ISSN: 2583-9365

Phytochemical evaluation and HPTLC fingerprint profile of various extracts of *Cassia tora*

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ISSN: 2583-9365

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Abstract

The objective of the present study is to evaluate phytochemical composition and the high-performance thin layer chromatography (HPTLC) fingerprint profile of methanol, ethanol, ethyl acetate, acetone, and aqueous extracts of medicinally useful plant *Cassia tora*. The CAMAG HPTLC system was used for the fingerprint profiling of various extracts of *C. tora* using the mobile phase toluene: ethyl acetate: glacial acetate (55: 45: 3 v/v/v). The profile showed that the various extracts of *C. tora* exhibited several peaks with different Rf values when visualized at 254 nm and 366 nm. The results from HPTLC fingerprint scanned at wavelength 550 nm revealed the presence of 18, 13, 15, 12 and 14 phytoconstituents in methanol, ethanol, ethyl acetate, acetone, and aqueous extracts, respectively. The result of HPTLC analysis of various extracts of *C. tora* shows that the maximum number of chemical constituents present in methanolic extract in comparison to ethanol, ethyl acetate, acetone, and aqueous extracts of *C. tora* in given solvent system of toluene, ethyl acetate and glacial acetate. Further bioactivity guided fractionation and analysis of isolated chemical entity can reveal the active constituents in the various extracts of *C. tora*. Phytochemical analysis revealed the presence of carbohydrates, proteins. glycosides, sapnonins, flavonoids, phenolics and tannins, phytosterols and triterpenoids.

Key words: *Cassia tora*, Phytochemical analysis, HPTLC, Fingerprint.

Introduction

Biodiversity of natural resources like plants, animals, microbes, minerals and marine sources has served not only the primary human needs but also health care since time immemorial (Patwardhan *et al*., 2004). India is a varietal emporium of medicinal plants and is one of the richest countries in the world as regards to genetic resources of medicinal plants. All known types of agro climatic, ecologic, and edaphic conditions are met within India. The biogeographic position of India is unique which makes India rich in all the three levels of biodiversity such as species diversity, genetic diversity, and habitat diversity (Krishnaraju *et al*., 2005).

Herbs being easily available to human beings have been explored to the maximum for their medicinal properties. Products of primary metabolism such as amino acids, carbohydrates and proteins are vital for the maintenance of life processes, while others like alkaloids, phenolics, steroids, terpenoids are products of secondary metabolism and have toxicological, pharmacological and ecological importance (Sharma and Bhagwan, 1996).

The phytochemical evaluations of plants which have a suitable history of use in folklore have often resulted in the isolation of principles with remarkable bioactivities (Kaviraj, 1993). Identification and quality evaluation of crude herbal extracts is a fundamental requirement. It is an accepted fact that the qualitative analysis of crude herbal extracts constitutes an important and reliable part of quality control protocol as any change in the quality of extract directly affects the constituents (Murugesan and Bhuvaneswari, 2016).

High performance thin layer chromatography (HPTLC) is a more efficient faster method, and the results are more reliable and reproducible. Combined with digital canning profiling, HPTLC also provides accurate and precise retention factor (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 along with 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 (Attimarad *et al.,* 2011). HPTLC plates has higher surface area thereby allowing for quicker and clearer

sample separation due to extra consistent and considerably smaller particle size of the adsorbent (Spangenberg *et al.,* 2011).

Chromatographic fingerprint is a logical option to meet the need for more effectual and powerful quality assessment to Chinese traditional herbal medicine (TCHM) and Indian Traditional Medicine (ITM). The optimized chromatographic fingerprint is not only an alternative analytical instrument for authentication, but also an approach to express the assorted patterns of chemical ingredients disseminated in the herbal drugs. HPTLC fingerprint analysis has developed into the most important assessment technique for quality control of herbal medicines because of its reliability and simplicity. It can use as an instrument for authentication, identification, and quality control of herbal drugs (Mauji *et al*., 2011).

MATERIALS AND METHODS

Collection and Identification of Plant Material

The fresh aerial part of the plant materials was collected in the month of October and November 2015 from Heggadadevana Kote Taluk of Mysore District, Karnataka. The taxonomic identification of the plant was confirmed by Dr. K. G. Gopalakarishna Bhat, Professor and Head, Department of Botany, Poornaprajna College, Udupi.

***C. tora* Leaves**

***C. tora* - at sample collection**

Preparation of Plant Extract

The collected plant materials were washed under running tap water and were allowed to drain before air drying under shade for two weeks. The leaves together.with the stem and the small branches were then grounded mechanically using the electric blender and the obtained coarse powder was kept in airtight containers for further use.

Solvent extraction

The coarse powder of *C. tora* (500 g each) was macerated separately with various solvents viz. methanol, ethanol, ethyl acetate and acetone of volume 2.5L each in closed conical flasks at room temperature. The flasks were shaken at a periodic interval during the first 6 h using orbital shaker and allowed to stand for 48 h. After 48 h, the contents were filtered through Buchner's funnel and Whatman No. 1 filter paper. The resultant filtrates were further concentrated under reduced pressure by using rotary flash evaporator at 39- 40°C till the solvent got completely evaporated and extract settled down to bottom. The residual solvent from the extract was evaporated after keeping the extract in a vacuum oven at 25 psi pressure at 60°C.

Aqueous extraction

Aqueous extract of this plant was prepared by boiling the coarse powder (50 g) with 200 ml of distilled water for 45 min and the extract was filtered through Whatman no. 1 filter paper. The obtained extract was evaporated on water bath to get the semi dried residue.

All the obtained semi solid extracts were stored in labelled airtight containers and kept in refrigerator at 4°C for further use.

Calculation of yield

The % yield (dry weight of extract) calculated after solvent and aqueous extraction of plant materials by using formula given below.

$$\% \text{ Yield} = \frac{\text{Final weight of dried extract}}{\text{Initial weight of powder}} \times 100$$

Qualitative Phytochemical Analysis

Physicochemical Analysis

Physicochemical constants like loss on drying, ash values (total, water soluble and acid insoluble), moisture content, extractive values and behaviour of the crude powder and pH of the various extracts of *C. tora* were carried using standard protocols (AOAC, 2005; Baravalia, 2010).

Organic Analysis

The extract obtained was then subjected to qualitative chemical tests for identification of various phytoconstituents using standard protocols (Finar, 1959; Harborne, 1998; Hasan *et al*., 2013; Khandelwal, 2004; Kokate, 2007; Middeltone, 1956; Peach and Tracey, 1955; Raju, 2014; Rosenthaler, 1930).

