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Standardization, Phytochemical and Pharmacological evaluation of *Cassia tora* seed extracts

Roopashree T.S*, Raman dang [1], Shobha Rani R.H[1] and Narendra C[2]

**Nargund College of Pharmacy, Bangalore, Karnataka, India*
[1] Al-Ameen college of pharmacy, Bangalore, Karnataka, India
[2]Visveshwarapuram Institute of Pharmaceutical Sciences, Bangalore, Karnataka, India

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ABSTRACT

Pharmacognostic investigation of the fresh, powdered and anatomical sections of the seeds of Cassia tora was carried out. The seeds were subjected to extraction and extract was standardized as per the guidelines and methods set by world health organization for herbal drugs. Antimicrobial activity of the standardized extract was carried out against both G $^{+ve\ and}$ G $^{-ve}$ organisms by cup-plate technique. Pharmacognostic evaluation confirmed the authenticity of the seeds, while phytochemical studies revealed the presences of carbohydrates, glycosides, saponins, triterpenes, tannins and some flavanoids in the extracts. The extract was identified by the presence of emodine in it by HPTLC. The extract was free from heavy metals and arsenic. The ash values were with in the acceptable limits. The saponin content of the extract was found to be 18.4%. Antibacterial evaluation revealed that aqueous extract was more effective and S.aureus was the most susceptible organism.

Keywords: Antimicrobial activity, *Cassia tora*, HPTLC, Standardization.

INTRODUCTION

There is much interest created by natural resources such as herbs for their therapeutic activity and treatment of diseases in man. *Cassia tora* (F: Cesalpinaceae) Linn is a small plant growing on dry soil in Bengal and throughout the tropical parts of India. Leaves, seeds and Root are used in internal as well as external preparations.[1] *Cassia tora* exhibits wide range of pharmacological properties [2-3]. Both leaves and seeds contain a glucoside resembling Chrysophanic acid and Emodine [4]. Seeds constitute valuable remedy in bacterial infections and skin diseases. It is reported to be highly useful in Psoriasis, Leprosy and sciatic pain in joints [5]. Seeds of the plant have been used in many ayurvedic formulations for both internal and topical usage. Standardization has been the key factor in the field of natural products and herbal drugs for their safe and effective use. For these reasons we report pharmacognostic parameters for the identification of seeds of *Cassia tora* and standardization

of its aqueous extract by using physical, phytochemical, analytical and microbiological methods [6]. To potentate the usefulness of the herb in treating infections antibacterial activity was evaluated against gram positive and gram negative organism. With growing resistance of various microbes against most of the existing antibacterial agents, especially for treating skin diseases, there is thrive for discovering new antibiotics. Herbs with their diverse phytoconstituents, new mode of action and pharmacological effect can be highly useful in controlling various infections and for treating dreaded skin diseases like psoriasis.

MATERIALS AND METHODS

Plant Material

Samples of *Cassia tora* seeds were obtained from Natural remedies, Bangalore and identified at Regional Research Institute, Bangalore.

Chemicals: All chemicals and solvents used were of analytical grade obtained from Rankem Pvt, Ltd and Himedia, India.

Extraction of *Cassia tora*

Dried seeds of *Cassia tora* were powdered and 30gms of powder was subjected to extraction in a soxhlet apparatus. *Cassia tora* was extracted with petroleum ether, benzene, chloroform, methanol and water successively. Before extraction with the next solvent the powder was air dried to remove the adhering solvent. Extracts were concentrated by evaporating the solvent in water bath.

Phytochemical analysis of the different extracts was carried out as per the standard procedures [7-8].

Antimicrobial activity of the different extracts

Activity was evaluated by Cup-plate technique using nutrient agar medium against *Staphylococcus aureus* (ML 267), *Bacillus subtillus* (ATCC 6633), *Pseudomonas aeruginiosa (*ATCC 25619*)* and *Escherichia coli* (ATCC 10536) collected from institute of microbial technology, Chandigarh, India.

Test Sample: 200 mgs of *Cassia tora* seeds aqueous extract was dissolved in 10 ml of DMSO to get the concentration of 20mg/ml.

