

STANDARDIZATION, PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *CASSIA TORA* SEED EXTRACT

T.S.Roopashree, Dang Raman, R.H. Rani Shobha, C. Narendra

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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 Roots are used in internal as well as external preparations. (Hatano T,1999) *Cassia tora* exhibits wide range of pharmacological properties (Kim YM, 2004 and Podolsky, 2003) Both leaves and seeds contain a glucoside resembling Chrysophanic acid and Emodin.(Acharya Tk, 1975) 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.(Tse TW, 2003) Seeds of the plant has 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. 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* and 30gms of powder was subjected to extraction in a soxhlet apparatus. *Cassia tora* was extracted with petroleum ether, benzene, chloroform, methanol and water. 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(Evans WC, 1996, Brain KR, 1975 and British Pharmacopoeia,1980)

Antimicrobial activity of the different extract

Procedure

Activity was evaluated by Cup-plate technique using nutrient agar medium against *Staphylococcus aureus* (ATCC 2267), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (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 1.5×10^8 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 at 121°C for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160°C 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°C) 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°C 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°C for 24 hours in a BOD incubator and zone of inhibition was observed and measured using a scale. (Ellen JB, 1990)

Standardization of aqueous extract of *Cassia tora* (WHO publication, 1998)

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: Emodin 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. HNO₃, 10 ml of conc. H₂SO₄ and 40 ml of 60% HClO₄ 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°C to 200°C until dense white

fumes of H₂SO₄ : HClO₄ were evolved. The digestion was continued at 180°C to 200°C until the acid was largely volatilized and the residues in the flask were clear white and only slightly moist with H₂SO₄. 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. 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 (5 gms) 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.

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.

For 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°C, and transferred aseptically into a sterilized petriplate and incubated at 30±5°C for 5 days and the number of colonies formed observed.

For 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°C, and transfer this aseptically into a sterilized petriplate. Incubate the petriplate in an incubator at 20±5°C for 5 days and observed for the colonies formed . **Determination of saponins:** Determination of the saponin contents was carried out as per the method described by Rajpal (Rajpal, 2002).

RESULTS

Pharmacognostic evaluation

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 rhombohedral 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. (Nadkarni, 1982)

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.

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 *Bacillus subtilis*. *S.aureus* was the most susceptible to the aqueous extract. Table.No.2. comparative antibacterial activity of different extracts against different organisms is given in Fig.1.

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 bluish brown crystalline powder with agreeable and characteristic agreeable odor and taste.

Identification: 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.

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.1. Aqueous extract was completely soluble in water, partially 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.

DISCUSSIONS

Cassia tora is a road side weed commonly found all over India. The herb has wide range of medicinal uses due to its diverse pharmacological activity. Apart from being used as antibacterial and antioxidant agent the plant has usefulness in treating various skin ailments such as psoriasis, leprosy, atopic dermatitis etc.(Hatano T, 1999, Kim YM, 2004 and Patel RP, 1957).

Identification of the crude drug is the most important aspect to begin any study with regards to the herbal drugs. In this regards morphological, microscopical and proximate analysis of the seeds of the herb will help to ensure the authenticity and quality of the herb.

Cassia tora seeds were evaluated for the morphology, gross microscopy and powder microscopy. The results were found to be similar to that reported in the literature indicating the authenticity of the test material.(Nadkarni KM, 1982)

Proximate analysis : The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth. Equally important in the evaluation of crude drugs, is the ash value and acid-insoluble ash value determination. The total ash is particularly important in the evaluation of purity of drugs, i.e. the presence or absence of foreign inorganic matter such as metallic salts and/or silica. (WHO guidelines. 1998).

Phytochemical evaluation: Different chemical compounds such as glycosides, tannins, flavonoids, saponins among others were detected in *Cassia tora*, which could make the plant useful for treating different ailments and having a potential of providing useful drugs of human use. This is because the pharmacological activity of any plant is usually traced to a particular compound(s). Table.No.1. The presence of tannins, saponins and other phenolic compounds, which have antiseptic properties, could explain the use of this plant for the treatment of sores and as anti-psoriatic agent.

Antimicrobial activity: Different extracts of *Cassia tora* was evaluated for its antibacterial activity. Among the extracts aqueous extract was found to be most effective as compared to other extracts. Among the organisms tested *S.aureus* was most susceptible.

Aqueous extracts of *Cassia tora* have been reported to be used as decoctions and infusions for treating skin infections and other skin diseases such as psoriasis (Patel RP, 1957).