Quantitative phytochemical analysis

Determination of total phenol content

The content of total phenol was quantified from various extracts of *C. tora* by Folin-Ciocalteu reagent method (Singleton *et al*., 1999).

a) Sample preparation

Various extracts of *C. tora* were weighed (10 mg each) and dissolved in 5 ml of methanol separately by using vortex mixture. The extracts solution was filtered to remove solid residue and final solution volume was adjusted to 10 ml with methanol.

b) Procedure

An aliquot of 0.5 ml of each extracts solution (concentration from 1 mg/ml to 10 mg/ml) were transferred to a test tube, then 5 ml 10% of Folin-Ciocalteu reagent and 4 ml of 1M Na2CO3 solution were added, and the final reaction mixtures were thoroughly mixed and incubated at room temperature for 15 min. After incubation, the absorbance was taken at 765 nm and readings were recorded. The samples were prepared in triplicate for each analysis and the mean value of absorbance was recorded.

Total phenol content in various extracts of *C. tora* was computed from the gallic acid standard graph and the quantity was expressed as in mg/g gallic acid equivalent (GAE) or % w/w of the extractives.

Determination of flavonoid content

The content of flavonoids, expressed as flavones and flavonols were quantified from various extracts of *C. tora* by colorimetric method using AlCl3 (Raju, 2014).

a) Sample preparation

Various extracts of *C. tora* were weighed (10 mg each) and dissolved in 5 ml of methanol separately by using vortex mixture. The extracts solution was filtered to remove solid residue and final solution volume was adjusted to10 ml with methanol.//

b) Procedure

An aliquot of 0.5 ml of each extracts solution (concentration from 1 mg/ml to 10 mg/ml) was added with 1.5 ml methanol, 0.1 ml of 1M potassium acetate, 2.8 ml of distilled water and 0.1 ml of AlCl3 (10%). Later, the absorbance was read at 415 nm and readings were recorded. The samples were prepared in triplicate for each analysis and the mean value of absorbance was recorded. Total flavonoids content in various extracts of *C. tora* were computed from the quercetin standard graph and the quantity was expressed as in mg/g quercetin equivalent (QE) or % w/w of the extractives.

HPTLC Profile (High Performance Thin Layer Chromatography)

HPTLC studies were carried out by following the method of Harborne, 1998 and Seasotiya *et al*., 2014.

Chromatography set up

Instrument	:	CAMAG Linomat V with WINCATS software
Stationary phase		
Plate size (X x Y)	:	10.0 x 10.0 cm
Thickness	:	20 mm
Material	:	HPTLC silica gel plates 60 F254
Manufacturer	:	E. MERCK, Germany
Linomat 5 application parameters		
Spray gas	:	Inert gas
Dosage speed	:	150 nl/s
Sample solvent type	:	Methanol
Pre dosage volume	:	0.2 µl
Sequence		
Syringe size	:	100 µl
Number of tracks	:	5
Application position Y	:	12.0 mm
Band length	:	10 mm
Distance between two tracks	:	20 mm
Chamber type	:	Twin through chamber 20 x 10 cm
Sample application	:	10 µl
Solvent system	:	Toluene: ethyl acetate: glacial acetate (55:45:3 v/v/v)
Drying device	:	Oven
Temperature	:	105℃
Detection	:	CAMAG TLC Scanner 3

Sample preparation and application

The various extracts of *C. tora* (50 mg each) were weighed and mixed with 10 ml of methanol of chromatographic grade in a volumetric flask fitted with a glass stopper. The contents were sonicated for 10 min using a sonicator and allowed to stand for 5 min. The mixture was then filtered through Millipore membrane filter and used for further analysis.

Prepared samples of various extracts (10 µl) were spotted as sharp band of 10 mm width using spray on technique with a Hamilton 100 µl HPTLC syringe on pre coated silica gel aluminum plate 60 F254, (10x10) using a CAMAG Linomat V automatic sample applicator set at a speed of 150 nl/sec. The bands were applied at a distance of 10 mm from the bottom edge of the plate and distance between two bands was 20 mm.

Developing solvent system

A number of solvent systems were tried for each extract, for better resolution and for maximum number of spots. But the satisfactory resolution was obtained in the solvents toluene: ethyl acetate: glacial acetic acid (55:45:3 v/v/v).

Development of chromatogram

Prior to chromatography procedure, TLC plate was activated by wash with methanol and kept at 60oC for 5 min. The mobile phase was added to TLC twin trough developing chamber. TLC developing chamber was closed with lid and allowed for saturation of solvent vapors. The sample loaded plate was kept in mobile phase up to the distance of 80 mm. The toluene: ethyl acetate: glacial acetate (55:45:3 v/v/v) used as mobile phase. The developing chamber assemble was kept aside for saturation and development of chromatogram for.30 min at room temperature. After 30 min, developed plate was dried using oven.

Scanning and detection of spots

Photo documentation chamber was used for photo documentation of plate at UV 254. The oven dried plate was viewed in UV radiation to mid-day light. Spots were visible without derivatization at 254 and 366 nm wavelengths but best results were shown when TLC plate was sprayed with detection reagent i.e. anisaldehyde sulfuric acid reagent. The plate was heated at 110°C for 5 min and then visualized in visible light range 400-600 nm. Scanning was performed by CAMAG HPTLC Densitometer (Scanner 3) in absorbance mode at both 254 and 366 nm; the extracts were also scanned at 400-600 nm using deuterium and tungsten lamp. The Rf values and color of the resolved bands were recorded.

Results

Qualitative Phytochemical Analysis

Physical nature of the extracts

Physical nature and % yield of various extracts of *C. tora* are presented in Table 1. The amount (% w/w) of aqueous extract was greater than that of solvent extracts.

Physicochemical analysis

The result of proximate analysis, extractive values, and behavior analysis of crude powder of *C. tora* are presented in Table 2 and 3. The average values are expressed as percentage of air-dried material. The loss on drying was 5.85±0.71%. Dry matter was 92.47±0.05 %. Total ash was 12.72±0.14 %, acid insoluble ash was 3.42±0.08 % and water-soluble ash was 9.30±0.06 %. The extractive value of crude powder was maximum in water (13.79±0.40 %), followed by methanol (9.98±0.23 %) and minimum was in petroleum ether (1.71±0.01%).

The behaviour of the aerial powder upon treatment with different chemical reagents was also observed and presented in Table 3. When the powders were treated with chemicals like HCl, HNO3, iodine solution, etc. various colours were obtained.

The pH of solvent extracts of *C. tora* was acidic, whereas aqueous extract was basic and results were presented in Table 4.

