The evaluation and quality control of essential herbal medicines is primarily based on phytochemical, pharmacological, and composite methods that include various metallurgical techniques such as microscopy, chromatography, etc. In the present study, we used HPTLC for verifying and identifying the phytoconstituents of *C. tora*. The World Health Organization (WHO) has also emphasized the need to ensure the quality of medicinal plant products using modern controlled methods and the application of appropriate standards. HPTLC fingerprints have a better fixation and the measurement of active components is done with reasonable accuracy in the short term.

Materials and Methods

Collection of Plant material

Different plant parts of *Cassia tora* such as root, stem, leaves, and seeds were collected from nearby local fields of Nanded. Root, stem, leaves, and seeds were dried separately. The dried plant material was made powder using an electric mixture grinder.

Preparation of ethanolic extract

A 20-gram powder was taken from 250 ml of Soxhlet extractor and extracted using 80% ethanol for about six hours and obtained extracts evaporated under reduced pressure. Finally, the extract was stored at a low temperature in the refrigerator for further reading.

HPTLC Analysis of Extracts

HPTLC fingerprints are made in the manner described by Patil et. al. (2014) with minor modifications. Two microliters of ethanolic extracts (belt length -6.0 mm) were used on the pre-fabricated TLC sheet of silica gel G60 F254 for a 200 μm- 05 x10cm thick plate (Merck, Mumbai) using Linomat V. The TLC applicator (Camag, Muttenz, Switzerland) is fitted with a 100-μL syringe. Prior to application, the plate was pre-washed with methanol AR and dried at 60 ° C. TLC plates are developed using the cellular category Toluene: ethyl acetate: Formic Acid (2.5: 2.0: 0.3) in the trough chamber of the Camag HPTLC (10x10cm). The room was filled with filter paper for 15 minutes and plate measurement was done for 10 minutes. The plate is built up to 85.0 mm and dried under air flow. Split bands are measured with HPTLC densitometric scanner using Camag TLC Scanner 4 in absorption mode using Win CATS software (version 1.4.8). After scanning the spectra and the obtained tables are analyzed to interpret the results.

Antibacterial Activity of Extracts

The ability to kill or inhibit the growth of bacteria causes redness by testing the antibacterial test of the extracted plant. This is done in the form of a disc dispersion and the barrier area (ZOI) is measured in mm for each bacterium. The various bacteria used in the study were *E. coli, S. aureus, S. epidermidis, S. pyogenes, S. pneumoniae,* and *P. aeruginosa*. Activated bacterial strains are incorporated into sterile nutrient sources. Sterile 6 mm discs were dipped in each extract and placed on seeded culture. After twenty-four-hour of incubation zone of inhibition in mm was measured against standard antibiotic ciprofloxacin (Sahu, *et al* 2017) [12].

Results and Discussion

The development of chromatographic and spectral fingers plays an important role in regulating the quality of complex pharmacological agents (Gong, *et al*., 2005) [2]. The chemical fingerprints obtained by chromatographic techniques are strongly recommended in the control of the quality of herbal medicines as they effectively represent the chemical integrity of herbal medicines and their products and are therefore used to verify the authenticity and identification of medicinal plants (Liang *et al*., 2004) [2, 5]. HPTLC works very well, is fast and results are reliable and reproducible. Consistent with the digital scanning profile, HPTLC also provides accurate and precise Rf values and sample mass analysis with in situ scanning densitometry assisted in the formation of alternatives that are easily detected by rear chromatographic chemical reactions as required, and chromatomatic fragmentation records as absorption, Rf, length, and location (Moffat, 2001) [6]. HPTLC fingerprints can be used in the proper identification of medicinal plants, as an analytical tool important in the control and suspension of drug paraphernalia, as a chemotaxonomical tool in the plant systematically and in determining the bioactive components of herbal medicine (Goodarzi *et al*., 2013) [3]. The current work also illuminates HPTLC studies with roots, stems, leaves, and seeds extracted from *C. tora*.

