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Phytochemical Screening and Antimicrobial activity of *Cassia fistula* Linn.

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ABSTRACT

The present communication deals with the Phytochemical Screening and Antimicrobial activity on the different parts of *Cassia fistula* Linn. Family-Caesalpiniaceae. Physiochemical values such as the Moisture contents, percentage of total ash, acid insoluble ash, acid soluble ash, water soluble ash, extractive values as petroleum ether-soluble extractives, ethanol-soluble extractives, methanol-soluble extractives and water-soluble extractives were calculated as well as colour reactions of powder and extract with different chemicals were performed to observe fluorescence analysis. The extracts were subjected to qualitative screening test for various constituents. This revealed the presence protein, glycosides, alkaloids, tannins and phenolic compound, steroid reducing sugars and saponin glycosides. Antimicrobial activity of different parts extract was evaluated on microbial strains like Gram positive species *Staphylococcus aureus* and *Bacillus subtilis* and Gram negative species *Escherichia coli* and *Pseudomonas aeruginosa.* These observations will help in the Pharmacognostical identification and standardization of the drug in the crude form and also to distinguish the drug from its adulteration.

Key words: Antimicrobial, *Cassia fistula*, Caesalpiniaceae, Phytochemistry, Pharmacognosy.

INTRODUCTION

Botanical Name : *Cassia fistula* Linn.
Family : Caesalpiniaceae
Common Name : Bahava.
Parts Used : Root, Leaves, flower and Fruit.

Cassia fistula is a medium sized deciduous tree, 10 m tall with a straight trunk to 5 m, 1 m diameter and spreading branches. Stem bark pale grey, smooth and slender when young and dark brown and rough when old. Leaves alternate, pinnate, 30-40 cm long, with 4-8 pairs of ovate leaflets, 7.5-15 cm long, 2-5 cm broad, entire, the petiolules 2-6 mm long. Flowers bright yellow in terminal, drooping racemes, 30-60 cm long; calyx oblong, obtuse, pubescent; corolla with five subequal, obovate, shortly clawed petals, to 3.5 cm across; stamens 10, upper three with erect filaments to 0.7 cm long and with basifixed anthers; lower three curved and filaments with dorsifixed anthers and the median four stamens with erect filaments, to 1 cm long and with versatile, curved anthers; pistil sessile or stalked, ovary pubescent, style to 0.5

cm long and with terminal stigma. Fruit an indehiscent pod, 40-60 cm long by 1-2 cm diameter, cylindrical, pendulous and terete, containing 25-100 seeds. The pod develops numerous transverse septa between the seeds. When fresh the pods contain a black pulp which on drying adheres to the septa (Orwa, *et al*,. 2009).

Fig.1: Plant habit *Cassia fistula*

Medicinal Properties and Uses:
Root useful in skin diseases, leprosy, tuberculosis gland, syphilis, cures burning sensation. Leaves antiperiodic, heal ulcer, used in rheumatism, juice given in erysipdas. Flower improves taste, laxative, antipyretic; cure "kapha" biliousness; cooling astringent, cause flatulence. Fruit digestible, cooling purgative, cure disease of heart and abdominal pains. Seeds- oily, carminative, improves appetite. Root is generally given as tonic and febrifuge. It has been found to act as strong purgative. In Konkan juice of young leaves is used to cure ring worm. In Hindu medicine fruit pulp is used as cathartic. It is mild laxative. Safe for children and pregnant women (Agharkar, 1991).

Cassia fistula is no exception it is often used as a highly effective moderate laxative that is safe even for children. However, in large doses, the leaves and bark can cause vomiting, nausea, abdominal pain and cramps. *C. fistula* is also employed as a remedy for tumors of the abdomen, glands, liver, stomach, and throat, for burns, cancer, constipation, convulsions, delirium, diarrhea, dysuria, epilepsy, gravel, hematuria, pimples, and glandular tumors. In Ayurvedic medicine systems, the seeds are attributed with antibilious, aperitif, carminative, and laxative properties while the the root is used for adenopathy, burning sensations, leprosy, skin diseases, syphilis, and tubercular glands. The leaves are employed there for erysipelas, malaria, rheumatism, and ulcers. In Brazilian herbal medicine, the seeds are used as a laxative and the leaves and/or bark is used for pain and inflammation.