On preliminary phytochemical analysis of various extracts of *C. tora*, it was observed that, the extract was positive for presence of phytoconstituents like carbohydrates, proteins, alkaloids, flavonoids, tannins, phenolics, glycosides, anthraquinones, phytosterols and triterpenoids. And absence of saponins and fixed oils. The results are depicted in Table 5.

Quantitative phytochemical analysis

Total phenol content

Total phenol content present in all the extracts were estimated by using Folin- Ciocalteu reagent method. The total phenol quantity in various extracts of *C. tora* were presented as mg/g of gallic acid equivalent (GAE) ± SEM (n=3) and presented in Table 6.

All the extracts of *C. tora* were tested at 100, 250, 750 and 1000 µg/ml and the positive control (gallic acid) at 25, 50, 100, 150, 200, 250 and 300 µg/ml. Higher concentration of phenol was found with methanol extract and its concentration was least in aqueous extract (Fig. 1).

The order of total phenol content with respect to various extracts of *C. tora* was methanol >ethanol >ethyl acetate >acetone >aqueous. The quantification range was from 0.88±0.021 to 0.002±0.000 µg/ml were calculated from the calibration curve.

Total flavonoid content

Total flavonoid content present in various extracts of *C. tora* were estimated by using AlCl3. The total flavonoids quantity in various extracts of *C. tora* were presented as mg/g of quercetin equivalent (QE) ±SEM (n=3) and presented in Table 7.

All the extracts of *C. tora* were tested at 100, 250, 750 and 1000 µg/ml and the positive control (quercetin) at 5, 10, 20, 30, 40, 50 and 100 µg/ml. Higher concentration of flavonoid was found with ethyl acetate extract and its concentration was least in aqueous extract (Fig. 2).

The order of total flavonoid content with respect to various extracts of *C. tora* was ethyl acetate > ethanol >methanol >acetone >aqueous. The quantification range was from 1.251±0.001 to 0.002±0.001 µg/ml was calculated from the calibration curve.

Total phenolic and flavonoids content

The amount of total phenolic and flavonoid contents in the various extracts of *C. tora* were summarized in Table 8.

HPTLC fingerprinting of various extracts of C. tora

The results showing number of peaks and the Rf values are presented in Table 9.

Table 1: Physical nature and % yield of various extracts of *C. tora*

Sl. No.	Solvent name	Colour	Consistency	% Yield (w/w)
1	Methanol	Dark brown	Sticky semi solid	3.8
2	Ethanol	Reddish brown	Sticky semi solid	4.1
3	Acetone	Dark brown	Sticky semi solid	3.7
4	Ethyl acetate	Brown	Sticky semi solid	3.3
5	Aqueous	Light brown	Semi solid	6.2

Table 2: Proximate parameters and extractive values of crude powder of *C. tora*

Sl. No.	Evaluation parameter	Value (%) w/w			Mean ± SEM(%) w/w
1	Loss on drying	6.23	6.85	4.48	5.85±0.71
2	Dry matter	92.54	92.38	92.48	92.47±0.05
3	Moisture content	7.46	7.62	7.52	7.53±0.05
4	Total ash	12.88	12.84	12.45	12.72±0.14
5	Acid insoluble ash	3.52	3.47	3.26	3.42±0.08
6	Water soluble ash	9.36	9.37	9.19	9.31±0.06
7	Petroleum ether soluble extractive value	1.72	1.74	1.69	1.71±0.01
8	Hexane soluble extractive value	1.83	1.91	1.85	1.86±0.02
9	Ethyl acetate soluble extractive value	2.25	2.31	2.27	2.28±0.02
10	Acetone soluble extractive value	3.53	4.10	3.87	3.83±0.17
11	Methanol soluble extractive value	9.64	9.89	10.42	9.98±0.23
12	Water soluble extractive value	13.03	13.90	14.43	13.79±0.41

Table 3: Behavior analysis of crude powder of *C. tora*

Sl. No.	Reagents	Color/precipitation
1	Powder as such	Green
2	Conc. H2SO4	Deep brown
3	Conc. HCl	Greenish black
4	Conc. HNO3	Creamy orange
5	Aqueous ferric chloride (5%)	Blackish brown
6	Aqueous KOH solution (5%)	Greenish
7	Aqueous NaOH solution (1N)	Greenish
8	Aqueous NaOH solution (40%) + Lead acetate	Creamy
9	Picric acid	Yellowish green
10	Aqueous silver nitrate solution (1%)	Greenish

Table 4: pH of various extracts of *C. tora* Organic analysis

Parameter	Methanol	Ethanol	Ethyl acetate	Acetone	Aqueous
pH (Mean±SEM)(n=3)	4.8±0.06	5.38±0.003	3.66±0.005	5.19±0.003	7.26±0.003

Table 5: Preliminary phytochemical analysis of various extract of *C. tora*

Sl. No.	Tests	Methanol	Ethanol	Ethyl acetate	Acetone	Aqueous
1	**Test for carbohydrates**					
	a. Molisch's test	+	+	+	+	+
	b. Fehling's test	+	+	+	+	+
2	**Test for proteins**					
	a. Biuret test	+	+	+	+	+
	b. Xanthoproteate test	+	+	+	+	+
3	**Test for saponins Foam test**	-	-	-	-	-
	a. Sodium bicarbonate	-	-	-	-	-
4	**Test for alkaloids**					
	a. Mayer's test	+	+	+	+	-
	b. Dragendrodroff's test	+	+	+	+	-
	c. Hager's reagent	+	-	-	+	+
	d. Wagner's test	+	+	-	-	+
5	**Test for flavonoids**					
	a. Aqueous NaOH test	+	+	+	+	+
	b. Sulphuric acid test	+	+	+	+	+
	c. Shinoda test	+	+	+	+	+
6	**Test for tannins and phenolic compounds**					
	a. Ferric chloride test	+	+	+	+	+
	b. Lead acetate	+	+	+	+	+
	c. Test for tannins	+	+	+	+	+
7	**Test for fixed oils and fats**	-	-	-	-	-
	a. Oily spot test					
8	**Test for glycosides**					
	a. Keller Killiani test	+	+	+	+	+
	b. Legal's test	+	+	-	+	+
9	**Test for anthraquinones**					
	a. Borntrager's test	+	+	+	+	+
10	**Test for phytosterols and triterpenoids**					
	a. Leiberman-Bucharat test	+	+	+	+	+
	b. Salkowaski test	+	+	-	+	+