S.aureus has been found to be the causative organism for various infections and is known to aggravate skin conditions like psoriasis, atopic dermatitis, erythroderma etc.(Tomi NS, 2005) According to American association of Dermatology, antibacterial therapy is included in treating psoriasis and many of the antibacterial agents, including those

obtained from the herbal source are used for treating different skin diseases like psoriasis (AAD Bulletin, 1991).

The study supports the use of aqueous extract of seeds of *Cassia tora* in treating various skin diseases and other bacterial infections.

Standardization being one of the one of the biggest challenges in the field of natural products, different people have different meanings to the word standardization. As per the latest edition of European Pharmacopoeia, standardization means “adjusting the herbal drug preparation to a defined content of a constituent or a group of substances (total saponins, total bitters, total alkaloids, total glycosides, total flavanoids, phenols etc). These compounds exert therapeutic effect and account for medicinal property of the medicinal herb. Most of the herbal extracts are made from crude herbs and can vary in percentage of active constituents, which further influences the therapeutic activity of herbs. Depending upon the source, the variation in the chemical composition of the medicinal plants is enormous, a possible cause of inconsistent therapeutic effects of herbal products. Thus, standardization involves creating clear links between a plant’s chemistry to its biology by using physical, phytochemical, analytical and microbiological methods.

As the Aqueous extract of seeds of *Cassia tora* exhibited highest antibacterial activity further standardization was carried out as per WHO guidelines. Emodin has been reported to be a marker compound for *Cassia tora*, hence it was used for the identification (Ido I, 2002)

Saponins in *Cassia tora* have been reported to have, anthelmintic, antipsoriatic, antioxidant, astringent and antibacterial properties. Many ayurvedic marketed preparations with *Cassia tora* as one of the ingredient are standardized with respect to its saponin content hence, saponin content was determined.

Determination of arsenic and heavy metals in herbal drugs is of major importance with respect to increasing concerns about heavy metal induced toxicity. Absence of heavy metals and arsenic ensures the safety of the extract. *Cassia tora* decoction is used as tea for its antibacterial and antifungal, anthelmintic activity in traditional system of medicine. (Nadkarni, 1982) The earlier experimental studies also indicate the antibacterial properties of the herbal extract, which might be the reason for the absence of bacteria and fungi in the extract.

ABSTRACT

The study was designed to standardize and evaluate the antibacterial activity of aqueous extract of seeds of *Cassia tora* (Cesalpinaceae). 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 the aqueous extract of *Cassia tora* seeds was standardized as per the guidelines and methods set by world health organization for herbal drugs. The standardized extract was subjected to phyto-chemical analysis followed by antimicrobial evaluation using cup-plate technique. Pharmacognostic evaluation confirmed the authenticity of the seeds with respect to its morphology as well the microscopy. Phytochemical studies of the standardized extract revealed the presences of carbohydrates, glycosides, saponins, triterpenes, tannins and some flavanoids. 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 within 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. The present study supports the use of aqueous extracts of *Cassia tora* seeds for treating bacterial infection and skin diseases like psoriasis.

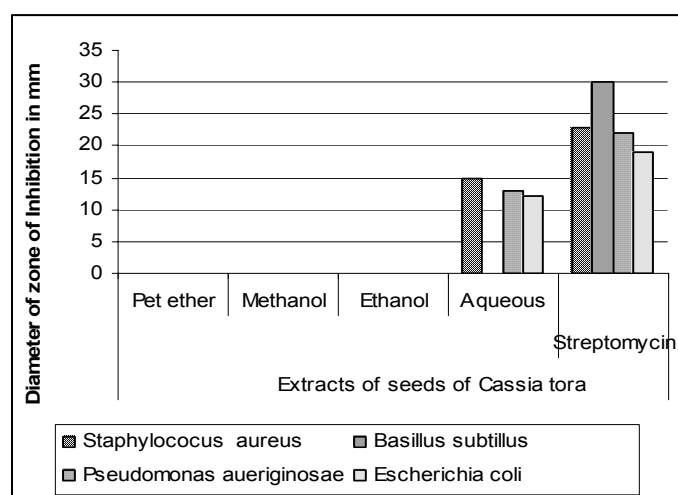


Fig.1.Comparative antibacterial activity of different extracts of seeds of *Cassia tora*

Table 1. Data showing phyto-constituents present in different extracts of *Cassia tora*