Distribution:
Cassia fistula is native to India, the Amazon and Sri Lanka, and is now widely cultivated worldwide as an ornamental tree for its beautiful showy yellow flowers.

MATERIAL METHODS

Sample collection and Authentication: The fresh, healthy, mature plants were collected from farm Dhule (M.S., India) away from pollution. The plant materials were identified using the Flora of Dhule and Nandurbar District (Patil, 2003) at Post–graduate Department of Botany, SSVP Sansthas, L.K.Dr.P.R. Ghogrey Science College, Deopur, Dhule-(M.S) India and herbarium were also preserved. The leaves, stem and fruits were washed and used for the present study. The dried plant materials were pulverized into fine powder using a grinder (mixer). About 1 kg of powdered material was prepared. After that powder were kept into air tight bags. Physiochemical values such as the percentage of total ash, acid insoluble ash, acid soluble ash, extractive values as petroleum ether-soluble extractives, etahnol-soluble extractives, methanol-soluble extractive, and water-soluble extractives were calculated according to the methods described in the Indian pharmacopoeia (Anonymous 1966; 1985).

Preparation of extract:
The dried plant material was pulverized into fine powder using a grinder (mixer). Phytochemical studies such as qualitative examination were done on the dried powdered material. About 5 g of powdered material was extracted in soxhlet extraction apparatus with 250 ml of each of the following solvents; petroleum ether, chloroform, and alcohol[9]. The extracts obtained with each solvent were filtered through Whatman filter paper No. 1 and the respected solvents were evaporated (at 40ºC) with the help of heating mantle. The sticky greenish-brown substances were obtained and stored in refrigerator for prior to use[10]. Some of the extracts of each solvent were used for the qualitative phytochemical screening for the identification of the various classes of active chemical constituents, using standard prescribed methods (Harborne, 1984; Trease and Evans,1987; Ajaiyeoba, 2000; Edeoga, *et al*., 2005). The positive tests were noted as present (+++) appreciable amount, (++) moderate amount, (+) trace amount and (-) completely absent.

Preparation of microorganism:
Isolation of bacterial species of Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Echerichia coli* and *Pseudomonas aeruginosa*) takes place. The cultures of these bacteria were checked for purity by doing gram staining and biochemical test and they were grown in nutrient broth at 37°C and maintained in nutrient agar slants at 2-8°C. Nutrient agar medium was used as bacterial culture medium in the antibacterial assays.

Selection of Reference antibiotic:
Reference antibiotic Amphicillin was obtained from authorized medical shop Dhule (M.S.). The purity of the antibiotic is 99.8%.

Dilutions and Inoculum preparations:
The dried plant extracts of *C.fistula* and antibiotic Amphicillin were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 10, 20 mg/ml. The inoculums of *S. aureus*, *B. subtilis*, *E. coli* and *P. aeruginosa* were prepared in nutrient broth medium and kept incubation at 37°C for 8 hours. After growth was observed, the cultures are stored in the refrigerator at 2-8°C for analysis.

Procedure for performing the Disc Diffusion test:
The required amount of Petri plates is prepared and autoclaved at 121°C for 15 minutes. They were allowed to cool under Laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dishes and allowed to solidify. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded with different concentrations of about 10, 20 mg/ml of plant extract of *C. fistula* and antibiotic Amphicillin into each separate disc of about 40 µg/ml. The paper diffuse discs were placed on the medium suitably apart and the plate were incubated at 5°C for 1 hour to permit good diffusion and then transferred to an incubator 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) (Bayer, 1966).

