



Antimicrobial Activity and Phytochemical Analysis of Medicinal Plant *Cassia tora*

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Abstract: Present study was carried out to investigate *in vitro* anti-bacterial and anti-fungal activity from the seeds of an Indian traditional medicinal plant *Cassia tora*. Plant material was separated and successively extracted with various organic solvents. Extracts were evaluated for solubility, moisture content, melting point, FTIR and other qualitative analysis for photoconstituents. *In vitro* antibacterial and antifungal studies were carried out by disc diffusion method. Two test samples were prepared in concentrations of 100mg/ml. Extract was found efficacious against various strains of bacteria and fungal species. 100mg/ml test sample shows better zone of inhibition in bacterial strains. It was obtained in the range of 16.67 to 23.00 among them maximum inhibition was observed in *Pseudomonas aeruginosa* (gram positive bacteria) and last inhibition was observed in *E. coli* (gram negative). It has also shown satisfactory inhibition in fungal species like *Candida albicans* and *Candida glabrata*. Thus, *Cassia tora* can be considered as a safer and efficacious herbal candidate for antimicrobial formulation.

Keywords: *Cassia-tora*, Vitro Study, Anti-bacterial, Anti-fungal, Isolation

1. Introduction

Cassia tora is a small shrub that grows widely as a weed in almost all the Asian countries [1-5]. The leguminosae [6, 7] commonly known as the legume, pea or bean family, are a large and economically important family of flowering plants. Legumes are a type of species in which the seeds grow to develop in to pods [8, 9]. Legumes are a good source of starch, dietary fiber, protein, minerals; legumes are a valuable part of a healthy diet [10-14]. As a group, nutrient composition of legumes makes them ideal animal foods [15] to meet dietary recommendation. Legumes have been recognized as functional food that promote good health and have therapeutic properties [16, 17]. *Cassia tora* is legume in the sub-family *Casual pinoideae* [18]. Its name has been derived from Sinhala languages, in which it is called *Tora* s an annual herb, 30-90 cm high which occurs as wasteland rainy season wild plant in India. *Cassia tora* are wild crop that grows in most parts of India as a weed [19]. The main useful parts of *Cassia tora* are leaves, roots and seeds. Gaur gum, also called guaran, is a gelling agent. Gaur

gum is extracted from a legume, the gaur bean. Gaur gum is widely used in food industry- in baked goods to increase dough yield and improve texture and shelf life, in dairy industry as a stabilizer, in meats as a lubricant, in desserts, frozen food items etc. It has also been considered of interest with regards to both weight loss and diabetic diets [20, 21]. *Cassia tora* has been reported to contain many active substances, including Anthraquinone, Quercetin, chrysoferol, emodin, rhein, etc. *Cassia tora* has been reported to exhibit significant antimutagenic activity [1, 2, 22]. Anthraquinone act as a fluorescence sensor [23-26] or fluorophores therefore this plant also shown sensing properties. It constitutes an Ayurvedic preparation "Dadhughnavati" which is one of the successful antifungal formulations [27].

India has rich heritage of Ayurveda and other alternative systems of medicines. The knowledge of medicinal plants was already implied by prehistoric people since times immemorial. Various ancient Indian literature like Ayurveda, *Shushrutsamhita* and *Charaksamhita* have variety of details regarding herbs, extracts, surgery and transplant. Discovery of various antibiotics like Penicillin, *Cafalosporins*,

Aminoglycosides, Macrolides, quinolones etc has created a revolution in medical science by curing wide range of disease. It is reported that on a revolution in medical science by curing wide range of diseases [28]. It is reported that, on average, two or three antibiotics derived from microorganisms are launched each year. After a downturn in that pace in recent decades, the pace is again quickening as scientists realize that the effective life span of any antibiotic is limited. Worldwide pending on finding new anti-infective agents (including vaccines) is expected to increase 60% from the spending levels in 1993. New sources, especially plant sources, are also being investigated. Second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. Therefore, there is a need to revive ancient knowledge of herbs for a safer and economics treatment [29].