No	Phyto constituents	Name of the chemical Test	<i>Cassia tora</i>			
			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 2. Antibacterial activity of different extracts of *Cassia tora*

No	Name of the Organism	Diameter of zone of Inhibition in mm*				
		<i>Cassia tora</i> extracts				Streptomycin
		Pet ether	Methanol	Ethanol	Aqueous	
1	<i>Staphylococcus aureus</i>	--	--	--	15±0.69	23±0.56
2	<i>Basillus subtilus</i>	--	--	--	--	30±0.48
3	<i>Pseudomonas aeruginosae</i>	--	--	--	13±0.54	22±0.58
4	<i>Escherichia coli</i>	--	--	--	12±0.85	19±0.64

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

Table 3. Standardization data of vacuum dried aqueous extract of seeds of *Cassia tora*

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
		Alcohol 95%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

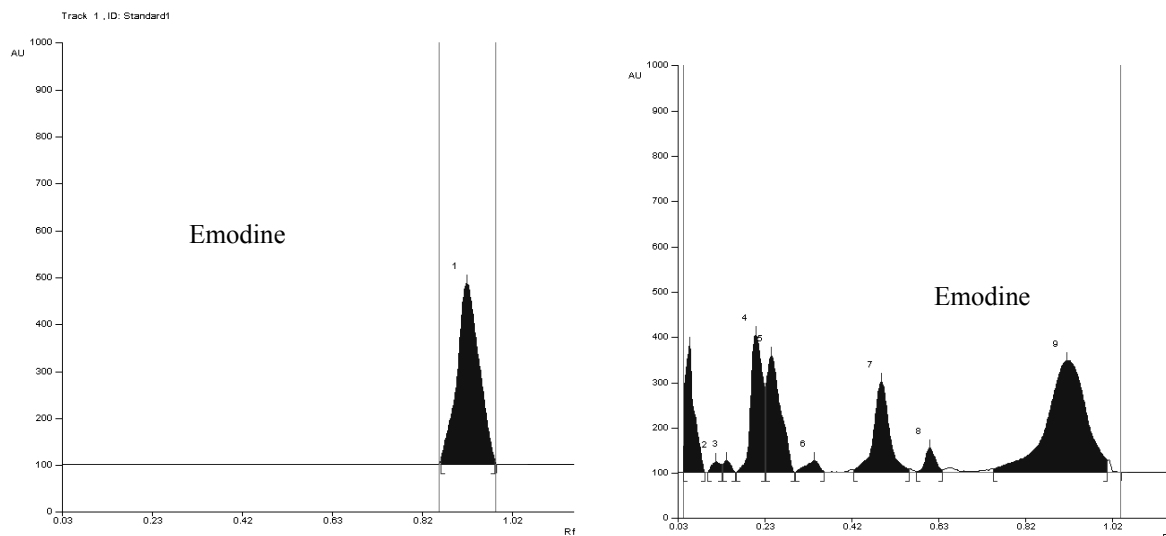


Fig. 2. HPTLC chromatogram of a)reference compound exhibiting single peak (Rf 0.70)and b) aqueous extract of *Cassia tora* exhibiting presence of reference compound (0.70)along with other phyto constituents
X-axis =Rf values, Y-axis = concentration of the constituent

CONCLUSIONS

Cassia tora seeds were authenticated and pharmacognostical standards were established. Phyto-chemical analysis confirmed presence of carbohydrates, glycosides, saponins, triterpenes, tannins and flavanoids. The aqueous extract was standardized as per WHO guidelines. Among the other extracts, aqueous extract *Cassia tora*

exhibited highest antibacterial activity against *S.aureus*. This study supports the use of *Cassia tora* in the form of decoction in traditional system of medicine for treating infections and other skin diseases.

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AUTHORS' ADDRESS

ROOPASHREE T.S. - Department of Pharmacognosy, Al-Ameen College of Pharmacy, Near lal bagh main gate, Hosur road, Bangalore-27.

DR.RAMAN DANG - Professor, Department of Pharmacognosy, Al-Ameen College of Pharmacy, Near lal bagh main gate, Hosur road, Bangalore-27.

SHOBHA RANI R.H. - Department of Pharmacy practice, Al-Ameen College of Pharmacy, Near lal bagh main gate, Hosur road, Bangalore-27.

NARENDRA C. - Asst. Prof, Dept of Pharmaceutics Visveswara pura Institute of Pharmaceutical Sciences, Bangalore. Corrospoding Author : roopa_tsr@yahoo.co.in.