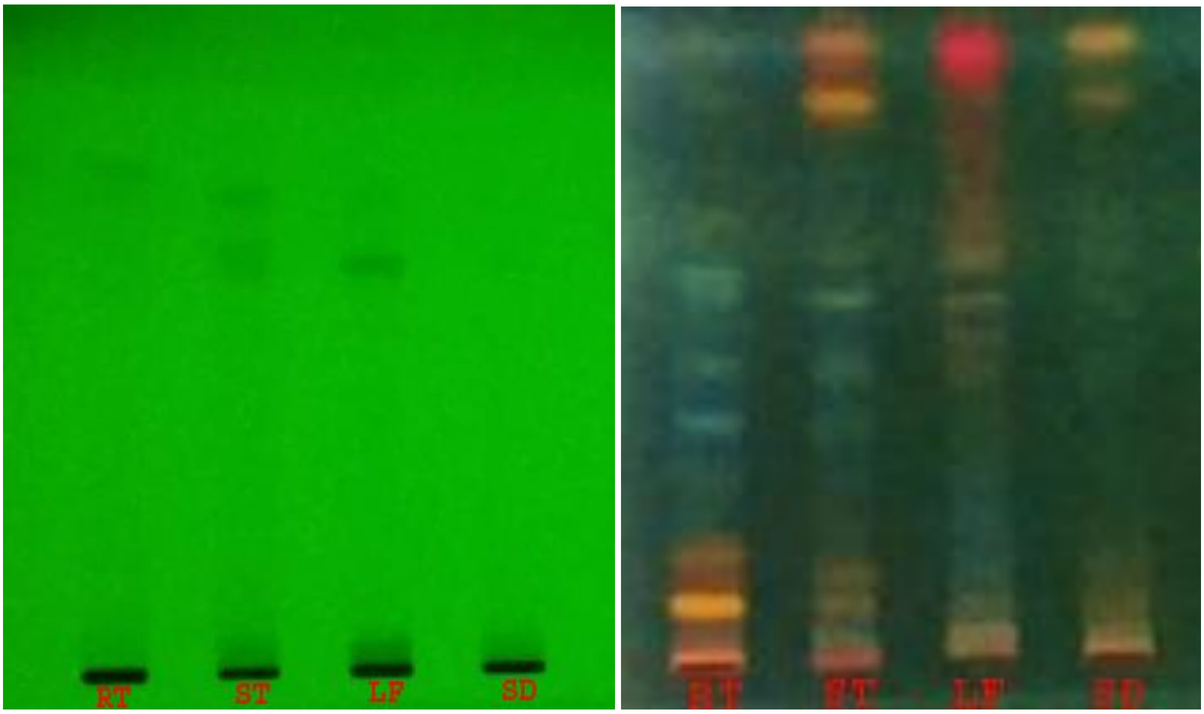

Fig 1: HPTLC Plates at 254 and 366 showing different phytochemicals (RT=Root; ST=Stem; LF=Leaf; and SD=Seed)