RESULTS & DISCUSSION

The first step towards ensuring quality of starting material is authentication. Thus, in recent years there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. The result of this study as follows:

Qualitative Phytochemical screening
The microchemical screening for the phytoconstituets shows the presences of protein, glycosides, alkaloids, tannins, phenolic compound, steroid reducing sugars and saponin glycosides (Table 1).

Fluorescence analysis
The fluorescence analysis is sensitive and enables the precise and accurate determination over a satisfactory concentration. The fluorescence colour is specific for each compound. A non fluorescent compound may fluorescence if mixed with impurities that are fluorescent. The colour of the extract from organic and inorganic solvents was observed under ordinary light (Table 2).

Physical constants
Results of moisture contents, ash analysis and extractive values of the dried leaves, stem and fruit have been presented in Table 3.

Table 1: Qualitative Phytochemical of *Cassia fistula*

Sr. No.	Test	Fruit Ex. In ethanol	Stem Ex. In ethanol	Leaves Ex.in ethanol
1	Protein	++	+++	+++
2	Glycosides	+++	+	++
3	Alkaloids	+	+	+
4	Tannins and Phenolic compd.	++	++	++
5	Steroid	+++	++	+
6	Saponin glycosides	+++	++	++
7	Reducing sugar	+++	+++	++

Abbreviation: Ex. = Extract; (+++) appreciable amount; (++) moderate amount; (+) trace amount and (-) completely absent

Table 2: Flourusence Analysis of *Cassia fistula*

Sr.No.	TEST	STEM	LEAF	FRUIT
1	Powder	Light green	Green	Dark brown
2	Pd+Iodine	Reddish brown	Reddish brown	Orange red
3	Pd+5%$FeCl_3$	Greenish orange	Greenish yellow	Yellowish orange
4	Pd+1N NaOH	Yellowish brown	Light brown	Red
5	Pd+Acetic acid	Yellow	Yellowish green	Whitish yellow
6	Ext+A.A+50%H_2SO_4	Brown	Buff green	Pinkish red
7	Pd+50%H_2SO_4	Brown	Light brown	Brown
8	Pd+50%conc HCl	Yellowish brown	Greenish yellow	yellow
9	Pd+Ammonia	Brown	Brown	Light orange
10	Ext+4%NaOH+1%$CuSO_4$	Greenish brown	Green	Dirty green
11	Ext+40%NaOH+1%Lead acetate	Dark orange	Dark green	Faint orange
12	Pd+50%HNO_3+Picric acid	Yellow	Dark yellow	Yellow
13	Pd+Satu.Picric acid	Yellow	Dark yellow	Yellow

Abbreviations: Pd= Powder A.A=Acetic Acid, Ext=Extract

Table 3: Physical Evaluation (% W/W) of *Cassia fistula*

Sr. No.	Parameter	Value (%w/w)			
		Leaves		**Stem**	**Fruit**
1.	Moisture content	10.10		9.50	7.30
2.	Extractive values				
	a) Petroleum Ether	15.08		20.00	8.20
	b) Ethanol	35.80		26.66	34.60
	c) Methanol	30.56		23.65	26.20
	d) Water	33.38		25.50	28.85

Table 4: Antibacterial efficacy of different solvent extracts of *Cassia fistula* leaf

Sr. No.	Microorganism	Strain +/-	Concentration (mg/ml)	Zone of inhibition (mm)			
				Petroleum ether	Chloroform	Alcohol	Amphicillin (40 µg/ml)
1	*Escherichia coli*	-ve	10	2	5	6	15
			20	4	7	11	
2	*Pseudomonas aeruginosa*	-ve	10	3	4	6	18
			20	6	8	15	
3	*Staphylococcus aureus*	+ve	10	5	5	7	22
			20	8	11	16	
4	*Bacillus subtilis*	+ve	10	3	3	5	13
			20	5	8	10	