+ Present - Absent

Table 6: Total phenolic content in various extracts of *C. tora*

Concentrationof extracts (µg/ml)	Total phenol content in mg/g (GAE)				
	Methanol	Ethanol	Ethyl acetate	Acetone	Aqueous
100	0.053±0.001	0.049±0.003	0.047±0.003	0.045±0.003	0.002±0.000
250	0.180±0.012	0.156±0.002	0.148±0.003	0.143±0.002	0.003±0.000
500	0.457±0.002	0.407±0.001	0.396±0.002	0.343±0.020	0.006±0.001
750	0.608±0.002	0.553±0.003	0.544±0.002	0.533±0.002	0.008±0.001
1000	0.880±0.021	0.794±0.001	0.727±0.002	0.647±0.003	0.013±0.001

Table 7: Total flavonoid content in various extracts of *C. tora*

Concentrationof extracts (µg/ml)	Total flavonoid content in mg/g (QE) in various extract of *C. tora*				
	Methanol	Ethanol	Ethylacetate	Acetone	Aqueous
100	0.042±0.003	0.098±0.003	0.123±0.002	0.013±0.001	0.002±0.001
250	0.105±0.002	0.224±0.003	0.313±0.003	0.032±0.002	0.003±0.001
500	0.228±0.003	0.502±0.002	0.636±0.003	0.068±0.003	0.005±0.001
750	0.375±0.002	0.795±0.001	0.960±0.002	0.098±0.002	0.008±0.001
1000	0.557±0.028	1.090±0.001	1.251±0.001	0.148±0.001	0.010±0.002

Table 8: Total phenolic and flavonoid content in various extracts of *C. tora* at conc. 1000 µg/ml

Sl. No.	*C. tora* extracts	Total phenolic content in mg of GAE/g of extract	Total flavonoid contentin mg of QE/g of extract
1	Methanol	7.56±0.30	7.65±0.02
2	Ethanol	7.02±0.05	17.86±0.03
3	Ethyl acetate	6.73±0.05	21.87±0.05
4	Acetone	6.44±0.04	2.37±0.02
5	Aqueous	0.42±0.02	0.364±0.03

Values are expressed as mean ±SEM, (n=3).

Table 9: HPTLC fingerprint of various extracts of *C. tora* at 550 nm

Extract	Solvent system	No. of peaks	Rf Value
Methanol	Toluene: Ethylacetate: Glacial	18	0.10, 0.12, 0.18, 0.25, 0.28, 0.30, 0.39,0.40, 0.50, 0.60, 0.70, 0.80
Ethanol	acetate (50: 45: 3 v/v/v)	13	0.10, 0.12, 0.18, 0.25, 0.28, 0.30, 0.39, 0.40, 0.50, 0.60, 0.70, 0.80
Ethyl acetate		15	0.10, 0.12, 0.18, 0.25, 0.28, 0.30, 0.39, 0.40, 0.50, 0.60, 0.70, 0.80
Acetone		12	0.18, 0.28, 0.40, 0.50, 0.60, 0.70, 0.80
Aqueous		14	0.18, 0.22, 0.39, 0.40

The TLC images (Fig. 3) indicate that all sample constituents were clearly separated without any tailing and diffuseness. A combined five tracts spot of various extracts of *C. tora* was showed in Fig. 4. Fingerprinting of various extracts of *C. tora* was carried out using HPTLC technique (Fig. 5 to 9). The various extracts of *C. tora* showed betters results in toluene: ethyl acetate: glacial acetate (55: 45: 3) solvent system. HPTLC spectral analysis of various extracts of *C. tora* was done in three different wavelengths, under UV at 254 and 366 nm, and white light. After scanning and visualizing the plates in absorbance mode at 254 nm, 366 nm, and visible light range (400-600 nm after spraying with anisaldehyde sulfuric acid reagent) better results were shown at 550 nm.

The results from HPTLC fingerprint scanned at wavelength 550 nm revealed the presence of 18, 13, 15, 12 and 14 phytoconstituents in methanol, ethanol, ethyl acetate, acetone, and aqueous extracts respectively (Table 9). The polyvalent phytoconstituents and corresponding ascending order of Rf values start from 0.1 to 0.8 for methanol, ethanol, and ethyl acetate extracts, for acetone extract Rf values ranged from 0.18 to 0.80 and for aqueous extract Rf values ranged from 0.18 to 0.40. TLC plate showed different color phytoconstituents in *C. tora* extracts (Fig. 3) by presence of greenish, purple, brown, violet, pink and light yellowish orange bands showing the presence of steroids, terpenoids and saponins after spraying with anisaldehyde sulfuric acid reagent.

Discussion

The present study was carried out to evaluate the phytochemical properties, antibacterial, anti-inflammatory activity, and safety feature of the *C. tora* extract in rats. The results of the present study are discussed as here under.

Phytochemical analysis

Physical nature of the extracts

The higher percentage yield of *C. tora* was found to be 6.2% (w/w) in aqueous extract followed by ethanol extract (4.1 %). This showed that, the aerial part of the plant *C. tora* contains higher concentration of polar constituents. The present finding is in accordance with the findings of Arya *et al*. (2010) who also reported higher extractive value of 6.8 % and 6 % in water extract and methanol extract respectively in the similar plant species *C. occidentalis*.

Physicochemical analysis

The physical constant evaluation of the powder is an important parameter in detecting adulteration or improper handling of plant extracts. The percentage of active chemical constituents in crude extract is mentioned on air-dried basis. Therefore, the loss on drying of plant material should be determined and the water content should also be controlled. In the present study, the moisture content of dry powder of *C. tora* was

7.53 % which is low, hence it would discourage bacteria fungi or yeast growth (Bhattacharya and Zaman, 2009). The total ash is particularly important in the evaluation of purity of plant extracts, i.e., the presence or absence of foreign inorganic matter such as metallic salts and/or silica (Musa *et al*., 2006; Chanda *et al*., 2010). In the present study, physical constant (total ash, acid insoluble ash and water soluble ash) values were found to be low, which is in accordance with the findings of Bhakta *et al*. (2014) who reported the similar level of physical constants, which indicate that the inorganic matter and non-physiological matter such as silica is less in *C. tora* dried powder. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent. High alcohol soluble and water soluble extractive values reveal the presence of polar substance like phenols, tannins and glycosides (Baravalia, 2010). The higher percentage of extractive value of crude powder of *C. tora* was found to be (13.79±0.41) in water extract. The present finding of the study is in accordance with the findings of Khan *et al*. (2016) who also reported the higher extractive value in water extract (9.87±0.83) of leaves of *C. tora* compared to other solvent (petroleum ether, chloroform and methanol) extracts.

Organic analysis

In the present study, various extract of the *C. tora* was found positive for carbohydrates, proteins, alkaloids, flavonoids, tannins, phenolics, glycosides, anthraquinones, phytosterols and triterpenoids.