Preparation of Inoculums: Suspension of organism was prepared as per McFarland nephelometer standard . A 24 hour old culture was used for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride(0.9% w/v) and the turbidity was adjusted such that it contained approximately 1.5X 10^8 cells/ml. It was obtained by adjusting the optical density of the bacterial suspension to that of a solution of 0.05ml of 1.175% of barium chloride and 9.95 ml of 1% sulphuric acid.

Procedure: The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at121^0C for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160^0C for 1 ½ hours. 30 ml of sterile molten agar medium was seeded by organisms (about 2 ml according to Mc Farland's standard), in semi hot conditions (40^0C) was poured aseptically in sterile Petri plate and allowed to solidify at room temperature. Bores were made on the medium using sterile borer and 0.1 ml of the extract was added to respective bore and 0.1ml of the standard Streptomycin at a concentration of 100 µg / ml was taken as standard. The Petri plates seeded with organisms, containing extracts and the

standard were kept in refrigerator at 4^0C for 1 hour to facilitate the diffusion of the extracts and the standard in to the media. After diffusion the Petriplates were incubated at 37 ± 1^0C for 24 hours in a BOD incubator and zone of inhibition was observed and measured using a scale [9].

Standardization of aqueous extract of *Cassia tora* [10].
Description: Description of the extract includes physical appearance of the extract by visual examination under diffused light.
Solubility: Solubility of the extract was observed with water, 95% and 50% ethanol.
Identification*:* By Thin Layer Chromatography; Adsorbent : Silica gel 60 F254,
Solvent system: Ethyl acetate: Methanol: Water 80:19:1.
Sample preparation: 1 gm of the extract is dissolved in 5 ml of DMF and
volume was made upto 50ml with methanol, filtered and used for TLC
Standard Preparation: Emodine 1mg/ml in methanolic DMF Solvent front run upto 8 cms.
Detection: UV - 366nm (Fig. 5).

Moisture Content: Moisture content of the extracts of all the three extracts was determined by IR moisture balance.
Ash values: The total ash, acid insoluble ash and water-soluble ash values were determined for the extracts using the standard procedure.

Test for Pb,Ni,Cd and Arsenic: As per the guidelines set by world health organization
Preparation of the extracts: The extracts were accurately weighed, finely powdered and taken up for the heavy metal analysis.

Preparation of digestion mixture: A ternary digestion solution of three acids was prepared by mixing 100 ml of conc. $HN0_3$, 10 ml of conc. H_2S0_4 and 40 ml of 60% $HCl0_4$ and allowed to cool.

Wet oxidation of Plant samples: The extracts were treated with the ternary acid mixture in a silica crucible without exerting pressure for the oxidation of the extracts. Blank digestions (in duplicate) were run on the reagents added in the same amounts as employed in the sample determinations.

Cd/Pb/Ni contents in the extracts: Sample was placed in digestion flask and then mixed with appropriate amount of the ternary acid mixture (5 ml for 1-2 gms of powdered extracts). Digestion was carried out at 180^0C to 200^0C until dense white fumes of H_2SO_4 : $HClO_4$ were evolved. The digestion was continued at 180^0C to 200^0C until the acid was largely volatilized and the residues in the flask were clear white and only slightly moist with H_2SO_4. The residue was diluted with glass distilled water and made up to definite volume in a volumetric flask. Then the solution was ready for the estimation of different toxic heavy metals like Cd, Pb and Ni.[10] These samples were analyzed with the help of Atomic Absorption Spectrophotometer (AAS) Perkin Elmer model AAnalyst 100.

Calculation: mg/kg of heavy metals (Cd, Pb and Ni) in plants = Sample dilution X AAS reading in mg/kg.