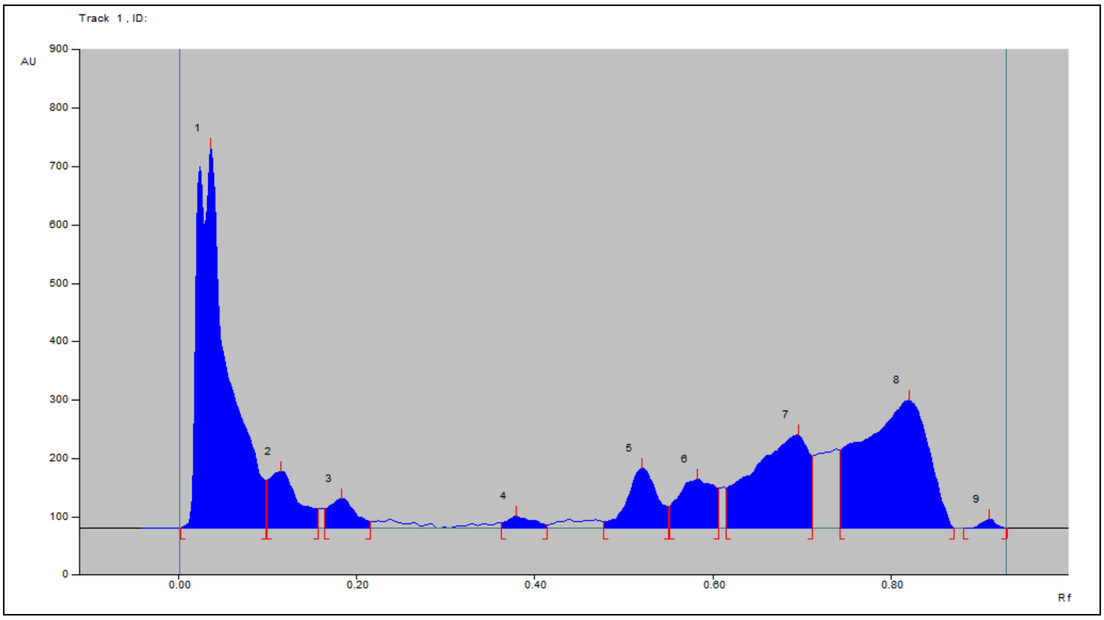

Fig 2: HPTLC profile (Peak Display) of *C. tora* roots at 254 nm

Table 1: HPTLC profile (Peak Table) of *C. tora* roots at 254 nm

Track 1, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	0.7 AU	0.04 Rf	650.3 AU	46.54 %	0.10 Rf	81.5 AU	18306.0 AU	36.14 %
2	0.10 Rf	82.0 AU	0.12 Rf	97.6 AU	6.99 %	0.16 Rf	32.6 AU	2697.3 AU	5.32 %
3	0.16 Rf	33.2 AU	0.18 Rf	50.4 AU	3.61 %	0.22 Rf	10.7 AU	1266.9 AU	2.50 %
4	0.36 Rf	8.7 AU	0.38 Rf	20.5 AU	1.47 %	0.41 Rf	5.5 AU	503.7 AU	0.99 %
5	0.48 Rf	11.2 AU	0.52 Rf	102.1 AU	7.31 %	0.55 Rf	37.5 AU	2822.7 AU	5.57 %
6	0.55 Rf	37.8 AU	0.58 Rf	83.5 AU	5.97 %	0.61 Rf	68.2 AU	2826.6 AU	5.58 %
7	0.62 Rf	69.3 AU	0.70 Rf	159.7 AU	11.43 %	0.71 Rf	23.6 AU	8384.2 AU	16.55 %
8	0.74 Rf	133.8 AU	0.82 Rf	218.7 AU	15.65 %	0.87 Rf	0.1 AU	13657.5 AU	26.96 %
9	0.88 Rf	0.0 AU	0.91 Rf	14.5 AU	1.04 %	0.93 Rf	0.1 AU	194.6 AU	0.38 %

Results from HPTLC for fingerprints scanned at wavelength 254 nm to detect ethanol extracted by *C. tora* root showed the presence of nine polyvalent phytoconstituents and a corresponding increase in Rf values ranged from 0.04 to 0.91 when the highest concentration of the compound was found to be 46.54% and its corresponding Rf value was 0.04. These are listed in Table 1. The HPTLC chromatogram is presented in Figure 2 showing nine peaks of phytoconstituents.