Flavonoids are the secondary constituents of many plants which contribute for the biological activity. In the present study, the phytochemical analysis revealed for the presence of flavonoids in the various extracts of *C. tora*. The present finding is in accordance with the findings of Khan *et al*. (2016) who also reported the presence of the flavonoids in the extract of *C. tora* which was reported to have hepatoprotective and anti-inflammatory activities.

Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh and Singh, 2007). Natural antioxidants mainly come from plants in the form of phenolic compounds such as flavonoids, phenolic acids, tocopherols etc. A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers (Ali *et al*., 2008). In the present study, the organic analysis of various extracts of *C. tora* revealed for the presence of phenolic compounds, which is in accordance with the findings of (Veerachari and Bopaiah (2012)) who reported the presence of phenolic compounds in methanol and ethanol extract of *C. sericea* and *C. occidentali*s, which were attributed to have anti-inflammatory, antioxidant, anticancer and antimicrobial activities.

Steroids in modern clinical studies have supported their role as anti- inflammatory and analgesic agents Singh (2006). In the present study, the plant extract was found to be positive for the presence of phytosterols and triterpenoids which is in support with the findings of Veerachari and Bopaiah (2012) who stated that, the methanol, ethanol and ethyl acetate extracts of *C. alata* and *C. occidentalis* had steroids in higher level.

In the present study, various extracts of *C. tora* found positive for anthraquinones which is in supportive with Sermakkani and Thangapandian (2013) who reported the presence of

Fig. 1: Total phenol content in various extracts of *C. tora*

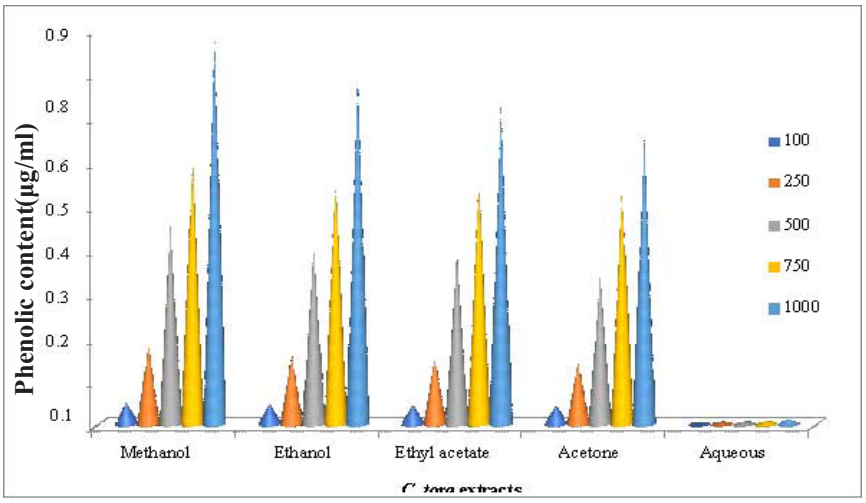


Fig. 2: Total flavonoid content in various extracts of *C. tora*

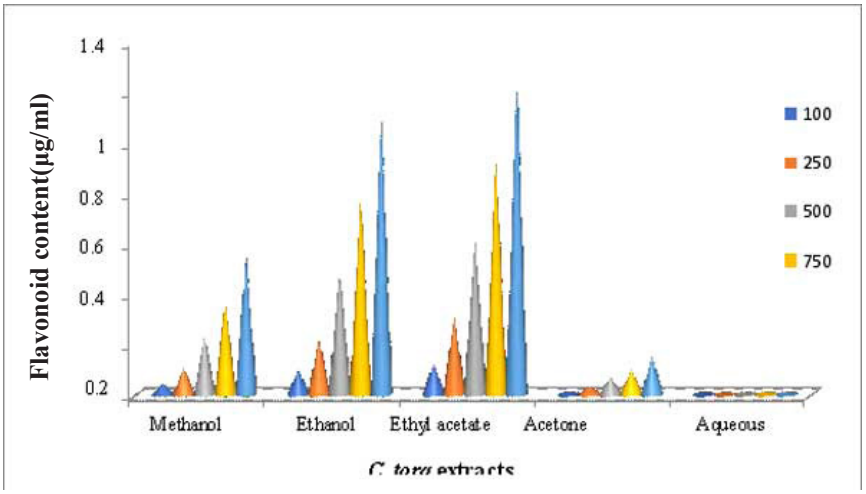
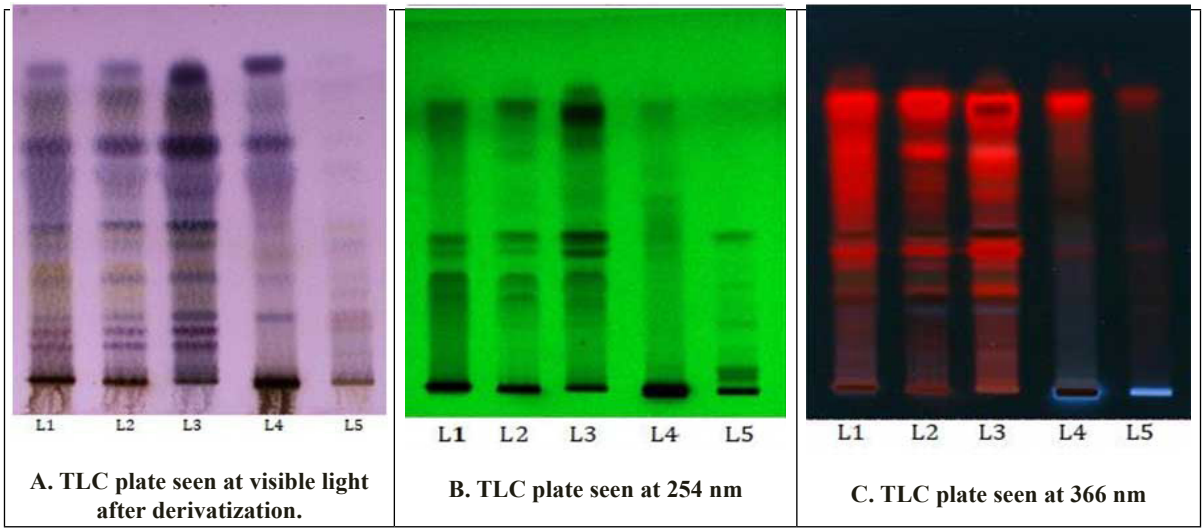


Fig. 3: TLC plates showing different bands for various extracts of C. tora



A. TLC plate seen at visible light after derivatization.

B. TLC plate seen at 254 nm

C. TLC plate seen at 366 nm

L1: Methanol extract, L2: Ethanol extract, L3: Ethyl acetate extract, L4: Acetone extract & L5: Aqueous extract.