Limit test for Arsenic: Preparation of the sample by acid digestion: Accurately weighed aqueous herbal extract (5gms) was powdered and treated with water (2.5ml) followed by nitric acid (5 ml) and sulphuric acid (2 ml) carefully. This was kept in a fuming cupboard with repeated addition of nitric acid until no further darkening takes place, until a clear solution with copious vapors of

sulphur dioxide was obtained indicating the complete removal of organic matter. The mixture was cooled and treated with a mixture of water (7.5) and 25g/l ammonium oxalate (2.5ml). This was further heated until fumes of sulfur trioxide were developed. This was cooled and transferred to 25ml volumetric flask, volume was adjusted with water and used for the limit test.
Preparation of the standard stain: Standard stain was prepared by mixing 10 ml of stannated hydrochloric acid and 1 ml of dilute arsenic to 50ml of water and treated as described in the general test, as per Indian Pharmacopoeia , which yields a stain on mercuric bromide paper AsR referred to as the standard stain. The intensity of the stain produced by the sample (extract) was compared to that of the standard[10].

Microbial test; Total viable aerobic count
Pretreatment of the extracts: One gram of the extracts was dissolved in 100 ml of buffered sodium chloride- peptone solution pH 7.0.

Bacteria: 1 ml of the pretreated extracts solution was mixed with 15 ml of sterilized liquefied *casein-soybean digest agar* at a temperature not exceeding 45^0C, and transferred aseptically into a sterilized petriplate and incubated at 30 ± 5^0C for 5 days and the number of colonies formed observed.

Fungi: 1 ml of the pretreated extracts solution was mixed with 15 ml of sterilized liquefied *Sabouraud glucose agar* at a temperature not exceeding 45^0C, and transfer this aseptically into a sterilized petriplate. Incubate the petriplate in an incubator at 20 ± 5^0C for 5 days and observed for the colonies formed [10].

Determination of saponins*:* Determination of the saponin contents was carried out as per the method described by Rajpal [11].

RESULTS AND DISCUSSION

Pharmacognostic evaluation of seeds of *Cassia tora were* observed to be Light brown in colour with mild and characteristic odour, mucilaginous and slightly bitter taste and were found to be rombohedral in shape ranging from 3.0 to 4.0 mm long and 2.0-3.0 mm thick. Microscopically, the cells of the epidermis consist of fragments of polyhedral to elongated parenchyma cells containing mucilage and starch granules. The endosperm showed presence of thick walled cells with numerous pits, oil globules, aleurone grains, brown matter and fragments of non-lignified cuticle. Chemo-microscopy revealed the presence of tannins, starch grains, lignin, cellulose, and mucilage, proteins, calcium oxalate crystals, fats and oils [12].

Phytochemical evaluation revealed presence of glycosides, tannins, flavanoids and saponins in different extracts. Table.No.1.

Percentage of moisture content, total ash, acid insoluble ash and water soluble ash was found to be 4.39±0.68, 3.4±0.54, 0.01±0.01 and 0.16±0.02 respectively [12].

Antibacterial activity: Cassia tora aqueous extract exhibited good antibacterial activity in terms of zone of inhibition as compared to other extracts against both gram positive and gram negative organisms except *Basillus subtillus*. *S.aureus* was most susceptible to the aqueous extract. Table.No.2. comparative antibacterial activity of different extracts against different organisms is given in Fig.1.

Table. No.1. Data showing phyto-constituents present in different extracts of *Cassia tora*.

Sl.No	Phyto constituents	Name of the chemical Test	*Cassia tora*			
			P. E	Met	Eth	Aq
1	Alkaloids	1. Mayer's test	-	-	-	-
		2. Dragendroff's test	-	-	-	-
		3. Wagner's test	-	-	-	-
		4. Hagers test	-	-	-	-
2	Carbohy-drates	1. Molisch's test	-	-	+	+
		2. Benedicts test	-	-	+	+
		3. Fehling's test	-	-	+	+
3	Glycosides	1.Modified Borntragers	-	+	+	+
		2. Legal test	-	+	+	+
4	Saponins	1. Foam test	-	-	+	+
		2. Froth test	-	-	+	+
5	Triterpenes	1. Salkowski test	-	-	+	+
		2. Libermann Burchard	+	-	+	+
		3. Tschugajew test	+	-	+	+
6	Fats & Oil	1. Stain test	+	-	+	-
7	Resins	1. Acetone water test	-	-	-	-
8	Phenols	1. Ferric Chloride test	-	-	-	-
9	Tannins	1. Alkaline Reagent	-	-	-	-
10	Flavanoids	1. Gelatin test	-	-	-	+
		2. Lead acetate test	-	-	-	+
		3. Shinoda test	-	-	-	+
		4. Zn-Hcl reduction	-	-	-	+
11	Proteins	1. Xanthoproteic test	-	-	-	-
		2. Ninhydrin test	-	-	-	-
		3. Biuret test	-	-	-	-
12	Diterpenes	1. Copper acetate test	-	-	-	-