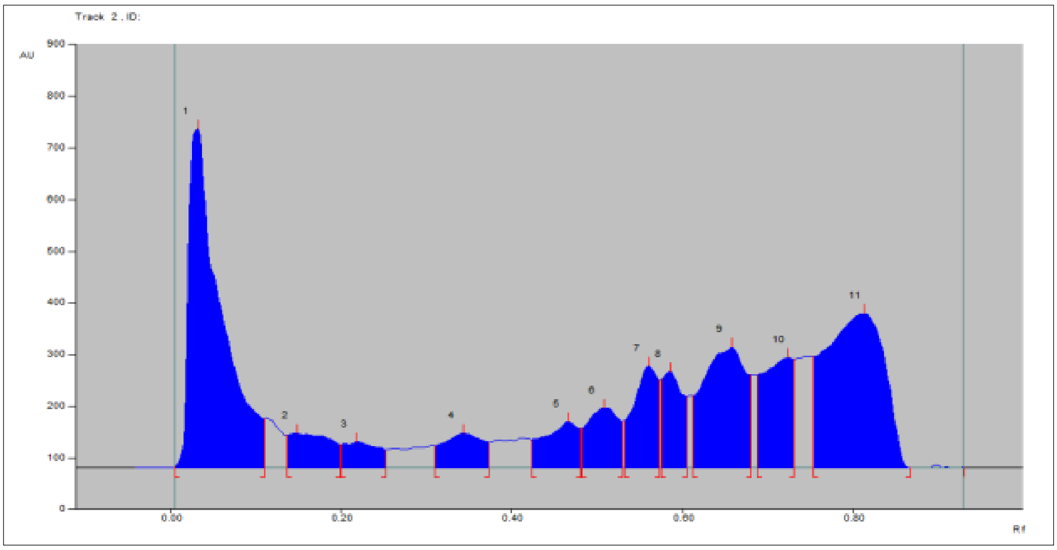

Fig 3: HPTLC profile (Peak Display) of *Cassia tora* stem

Table 2: HPTLC profile (Peak Table) of *C. tora* stem

Track 2, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	2.1 AU	0.03 Rf	656.2 AU	30.19 %	0.11 Rf	95.1 AU	20544.6 AU	26.91 %
2	0.14 Rf	62.4 AU	0.15 Rf	67.1 AU	3.09 %	0.20 Rf	44.7 AU	2767.8 AU	3.63 %
3	0.20 Rf	44.8 AU	0.22 Rf	49.9 AU	2.29 %	0.25 Rf	34.3 AU	1708.4 AU	2.24 %
4	0.31 Rf	42.7 AU	0.34 Rf	66.5 AU	3.06 %	0.37 Rf	49.2 AU	2621.9 AU	3.43 %
5	0.42 Rf	54.7 AU	0.47 Rf	90.0 AU	4.14 %	0.48 Rf	75.5 AU	2971.5 AU	3.89 %
6	0.48 Rf	75.7 AU	0.51 Rf	115.5 AU	5.31 %	0.53 Rf	89.7 AU	3614.0 AU	4.73 %
7	0.53 Rf	90.7 AU	0.56 Rf	197.1 AU	9.07 %	0.57 Rf	69.8 AU	4739.5 AU	6.21 %
8	0.57 Rf	170.1 AU	0.59 Rf	186.3 AU	8.57 %	0.61 Rf	38.1 AU	3803.6 AU	4.98 %
9	0.61 Rf	138.5 AU	0.66 Rf	233.5 AU	10.74 %	0.68 Rf	80.4 AU	9813.0 AU	12.86 %
10	0.69 Rf	181.0 AU	0.72 Rf	213.1 AU	9.80 %	0.73 Rf	10.2 AU	6328.3 AU	8.29 %
11	0.75 Rf	215.2 AU	0.81 Rf	298.5 AU	13.73 %	0.87 Rf	0.1 AU	17419.0 AU	22.82 %

Results from HPTLC for fingerprints scanned at wavelength 254 nm to obtain ethanol extracted for *C. tora* stem showed eleven phytoconstituents with a corresponding increasing order of Rf values ranging from 0.03 and 0.81. The highest concentration of the compound was found at 30.19% with a Rf value of 0.03. This is recorded in Table 2 and the HPTLC chromatogram is presented in Figure 3 showing eleven phytoconstituents.