Fig. 4: 3D diagram of combined five track spots of extracts of *C. tora* at 550 nm

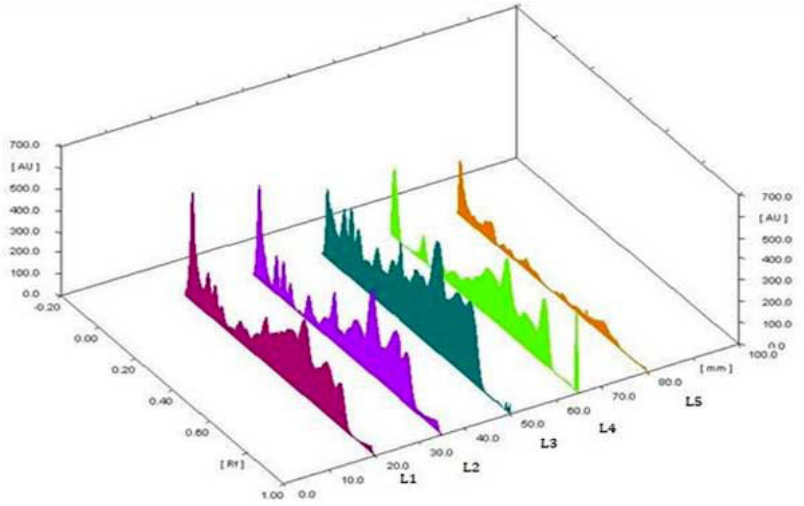


Fig. 5: Chromatogram of methanol extract of *C. tora* at 550 nm.

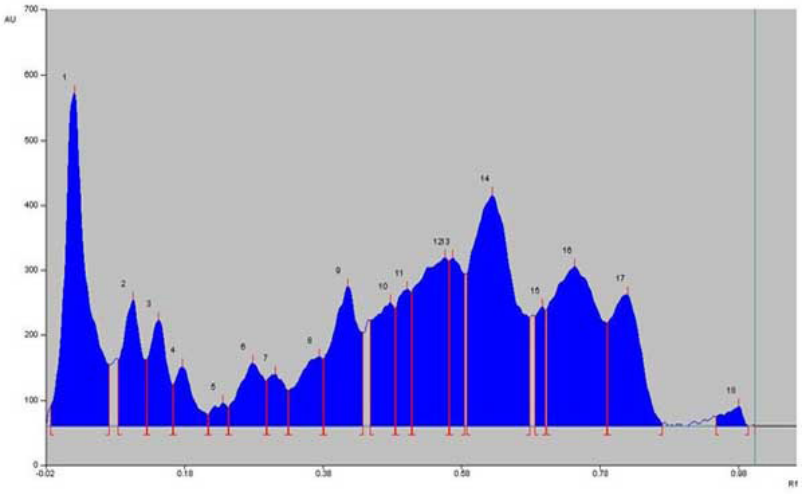


Fig. 6: Chromatogram of ethanol extract of *C. tora* at 550 nm.

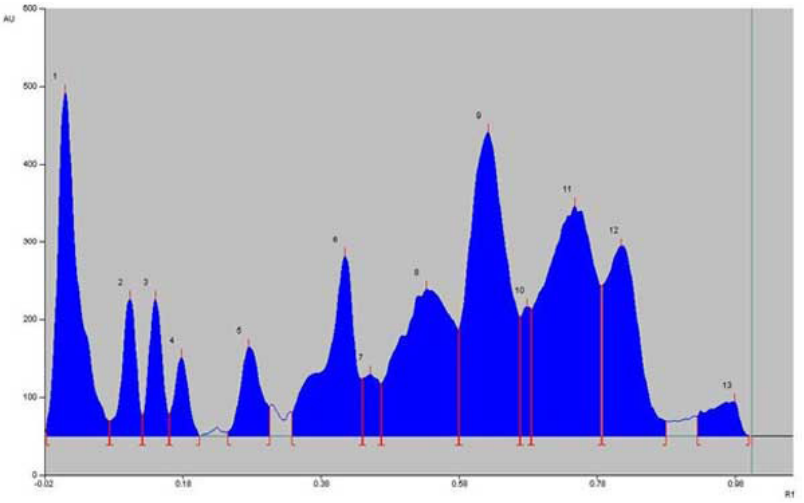


Fig. 7: Chromatogram of ethyl acetate extract of *C. tora* at 550 nm.

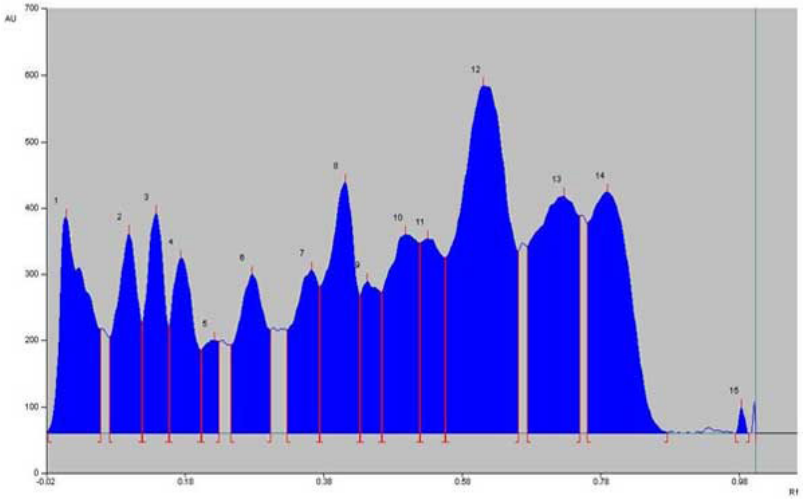


Fig. 8: Chromatogram of acetone extract of *C. tora* at 550 nm.