Table 5: Antibacterial efficacy of different solvent extracts of *C. fistula* Stem

Sr. No.	Microorganism	Strain +/-	Concentration (mg/ml)	Zone of inhibition (mm)			
				Petroleum ether	Chloroform	Alcohol	Amphicillin (40 µg/ml)
1	*Escherichia coli*	-ve	10	2	4	7	14
			20	4	8	12	
2	*Pseudomonas aeruginosa*	-ve	10	2	4	5	18
			20	7	9	13	
3	*Staphylococcus aureus*	+ve	10	4	5	8	23
			20	8	10	14	
4	*Bacillus subtilis*	+ve	10	3	4	6	14
			20	5	7	10	

Table 6: Antibacterial efficacy of different solvent extracts of *C. fistula* fruits

Sr. No.	Microorganism	Strain +/-	Concentration (mg/ml)	Zone of inhibition (mm)			
				Petroleum ether	Chloroform	Alcohol	Amphicillin (40 µg/ml)
1	*Escherichia coli*	-ve	10	3	6	9	15
			20	4	7	11	
2	*Pseudomonas aeruginosa*	-ve	10	3	5	7	17
			20	6	8	15	
3	*Staphylococcus aureus*	+ve	10	5	6	9	20
			20	7	9	17	
4	*Bacillus subtilis*	+ve	10	3	3	7	14

Antibacterial study

Petroleum ether, chloroform and alcohol extracts of *C. fistula* leaves and stem were tested against various Gram-negative and Gram-positive bacteria (Table 4). Among the extracts assayed, the alcohol leaf extracts of *C. fistula* exhibited good activity against *S. aureus* at 20 mg/ml for example, 16 mm was recorded as diameter zone of inhibition. This was followed by 15 mm *P. aeruginosa*, 11 mm *E. coli* and *B. subtilis* 10 mm respectively. The least activity of leaf is 2 mm against *E. coli*, whereas *S. aureus* and *S. aureus* shows 3 mm at 10 mg/ml was recorded by petroleum ether extracts.

The stem extracts (Table 5) of *C. fistula* exhibited good activity against *S. aureus* at 20 mg/ml for example, 14 mm was recorded as diameter zone of inhibition. This was followed by 13 mm *P. aeruginosa*, 12 mm *E. coli* and *B. subtilis* 10 mm respectively. The least activity of bark is 2 mm against *E. coli* and *P. aeruginosa*, whereas 3 mm *B. subtilis* at 10mg/ml was recorded by petroleum ether extracts. Activities of the various extracts were comparable to those of standard antibacterial agent Ampicillin.

The fruit extracts (Table 6) of *C. fistula* exhibited good activity against *S. aureus* at 20 mg/ml for example, 17 mm was recorded as diameter zone of inhibition. This was followed by 15 mm *P. aeruginosa*, 12 mm *E. coli* and *B. subtilis* 11 mm respectively. The least activity of bark is 3 mm against *E. coli* and *P. aeruginosa*, whereas 3 mm *B. subtilis* at 10mg/ml was recorded by petroleum ether extracts. Activities of the various extracts were comparable to those of standard antibacterial agent Ampicillin.

CONCLUSION

The physicochemical characters and antimicrobial efficiency reported in this work can serve as a valuable source of information for botanical study, quality control and provide suitable diagnostic tool for the authentication of the original drug, standardization as well as identification of adulterants from the drug. In the present investigation we observed the high extractive values in ethanol compared to other solvents used. The fluorescence colour is specific for each compound. Alcoholic extract of *C. fistula* fruit showed the most remarkable activity. The polarity of the solvent seems to play an important role in exhibiting potential antibacterial activity. It is used as enrichment for Ayurvedic pharmacopoeia and photographs can serve as standard reference material. Further phytochemical studies for identification and elucidation of active constituent in plant material tested in expected to serve as lead in the development of novel bioactive antimicrobial compound.

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