## **Pharmacological Research**

# *In vitro* antibacterial and antifungal activities of *Cassia fistula* Linn. fruit pulp extracts

#### Bhalodia N. R., Nariya P. B.<sup>1</sup>, Acharya R. N.<sup>2</sup>, Shukla V. J.<sup>3</sup>

Research Associate, ADL, Zydus Cadila Healthcare, Ahmedabad, <sup>1</sup>Research Scientist, ADL, Ramniklal Manikchand Dhariwal Research Centre, Valsad, <sup>2</sup>Associate Professor, Department of Dravyaguna, <sup>3</sup>Head, Pharmaceutical Chemistry Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar, Gujarat, India

#### Abstract

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Aim of the study is to assess the antimicrobial activity *Cassia fistula* fruit pulp extracts on some bacterial and fungal strains. Hydro alcohol and chloroform extracts of *Cassia fistula* fruit pulp were evaluated for the potential antimicrobial activity. The antimicrobial activity was determined in both the extracts using the agar disc diffusion method. Extracts were effective on tested microorganisms. The antibacterial and antifungal activities of solvent extracts (5, 25, 50, 100, 250  $\mu$ g/mL) of *C. fistula* were tested against two gram positive, two gram negative human pathogenic bacteria and three fungi, respectively. Crude extracts of *C. fistula* exhibited moderate to strong activity against most of the bacteria tested. The tested bacterial strains were *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coil*, *Pseudomonas aeruginosa*, and fungal strains were found to be dose dependent. The antibacterial activities of the *C. fistula* were due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

Key words: Antibacterial activity, Antifungal activity, Bacteria, Cassia fistula

### Introduction

Antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the healthrelated quality of human life since their introduction. Drugs derived from natural sources play a significant role in the prevention and treatment of diseases. In many countries, traditional medicine is one of the primary health care system.<sup>[1,2]</sup> Herbs are widely exploited in the traditional medicine and their curative potentials are well documented.<sup>[3]</sup> About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful especially in the areas of infectious disease and cancer.<sup>[4]</sup> Recent trends, however, shows that the discovery rate of active novel chemical entities is declining.<sup>[5]</sup> Natural products of higher plants, may give a new source of antimicrobial agents with possibly novel mechanisms of action.<sup>[6,7]</sup> The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts

Address for correspondence: Mr. Nayan R. Bhalodia, Panchavati Gaushala, 95-B, Saru Section Road, Opposite Gita Bungalow, Jamnagar, Gujarat, India. E-mail: nayanbhalodia@gmail.com of the world.  $^{[8]}$  Much work has been done on ethno medicinal plants in India.  $^{[9]}$ 

In the recent years, researches on medicinal plants have attracted a lot of attention globally. Evidences have been accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary, and alternative systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides etc, which have been found *in vitro* to have antimicrobial properties.<sup>[10,11]</sup>

Herbal medicines have been known to man since centuries. Therapeutic efficacy of many indigenous plants for several disorders have been described by practitioners of traditional medicine.<sup>[12]</sup> Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. Traditional medicine continues to be a valuable source of remedies that have been used by millions of people around the world to secure their health.<sup>[13]</sup> The pharmaceutical industries have produced a number of new antibiotics, resistance to these drugs by microorganisms has increased. In general, bacteria has the genetic ability to transmit and acquire resistance to synthetic drugs that are utilized as therapeutic agents.<sup>[14]</sup>

Therefore, actions must be taken to reduce this problem, such

as to minimize the use of antibiotics and to continue studies to develop new drugs, either synthetic or natural to control pathogenic microorganism. In an effort to expand the spectrum of antibacterial agents from natural resources, *Aragvadha* (Cassia fistula) belongs to Caesalpiniaceae, which is subfamily of Leguminosae<sup>[15–18]</sup> has been attempted in this study.