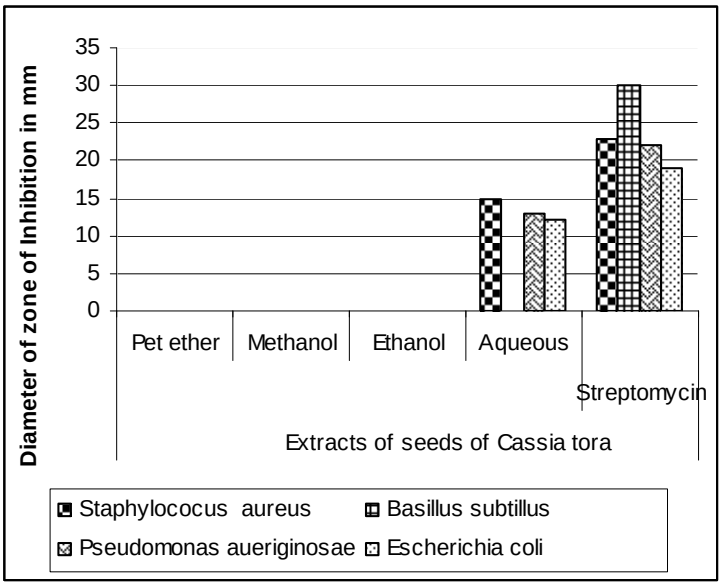
Table. No.2. Antibacterial activity of different extracts of *Cassia tora*

Sl.No	Name of the Organism	Diameter of zone of Inhibition in mm*				
		Cassia tora extracts				Streptomycin
		Pet ether	Methanol	Ethanol	Aqueous	
1	*Staphylococus*	--	--	--	15±0.69	23±0.56

	aureus					
2	*Basillus subtillus*	--	--	--	--	30±0.48
3	*Pseudomonas aueriginosae*	--	--	--	13±0.54	22±0.58
4	*Escherichia coli*	--	--	--	12±0.85	19±0.64

*Values are in terms of Mean±SEM of results done in triplicate, -- = Nil

Fig.No.1. Comparative antibacterial activity of different extracts of seeds of *Cassia tora*



Standardization of aqueous extract of Cassia tora: Complete standardization data for the aqueous extract of seeds of *Cassia tora* is given in Table.No.3. The extract was a dry blakish brown crystalline powder with agreeable and characteristic agreeable odor and taste. Reference standard emodine gave a single spot with a Rf of 7.0, while the extract also exhibited a spot with the Rf value of 0.7, which corresponds to that of the reference standard.

Aqueous extract was completely soluble in water, partically soluble in 50%v/v alcohol and it was completely insoluble in 95%v/v alcohol. Moisture content, total ash, acid insoluble and water soluble ash values of the extract was found to be 1.39±0.56, 0.46±0.02, 0.01±0.01 and 0.09±0.02 respectively. Total saponin content was found to be 18.4 ±1.25 %. Heavy metals such as lead, cadmium and nickel were found to be absent. The extract did not show presence of any bacteria or fungi.