2. Experimental Section

2.1. Collection and Identification

The plant material was collected in the month of September from Surendranagar district. It was identified by department of Pharmacognosy, C. U. Shah University and herbarium specimen was stored. Dirt particles were removed and it was subjected for shade drying for 15-20 days. After drying, legumes and it was washed thoroughly. Seeds were again subjected for drying in tray dryer legumes and it was washed thoroughly. Seeds were again subjected for drying in tray dryer at 60°C for 4-5 hours. After drying, seeds were collected in a tray and it was inspected visibly for solid impurities. After shifting in a sieve, grinding was done and seeds were converted to fine dry powder. The powder was stored in a air tight container for further use.

2.2. Qualitative Analysis of Plant Material

Solubility Study:

Solubility study was performed using solvent like water, methanol, ethanol, Chloroform, NaOH (0.1), & HCl (0.1). The procedure was followed as per IP (Indian pharmacopeia).

Moisture Content:

Take 1 gm extract in a bottle. Keep it for drying in hot air oven at 100°C for 6 hours. Take initial weight and final weight and calculate the Loss on Drying.

Melting point:

Melting point of the drug was determined by taking small amount of the drug in a capillary tube closed at one end and was placed in a melting point apparatus and the temperature at which the drug melts was noted. Average of triplicate reading was taken.

2.3. Collections and Identifications

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2.4. Method for Extraction

There are various methods of extraction for crude drug like infusion, decoction, digestion, percolation, maceration and hot continuous percolation (Soxhlet). Among all these methods, Hot continuous percolation i.e. Soxhlet method is most reliable and it gives maximum yield of extract. Drug is suitably comminuted. 150gm of powdered drug was packed in a thimble which was placed into extractor. In first stage, 1500ml Petroleum ether 60:80 was added into a round bottom flask. By this step, fatty materials and oils were removed. The continuous cycles were carried out for 5 hours a day for 3 days. After that, it was further extracted with methanol until the clear white solutions from the siphon tube were obtained. After the Soxhlet extraction, a thick dark solvent with extracted material is accumulated in the round bottom flask. Extra solvent was removed by suction pump. Extract was collected and dried in a porcelain dish and placed into desiccators. This extract was further used for experimental purpose.

3. In-Vitro Antimicrobial Activity

Preparation of test Sample

For antimicrobial studies of the leaves, the concentration in range of 1-4 mg/ml was prepared by dissolving solid extract in suitable solvent.

Dilutions and inoculums preparations

The dried extract was weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 50, 100mg/ml. The antibiotic Ciprofloxacin was weighed and dissolved in DMSO to prepare appropriate dilution to get required concentration of 1mg/ml. The inoculums of bacterium were prepared in nutrient broth medium and were incubated at 37°C for 8 hours.

Procedure for performing the disc Diffusion test

The required amount of petri plates is prepared and autoclaved at 121°C for minutes. And they were allowed to cool under laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dish and allowed to solidify. 1 ml inoculums suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The sterile discs were loaded with different concentrations of about 50, 100mg/ml of plant extract of Cassia tora and antibiotic (ciprofloxacin for antibacterial) into each separate disc of about 100µl. The paper diffuse discs were incubated at 37°C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm) [30, 31].

Antifungal activity

Dextrose medium was used. The required amount of Petri plates and autoclave at 121°C for 15 minutes and they were allowed to cool under laminar air flow. Aseptically transfer

about 20 ml of media into each sterile Petri dish and allowed to solidify. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass to get uniform distribution of the fungi. The sterile discs were loaded with different concentrations about 50, 100mg/ml of plant extract of *Cassia tora* and antifungal (Fluconazole for antifungal activity) into each separate disc of about 100 μ l. The paper diffuse discs were placed on the medium suitably apart and the plates were incubated at 37° for 24 hours. The antifungal activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm) [32-34].