*Cassia fistula* Linn. a semiwild Indian Labernum also known as the Golden Shower, is distributed in various countries including Asia, Mauritius, South Africa, Mexico, China,West Indies, East Africa, and Brazil as an ornamental tree for its beautiful branches of yellow flowers. Recognize by the British pharmacopoeia.<sup>[19]</sup> It is widely used for its medicinal properties, its main property being that of a mild laxative suitable for children and pregnant women. It is also a purgative due to the wax aloin and a tonic.<sup>[20]</sup> It has been reported to treat many other intestinal disorders like healing ulcers.<sup>[21,22]</sup> The plant has a high therapeutic value and it exerts an antipyretic and analgesic effect.<sup>[23]</sup>

In the Indian literature, this plant has been described to be useful against skin diseases, liver troubles, tuberculous glands and its use in the treatment of haematemesis, pruritus, leucoderma, and diabetes has been suggested.<sup>[24,25]</sup> C. fistula extract is used as an antiperiodic agent and in the treatment of rheumatism. It has been concluded that plant parts could be used as a therapeutic agent in the treatment of hypercholesterolemia partially due to their fiber and mucilage content.<sup>[26]</sup> Beside its pharmacological uses, the plant extract is also recommended as a pest and disease control agents in India.<sup>[27-29]</sup> This plant is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections.<sup>[30]</sup> It is used by Malaialis tribe in India to treat nasal infection.<sup>[31]</sup> The pulp of the ripen fruits has mild, pleasant purgative action and is also used as an antifungal drug.<sup>[32]</sup> The whole plant is used to treat diarrhea; fruits are used to treat skin diseases, fever, abdominal pain, leprosy by traditional people. C. fistula plant organs are known to be an important source of secondary metabolites, notably phenolic compounds.[33] C. fistula possesses pharmacological activities such as hypoglycemic, anticancer, abortifacient, anticolic, antifertility, estrogenic, laxative, antibacterial, antipyretic, anti-inflammatory, smooth muscle stimulant, antiarthritic, antitussive, purgative, analgesic, antifungal, antiviral, hepatoprotective, anti-implantation.<sup>[34,35]</sup>

*C. fistula* exhibited significant antimicrobial activity and showed properties that support folklore use in the treatment of some diseases as broad-spectrum antimicrobial agents.<sup>[36]</sup> Thus *C. fistula* is well anchored in its traditional uses and has now found widespread acceptance across the world.

The current investigation carried out a screening of hydro alcoholic and chloroform extracts of *C. fistula* against pathogenic bacteria and fungi in order to detect new sources of antimicrobial agents. Chloroform extracts were obtained after successive separation from hydro alcoholic extracts which were used for further isolation study. So, the microbial activity of this pure chloroform extracts also needed to check the better results against bacterial and fungal strains. This paper reports the results of the antibacterial and antifungal activity of hydro alcohol and chloroform extracts of fruit pulp.

#### **Materials and Methods**

### **Collection of plant materials**

The fresh and healthy pods of plants *C. fistula* were collected in June–Aug 2009 from various areas of Jamnagar district, Gujarat, India. Fruit pulp of plant has been collected according to the Ayurvedic classical references.<sup>[19,34,37,38]</sup> The plant specimens were identified in the Pharmacognosy Laboratory of I.P.G.T and R.A., Jamnagar. Plant parts were collected on the basis of the information provided in the ethnobotanical Survey of India. Each specimen/plant material was labeled, numbered, annoted, with the date of collection, locality, and their medicinal uses were recorded.

#### Extraction

The extraction of the C. *fistula* fruit pulp was carried out using known standard procedures.<sup>[39]</sup> The fresh pulp of C. *fistula* pod (25.0 g) was extracted with 900 ml of diluted methanol, filtered and evaporated to dryness on a hot water bath to yield a hydro alcoholic crude extract (9.7 g). In addition, further with chloroform in a Soxhlet apparatus, where chloroform is a hydrolysate extract and used for further isolation purpose. The extraction was continued until the extraction was exhausted. The extracts were then combined, filtered, and evaporated to dryness.