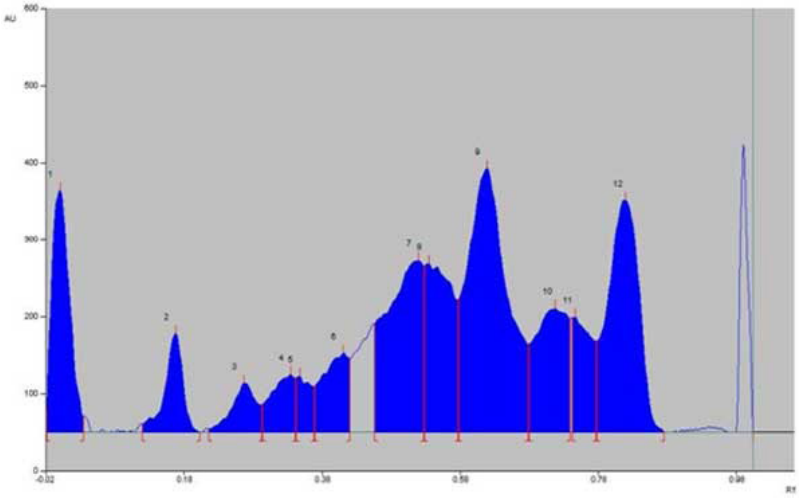
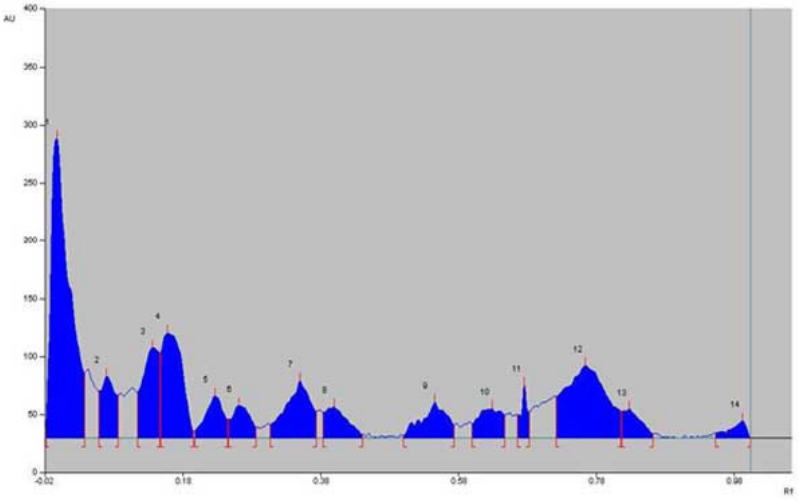


Fig. 9: Chromatogram of aqueous extract of *C. tora* at 550 nm.



anthraquinones in methanolic extract of *C. italica,* which was reported to exhibit both acute, chronic anti-inflammatory and anti-pyretic activity in rat models.

Quantitative phytochemical analysis

Quantitative phytochemical analysis of various extracts of *C. tora* for phenolic and flavonoid compound was conducted.

Total phenolic content

Phenolic compounds are most widely occurring groups of phytochemicals. These compounds are reported to have anticancer, antimicrobial, anti-inflammatory and anti- allergic activities (Eleazu *et al*., 2012).

In the present study, the extract was also found to have phenolic content (mg/g of GAE) in methanol extract at higher concentration i.e. 7.56±0.30 followed by ethanol extract 7.02±0.05 and least in aqueous extract 0.42±0.14, which is in support with the findings of phenolic content in the ethyl acetate extract of leaf and seed of *C. tora* were.1300 µg/ml and 900 µg/ml respectively (Sabyasachi *et al*., 2016) and in methanolic extract of leaves of *C. tora* had 10.67±1.46 mg/gm (Khan *et al*., 2016).

1.1.1. Total flavonoid content

Flavonoids are most widely occurring groups of phytochemicals and act as antioxidants. These compounds are reported to have anticancer, antimicrobial, anti- inflammatory and anti-allergic activities (Eleazu *et al*., 2012).

The present study, the extract of *C. tora* was also found to have flavonoid content (mg/g of QE) in ethyl acetate extract at higher concentration i.e. 21.87±0.05 followed by ethanol extract 17.86±0.03 and least in aqueous s extract 0.364±0.03, which is in support with the findings of Ekwueme *et al*. (2015) who reported, the presence of flavonoids in the leaf of Senna mimosoides (2.67±0.0013 mg/100g) could account for its use as an ant-inflammatory agent.

1.2. HPTLC fingerprinting of various extracts of *C. tora*

Developing HPTLC profile along with their Rf values, could serve as a reference standard for investigation of medicinal properties of plants (Sharma et. al., 2014). In the present study, TLC plate showed different color phytoconstituents in various extracts of *C. tora,* after spraying with anisaldehyde sulfuric acid reagent. The HPTLC profile confirmed the results of phytochemical screening by the presence of various coloured bands at different wavelengths with specific solvent systems, symbolizing the presence of particular phytoconstituents. Qualitative tests carried on various extracts of *C. tora* confirmed the presence of various pharmacologically important phytoconstituents like steroids and terpenoids. The result agreed with findings of Sujogya *et al*. (2011) who reported, the presence of alkaloids, flavonoids, triterpenoids, carbohydrates, glycosides, saponins, protein and amino acid in HPTLC fingerprinting of methanolic extract *C. fistula* leaves.

In the present study, the HPTLC fingerprinting of extracts of C. tora showed the presence of 18, 13, 15, 12 and 14 phytoconstituents in methanol, ethanol, ethyl acetate, acetone and aqueous extracts respectively at 550 nm with the solvent system toluene: ethyl acetate: glacial acetate (55: 45: 3). The present study is in accordance with the findings of Seasotiya *et al*. (2014) who reported the ethyl acetate and methanol extracts of *C. fistula* leaves showed maximum number of components 16 and 15 respectively at 400 nm with solvent system toluene: ethyl acetate: formic acid (5:4:1).

The present study was undertaken to verify and evaluate the various phytochemical, pharmacological and toxicological properties of the *C. tora* plant extract.The pharmacological properties like antibacterial, anti-inflammatory activities and toxicological properties like acute oral and dermal toxicity studies of methanol extract in rats were conducted. There are very few research reports on pharmacological properties and toxicological properties of *C. tora.*

Considering the need for systematic elucidation of pharmacological and toxicological properties of C. tora, this study was conducted to evaluate antibacterial anti-inflammatory activity and the acute oral and dermal toxicities of methanol extract of *C. tora* in Wistar albino rats.

The physical nature of the various extracts of *C. tora* revealed, higher per cent of yield in aqueous extract followed by ethanol.

The preliminary phytochemical analysis of various extracts of *C. tora* revealed the presence of various phytoconstituents like carbohydrates, proteins, alkaloids, flavonoids, tannins, phenolics, glycosides, anthraquinones, phytosterols and triterpenoids.

The quantitative phytochemical analysis of various extracts of *C. tora* for phenolic and flavonoid compounds revealed, higher concentration of phenolics in methanol extract followed by ethanol extract and for flavonoids ethyl acetate extract had higher concentration followed by ethanol.

HPTLC fingerprinting profile of various extracts of *C. tora* confirmed the results of phytochemical screening by the presence of various coloured bands at different wavelengths with specific solvent systems.