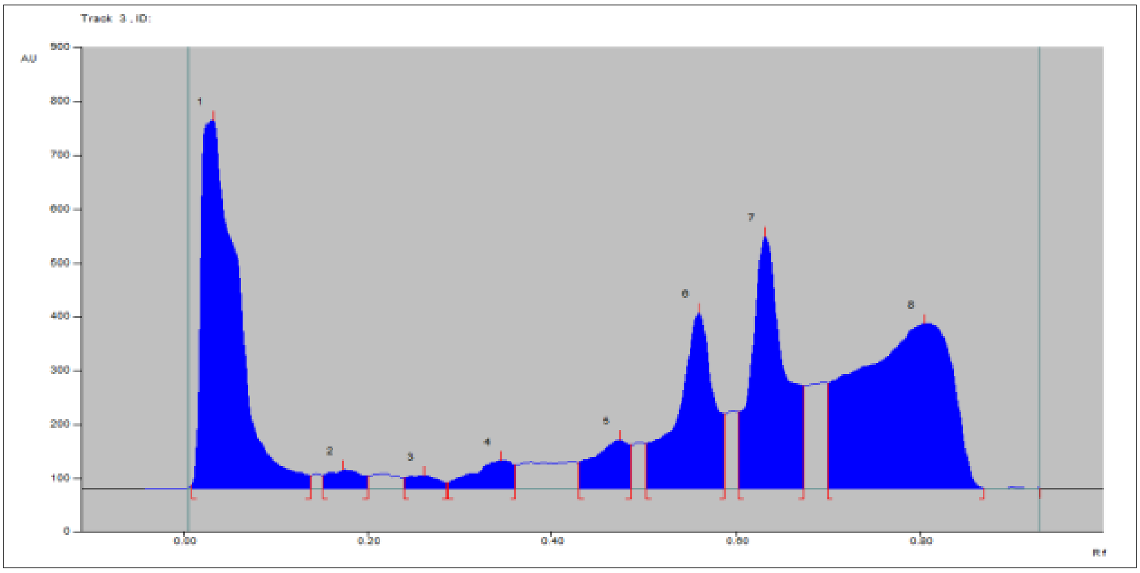

Fig 4: HPTLC profile (Peak Display) of *C. tora* leaf

Table 3: HPTLC profile (Peak Table) of *C. tora* leaf

Track 3, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	6.9 AU	0.03 Rf	684.1 AU	34.39 %	0.14 Rf	24.5 AU	22326.7 AU	27.88 %
2	0.15 Rf	24.7 AU	0.17 Rf	35.0 AU	1.76 %	0.20 Rf	23.8 AU	1060.1 AU	1.32 %
3	0.24 Rf	20.3 AU	0.26 Rf	24.8 AU	1.25 %	0.29 Rf	10.4 AU	705.5 AU	0.88 %
4	0.29 Rf	10.7 AU	0.34 Rf	52.9 AU	2.66 %	0.36 Rf	45.0 AU	1868.2 AU	2.33 %
5	0.43 Rf	48.9 AU	0.47 Rf	90.2 AU	4.54 %	0.49 Rf	81.0 AU	2986.3 AU	3.73 %
6	0.50 Rf	84.3 AU	0.56 Rf	326.9 AU	16.43 %	0.59 Rf	39.3 AU	10603.0 AU	13.24 %
7	0.60 Rf	143.1 AU	0.63 Rf	468.3 AU	23.54 %	0.67 Rf	90.9 AU	13986.1 AU	17.47 %
8	0.70 Rf	196.0 AU	0.81 Rf	307.0 AU	15.43 %	0.87 Rf	0.6 AU	26535.0 AU	33.14 %

Results from HPTLC for fingerprints scanned at a wavelength of 254 nm to detect ethanol extracted from leaf *C. tora* showed eight polyvalent phytoconstituents corresponding to the corresponding increase in Rf values from

0.03 to 0.81 where the highest concentration of the compound was found to be 34.39% and the corresponding Rf value was found to be 0.03. This is recorded in Table 3 and the HPTLC chromatogram is presented in Figure 4 showing eight peaks of phytoconstituents.