4. Results and Discussion

Table 1. Solubility of drug in solvents.

Sr. No.	Name of solvents	Solubility in grade
1	Water	++++
2	Methanol	+++++
3	Ethanol	+++++
4	Chloroform	++++
5	HCL (0.1)	+++
6	NaOH (0.1)	+++

Methanolic extract of *Cassia tora* was found to be highly soluble in Methanol and ethanol. Readily soluble in water and sparingly soluble in 0.1N HCL and 0.1N NaOH. Qualitative results of solubility by grade are shown in table 1.

4.1. Phytochemical Screening

The Phytochemical screening [35, 36] of the drug is a very sensitive aspect in the process of standardization and quality control because the constituents vary qualitatively and quantitatively not only from plant to plant but also in

4.2. Antibacterial & Antifungal Activity

Table 7. Antibacterial activity of standard drug Ofloxacin (1mg/ml).

Sr. No.	Strain	Zone of inhibition(mm)(Mean \pm SEM)				
		Stda	Stdb	StdC	Ofloxacin Mean	Std.Dev.
1	Escherichia coli	23	25	24	24.00	1.00
2	Salmonellatyphi	20	19	20	19.67	0.58
3	Pseudomonas aeruginosa	22	21	24	22.33	1.53
4	Staphylococcus aureus	23	24	21	22.67	1.53
5	Coagulase negative Staphylococci	19	18	21	19.33	1.53
6	Klebsiellapneumoniae	21	20	20	20.33	0.58

Table 8. Antibacterial activity of Test sample 1 (*Cassia tora* extract 100mg/ml).

Sr. No.	Strain	Zone of inhibition(mm)(Mean \pm SEM)				
		Testa	Testb	Testc	100mg/ml	Std.Dev.
1	Escherichia coli	16	17	17	16.67	0.58
2	Salmonellatyphi	18	19	18	18.33	0.58
3	Pseudomonas aeruginosa	22	23	24	23.00	1.00
4	Staphylococcus aureus	23	23	22	22.67	0.58
5	Coagulase negative Staphylococci	20	21	19	20.00	1.00
6	Klebsiellapneumoniae	22	20	21	21.00	1.00

different samples of the same species depending upon various atmospheric factors and storage conditions. The results are presents below:

Table 2. Test for Alkaloids.

Sr. No.	Tests	Observation	Inference
1.	Mayer's test	No precipitate	Alkaloid absent
2.	Wagner's test	No precipitate	Alkaloid absent

Table 3. Test for Carbohydrates.

Sr. No.	Tests	Observation	Inference
1	Moilsch's test	A violent ring at the junction	Carbohydrate confirmed
2	Fehling's test	Brick red precipitate	Carbohydrate confirmed
3	Barfoed's test	Red precipitate	Carbohydrate confirmed
4	Benedict's test	Red precipitate	Carbohydrate confirmed

Table 4. Test for Steroids.

Sr. No.	Tests	Observation	Inference
1.	Libermann Burchard test	Green colour	Steroid Present

Table 5. Test for Proteins.

Sr. No.	Tests	Observation	Inference
1.	Biuret test	Appearance of violet colour	Protein confirmed
2.	Millon's test	Pink colour	Protein confirmed

Table 6. Test for Tannins.

Sr. No.	Tests	Observation	Inference
1.	A small quantity of extract were treated with 10% lead acetate solution	No precipitate	Tannins absent

Table 9. Antibacterial activity of Test sample 2 (Cassia tora extract 100mg/ml).

Sr. No.	Strain	Zone of inhibition(mm)(Mean \pm SEM)				
		Testa	Testb	Testc	100mg/ml	Std.Dev.
1	Escherichia coli	13	14	14	13.67	0.58
2	Salmonelltyphi	14	14	15	14.33	0.58
3	Pseudomonas aeruginosa	16	17	16	16.33	0.58
4	Staphylococcus aureus	19	19	21	19.67	1.15
5	Coagulase negative Staphylococci	15	16	15	15.33	0.58
6	Klebsiellapneumoniae	16	16	18	16.67	1.15

Table 10. Antifungal activity of standard drug Fluconazole (1mg/ml).