References

1. Arya, V., Yadav, S., Kumar, S. and Yadav, J. P., (2010). Antimicrobial activity of *Cassia occidentalis* L (Leaf) against various human pathogenic microbes. *Life Sci. Med. Res.*
2. Association of Official Analytical Chemists (AOAC), (2005). Official *Method*s of Analysis. *Edn*. 18th., Washington D. C. USA
3. Attimarad, M., Ahmed, K. K., Aldhubaib B. E. and Harsha S., (2011). High-performance thin layer chromatography: A powerful analytical technique in pharmaceutical drug discovery, Pharm Methods, 2(2): 71–75.
4. Baravalia, Y., (2010). Evaluation of anti-inflammatory and hepatoprotective potency of a selected medicinal plant. Ph. D. thesis, Saurashtra University, Rajkot, India

5. Bhattacharya, S. and Zaman, M. K., (2009). Pharmacognostical evaluation of Zanthoxylum nitidum root. *J. Phcog.,* 1 (2): 15-21

6. Chanda, S., Nagani K. and Parekh, J., (2010). Assessment of quality of Manilkara hexandra (roxb.) dubard leaf (sapotaceae): Pharmacognostical and physicochemical profile. *J. Phcog.*, 2 (13): 520-524

7. Ekwueme, F. N., Oje, O. A., Ozoemena, N. F. and Nwodo, O. F. C., (2015). Qualitative and quantitative phytochemical screening of the aqueous leaf extract of Senna mimosoides: Its effect in in vivo leukocyte mobilization induced by inflammatory stimulus. I*nt. J. Curr. Microbiol. App. Sci*., 4 (5): 1176-1188

8. Eleazu, C. O., Eleazu, K. C., Awa, E. and Chukwuma, S. C., (2012). Comparative study of the phytochemical composition of the leaves of five Nigerian medicinal plants. *J. Biotechnol. Pharm. Res*., 3 (2): 42-46

9. Finar, I. L., (1959). Organic Chemistry. *Edn.* 2nd. The English Language Book Society, London, *pp* 280-431

10. Harborne, A. J., (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. *Edn.* 1st. Springers Science Business Media

11. Hasan, R. U., Prabhat, P., Shafaat, K. and Khan, R., (2013). Phytochemical investigation and evaluation of antioxidant activity of fruit of Solanum indicum Linn. *Int. J. Pharm. Pharm. Sci.*, 5 (3): 237-242

12. Kaviraj A. G., (1993). Astang Sangrah, Krishnadas Academy Orientalia Publishers and Distributors, Varanasi, *pp* 4-32.

13. Khandelwal, K. R., (2004). Practical Pharmacognosy Techniques and Experiments, Edn. 12th., Nirali Prakashan, New Delhi., *pp* 149-156

14. Krishnaraju, A. V., Rao, T. V. N., Sundararaju, D., Vanisree, M., Tsay, H. S. and Subbaraju, G. V., (2005). Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *Int. J. App. Sci. Eng*., 3 (2): 125-134

15. Kokate, C. K., (2007). Text Book of Pharmacognosy. Nirali publications, New Delhi., *pp* 1- 73

16. Mauji, R., Abdin, M. Z., Khan, M. A. and Prabhakar, J., (2011). HPTLC fingerprint analysis: A Quality control of Authentication of Herbal Phytochemicals. Springer Verlag Berlin Heidelberg 105.

17. Middletone, H., (1956). Systematic Qualitative Analysis. Edn. 3rd. Edward Arnnold Publishers Ltd., London, *pp* 91-94

18. Murugesan, S. and Bhuvaneswari, S., (2016). HPTLC fingerprint profile of methanol extract of the marine red alga Portieria hornemannii (Lyngbye) (Silva). *Int. J. Adv. Pharm*a. 5 (3): 61-65

19. Musa, K. Y., Katsayal, A, U., Ahmed, A., Mohammed, Z. and Danmalam, U. H., (2006). Pharmacognostic investigation of the leaves of *Gisekia pharnacioides. Afr. J. Biotechnol.*, 5 (10): 956-957

20. Patwardhan, B., Vaidya, A. D. B. and Chorghade, M. S., (2004). Ayurveda and natural product drug discovery. *Curr. Sci.*, 86 (6): 789-799

21. Peach, T. and Trancey, M. V., (1955). Modern Methods in Plant Analysis. Edn. 1st.Springer Verlog, Berlin, *pp* 387

22. Raju, A., (2014). Anticancer activity of certain Drosera L. species. Ph.D. thesis, Jawaharlal Nehru Technological University, Hyderabad, India

23. Rosenthaler, L., (1930). Chemical Investigations of Plants. Edn. 1st. G. Bell and Sons, London, *pp* 23-132

24. Seasotia, L., SIiwach, P., Malik, A., BAI, S., Bharti, P. and Dalal, S., (2014). Phytochemical evaluation and HPTLC fingerprint profile of *Cassia fistula. Int. J. Adv. Pharm. Biol. Chem*., 3 (3): 604-611

25. Sermakkani, M. and Thangapandian, V., (2013). Anti-inflammatory potential of *Cassia italica (*mill) Lam. ex. fw. andrews leaves. *Int. J. Pharm. Pharm. Sci.*, 5 (1): 18-22

26. Sharma R. K. and Bhagwan D., (1996). Charak Samhita. Edn 4, Vol. 2, Chowkhamba Sanskrit Series, Varanasi, *pp* 17-101

27. Sharma, N., Gupta, P., Singh, A. and Rao, C. V., (2014). Pharmacognostical, phytochemical investigations and HPTLC fingerprinting of Pentapetes phoenicea L. leaves. *Indian J. Nat. prod. Reso*. 5 (2): *pp* 158-163

28. Singh, A. P., (2006). Short review: Distribution of steroid like compounds in plant flora. *Pharmacogn. Mag*., 2 (6): 87-89

29. Singh, R. and Singh, S., (2007). Evaluation of antioxidant potential of ethyl acetate extract/fractions of *Acacia auricliformis. A. Cunn. Food Chem. Toxicol.*, 45 : 1216-1223

30. Singleton, V. L., Orthofer, R. and Lamuela-Raventos, R. M., (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin– Ciocalteau reagent. *Meth. Enzymol*., 299: 152–178

31. Spangenberg, B., Poole, C. and Weins, C., 2011. Quantitative thin layer chromatography: A Practical Survey. Springer, Berlin, Germany

32. Sujogya K. P., Padhi, L. P. and Mohanty, G., (2011). Antibacterial activities and phytochemical analysis of C*assia fistula* (Linn.) leaf. J *Adv Pharm Technol Res*., 2 (1): 62-67

33. Veerachari, U and Bopaiah, A. K., (2012). Phytochemical investigation of the ethanol, methanol and ethyl acetate leaf extracts of *six Cassia species. Int. J. Pharm. Bio. Sci.*, 3 (2): 260-270.