Table.No.3. Standardization data of vacuum dried aqueous extract of seeds of *Cassia tora*

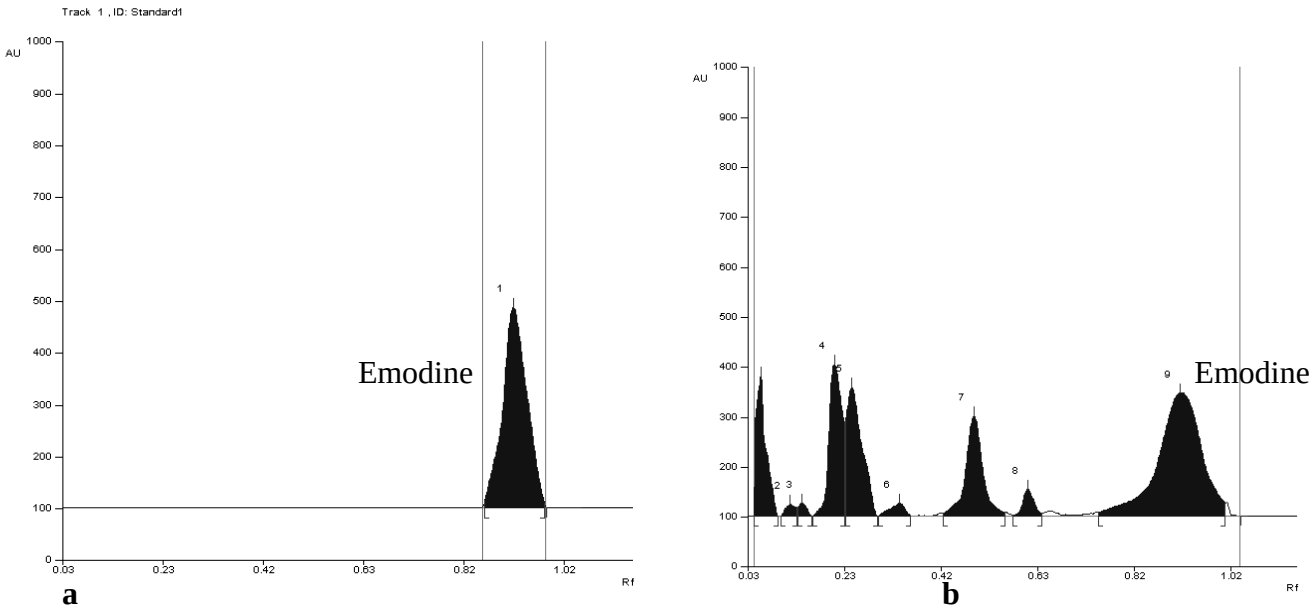
Sl.No	Parameters	Results
1	Color	Blackish brown
2	odour	Agreeable, characteristic

3	Nature		Crystalline
4	Taste		Bland
5	Solubility	Water	Soluble
		Alcohol 50%v/v	Partially soluble
		Alcohol95%v/v	Insoluble
6	Moisture content (%)		1.39±0.56*
7	Total ash(%)		0.46±0.02*
8	Acid insoluble ash(%)		0.01±0.01*
9	Water soluble ash(%)		0.09±0.02*
10	Total Saponins(%)		18.4 ±1.25*
11	Limit test for Arsenic		Passes
12	Heavy metals	Nickel	Nil
		Cadmium	Nil
		Lead	Nil
13	Microbiological test	Bacteria	Absent
		Fungi	Absent

*Values are mean±SEM of values done in triplicate

Apart from reference compound other nine phyto-constituents corresponding to Rf values of 0.8, 0.04, 0.09, 0.13, 0.16, 0.23, 0.29, 0.43 and 0.57 were detected in the HPTLC chromatogram. Fig.2

Fig.2. HPTLC chromatogram of a) Reference compound exhibiting single peak (Rf 0.70)and b) Aqueous extract of *Cassia tora* exhibiting presence of reference compound (0.70)along with other phyto constituents.



a **b**

X-axis =Rf values, Y-axis = concentration of the constituent.

CONCLUSION

Although *Cassia tora* is a very common plant found as a road side weed in most parts of India and has been used extensively for its medicinal use since ages, there are very few scientific studies conducted to support its usefulness. This systematic study gives a scientific proof with respect to its pharmacological and medicinal uses such as antibacterial and antipsoriatic agent.

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