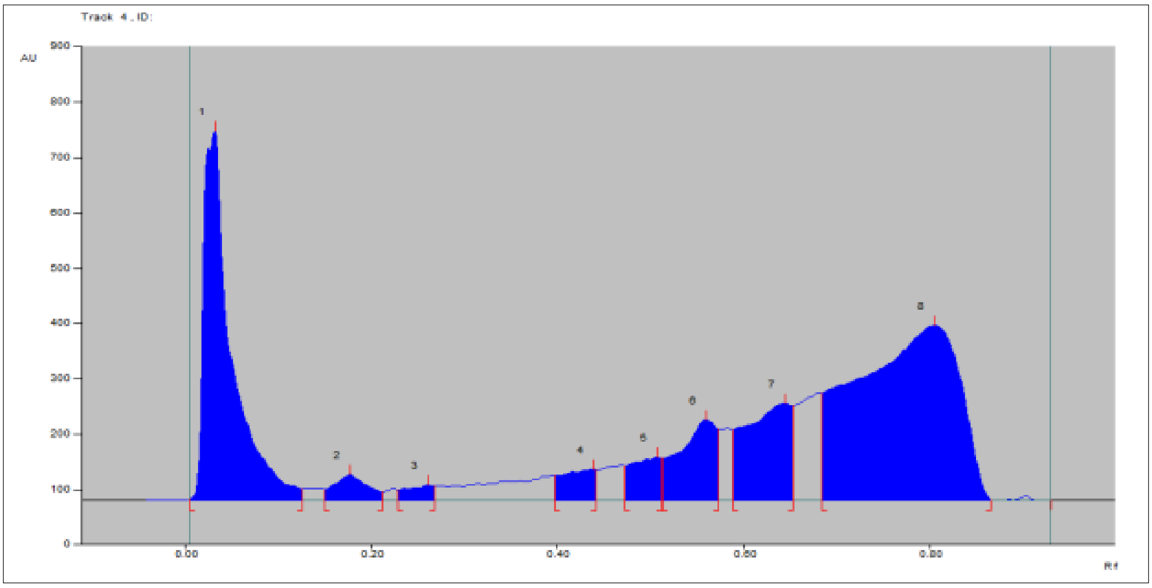

Fig 5: HPTLC Peak Display of *C. tora* seed

Table 4: HPTLC Peak Table of *C. tora* seed

Track 4, ID:

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.3 AU	0.03 Rf	667.7 AU	44.19 %	0.13 Rf	20.5 AU	16536.3 AU	26.02 %
2	0.15 Rf	19.4 AU	0.18 Rf	46.2 AU	3.06 %	0.21 Rf	15.1 AU	1375.2 AU	2.16 %
3	0.23 Rf	18.6 AU	0.26 Rf	27.6 AU	1.83 %	0.27 Rf	25.8 AU	667.3 AU	1.05 %
4	0.40 Rf	44.0 AU	0.44 Rf	56.4 AU	3.73 %	0.44 Rf	54.9 AU	1666.6 AU	2.62 %
5	0.47 Rf	62.1 AU	0.51 Rf	77.6 AU	5.14 %	0.51 Rf	75.6 AU	2121.5 AU	3.34 %
6	0.51 Rf	75.8 AU	0.56 Rf	144.6 AU	9.57 %	0.57 Rf	28.3 AU	4845.9 AU	7.62 %
7	0.59 Rf	127.2 AU	0.64 Rf	175.1 AU	11.59 %	0.65 Rf	70.2 AU	7113.6 AU	11.19 %
8	0.68 Rf	193.7 AU	0.81 Rf	315.5 AU	20.89 %	0.87 Rf	0.3 AU	29230.0 AU	45.99 %

Results from HPTLC for fingerprints scanned at wavelength 254 nm to detect ethanol extracted from *C. tora* seeds showed eight polyvalent phytoconstituents corresponding to a corresponding increase in Rf values from 0.03 to 0.81 where the highest concentration of the compound was found to be 44.19% and the corresponding Rf value showed 0.03. This is recorded in Table 4 and the HPTLC chromatogram is presented in Figure 5 showing the eight peaks of the phytoconstituents.