Sr. No.	Strain	Zone of inhibition(mm)(Mean \pm SEM)				
		TestA	Testb	Testc	Fluconazole Mean	Std.Dev.
1	Candida albicans	17	18	17	13.67	0.58
2	Candida glabrata	19	19	18	18.33	0.58

Table 11. Antifungal activity of Test sample 1 (Cassia tora extract 100mg/ml).

Sr. No.	Strain	Zone of inhibition(mm)(Mean \pm SEM)				
		TestA	Testb	Testc	Fluconazole Mean	Std.Dev.
1	Candida albicans	14	13	14	13.67	0.58
2	Candida glabrata	18	19	18	18.33	0.58

Table 12. Antifungal activity of Test sample 2 (Cassia tora extract 50mg/ml).

Sr. No.	Strain	Zone of inhibition(mm)(Mean \pm SEM)				
		TestA	Testb	TestC	Fluconazole Mean	Std.Dev.
1	Candida albicans	12	11	11	11.33	0.58
2	Candida glabrata	13	11	12	12.00	1.00

Table 13. Antibacterial & antifungal activity of methanolic extract of seeds of cassia tora.

Sr. No.	Strain	Ofloxacin			100mg/ml		50mg/ml		
1	Escherichia coli	24.00	\pm 1.00		16.67	\pm 0.58		13.67	\pm 0.58
2	Salmonelltyphi	19.67	\pm 0.58		18.33	\pm 0.58		14.33	\pm 0.58
3	Pseudomonas aeruginosa	22.33	\pm 1.53		22.33	\pm 1.53		16.33	\pm 0.58
4	Staphylococcus aureus	22.67	\pm 1.53		22.67	\pm 1.53		19.67	\pm 1.15
5	Coagulase negative Staphylococci	19.33	\pm 1.53		19.33	\pm 1.53		15.33	\pm 0.58
6	Klebsiellapneumoniae	20.33	\pm 0.58		20.33	\pm 0.58		16.67	\pm 1.15
				Fluconazole(1mg/ml)	100mg/ml			50 mg/ml	
1	Candida albicans	13.67	\pm 0.58		13.67	\pm 0.58		11.33	\pm 0.58
2	Candida glabrata	18.33	\pm 0.58		18.33	\pm 0.58		12.00	\pm 1.00

From the above data, it is observed that Cassia tora seed methanolic extract showed significant anti-bacterial and anti-fungal activity against various negative bacteria like Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Coagulase negative Staphylococci, Klebsiellapeumonia etc. It has also inhibited gram positive bacteria like Staphylococcus aureus and fungal species like Candida albicans and Candida glabrata. Ofloxacin is the fluoroquinolone derivative that is generally used for urinary tract infection, enteric fever and upper respiratory tract infection. Since it is working as a broad spectrum antibiotic, it is used as standard drug. Two test samples of Cassia toramethanolic extract was taken in two various concentrations of 100mg/ml and 50mg/ml. that showed significant activity with 1mg/ml if standard drug Ofloxacin. Among both the test samples, 100mg/ml sample has shown best

antifungal and antibacterial activity.

5. Conclusion

It is strongly believed that above detailed information from extensive literature survey, on various activity of *Cassia tora* might provide detailed evidence for the varied pharmacological and medicinal spectrum. Toxicity of plant leaves also was investigated so there is need of further research in regard; how to expel the toxicity of plant leaf. Thus, Cassia tora seed extract was proved to be efficacious against various bacteria and fungus species. It can be further formulated into a topical formulation to treat common skin disease like itching, rashes eczema and dermatitis. Further in vivo studies are necessary to corroborate the findings.

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