Table 5: Antibacterial activity of *Cassia tora* on nutrient agar.

Sr. No	Test Organism	Zone of Inhibition (in mm)				Standard Ciprofloxacin
		Root	Stem	Leaf	Seed	
1	*Escherichia coli*	08	10	11	07	18
2	*Staphylococcus aureus*	09	08	14	10	17
3	*Staphylococcus epidermidis*	12	10	15	09	19
4	*Pseudomonas aeruginosa*	11	09	12	07	19
5	*Streptococcus pyogenes*	06	08	11	07	17
6	*Streptococcus pneumoniae*	05	09	10	08	19

The results of the antibacterial activity of Cassia tora extracted from other red blood cells are shown in Table 5. From the results, it is clear that root extraction showed significant antibacterial activity compared to the standard antibiotic ciprofloxacin. The hydroalcoholic leaf extract was found to be more inhibitory against *Staphylococcus epidermidis* (15 mm) followed by root (12 mm), stem (10 mm), and seed (09 mm). Similarly, *Staphylococcus* *aureus* showed a maximum zone of inhibition in leaf extract (14 mm), seed (10mm), root (09 mm), and stem (08 mm). In comparison with standard Ciprofloxacin, *S. aureus*, and *E. coli*, *S. pneumoniae* showed less zone of inhibition. Among all four extracts, the leaf extract of *C. tora* was found to be more inhibitory against all these pus-forming bacteria. Therefore, in absence of antibiotics or any type of ointment leaf extracts of *C. tora* can be employed externally to cure skin infections caused by these bacteria. Flavonoids and steroids are known to be found in *C. tora* extracts reported to have antibacterial properties Shaikh and Syed (2015). Many mechanisms of antimicrobial action of phytochemicals have been proposed by various researchers. Phytochemicals may show different mechanisms of action against these types of bacteria ranging from disruption of phospholipid cell membranes, which results in increased penetration profile and loss of cellular components, damage to enzymes involved in cellular energy production and the integration of structural components, with the destruction or malfunction of genes (Saxena, *et al*. 2013) [13]. These findings support the earlier work of Sahu, *et al*. (2017) [12] in which they have reported Methanolic extract of *C. tora* was found to be inhibitory against these bacteria.

HPTLC fingerprints are an important quality testing tool for botanical experiments, allowing for the comprehensive analysis of a number of compounds both efficiently and inexpensively. HPTLC studies have shown that it is more flexible than conventional TLC methods as the spots are well resolved. The HPTLC method is simple, fast, accurate, reproducible, selective, and economical, and can be used to analyze quality control and pricing of plant material (Palani and Natesan, 2011). HPTLC is an equally powerful analysis method suitable for quality and quantitative analysis tasks. The HPLC plays an important role in today's analytical world, not in competition with the HPLC but in a coherent process. It combines the exciting art of chromatography with precision and speed with better separation and adjustment. The HPTLC approach interacts with high-quality and quantitative analytical applications such as pharmaceutical and dietary ingredients, nutraceuticals, and various types of medications (Jadhav, 2018) [4]. From earlier research, it is clear that *C. tora* is used in the preparation of different herbal medicines. Therefore, the HPTLC fingerprint profile of *C. tora* developed in this study will be useful in the correct identification of species before its use for herbal preparation and also for the detection of unwanted adulterations in the ayurvedic formulation.

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