Further, the crude hydro alcoholic and chloroform extracts were cooled and filtered. The concentrated hydro alcoholic and chloroform extracts were further subjected for its antimicrobial studies. The residence was dissolved with dimethyl sulfoxide (DMSO) with different concentrations and checked it for the antimicrobial activity.

#### Preliminary phytochemical screening

The extracts were subjected to preliminary phytochemical testing to detect the presence of different chemical groups of compounds. Air-dried and powdered plant materials were screened for the presence of saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, cumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids.<sup>[37,40-43]</sup>

#### Test microorganisms and growth media

The following microorganisms: S. aureus (MTCC 96), S. pyogenes (MTCC 442), E. coli (MTCC 443), P. aeruginosa (MTCC 424) and fungal strains A. niger (MTCC 282), A. clavatus (MTCC 1323), C. albicians (MTCC 227) were chosen based on their clinical and pharmacological importance.<sup>[44]</sup> The bacterial strains obtained from Institute of Microbial Technology, Chandigarh were used for evaluating the antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 h at 37°C on Nutrient Agar and Potato Dextrose Agar medium (Microcare lab., Surat, India) following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C(the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C) whereas the yeasts and molds were grown in sabourauddextrose agar (SDA) and potato dextrose agar (PDA) media, respectively, at 28°C. The stock cultures were maintained at 4°C.

#### Antimicrobial activity

#### Determination of zone of inhibition method

In vitro antibacterial and antifungal activity were examined for hydro alcoholic and chloroform extracts. Antibacterial and antifungal activities of plant extracts against four pathogenic bacteria (two gram positive and two gram negative) and three pathogenic fungi were investigated by the agar disk diffusion method.[45-47] Antimicrobial activity testing was carried out by using the Agar cup method. Each purified extracts were dissolved in DMSO, sterilized by filtration using sintered glass filter and stored at 4°C. For the determination of zone of inhibition (ZOI), two gram positive, two gram negative and three fungal strains were taken as a standard antibiotic for comparison of the results. All the extracts were screened for their antibacterial and antifungal activities against the E. coil, P. aeruginosa, S. aureus, S. pyogenes, and the fungi C. albicans, A. niger, and A. clavatus. The sets of five dilutions (5, 25, 50, 100, and 250 µg/mL) of C. fistula extract and standard drugs were prepared in double distilled water using nutrient agar tubes. Muller Hinton sterile agar plates were seeded with indicator bacterial strains (108cfu) and allowed to stay at 37°C for 3 h. The zones of growth inhibition around the disks were measured after 18 to 24 h of in incubation at 37°C for bacteria and 48 to 96 h for fungi at 28°C, respectively. The sensitivity of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

#### Results

#### Preliminary phytochemical screening

It was found that hydro alcoholic extracts of *C. fistula* fruit pulp contained tannins, flavonoids, saponins, triterpenoids, steroids, glycosides, anthraquinones, gum and mucilage, reducing sugars, carbohydrates, protein and amino acids, andchloroform extracts contained glycosides, phenolic compounds, tannins, and anthraquinones-type compounds in higher amount [Table 1].

#### **Microbial activity**

The antimicrobial activity of both the extracts of *C. fistula* were studied in different concentrations (5  $\mu$ g/ml, 25  $\mu$ g/ml, 50  $\mu$ g/ml, 100  $\mu$ g/ml, 250  $\mu$ g/ml) against four pathogenic bacterial strains two Gram positive (S. *aureus* MTCC 96, S. *pyogenes* MTCC 442), two Gram negative (*E. coli* MTCC 443, *P. aeruginosa* MTCC 424) and three fungal strains(A. *niger* MTCC 282, A. *clavatus* MTCC 1323, C. *albicians* MTCC 227).

Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in Tables 2-5.

The antibacterial and antifungal activity of the extracts increased linearly with increase in concentration of extracts ( $\mu g$ /ml). As compared to standard drugs, the results revealed that in both the extracts for the bacterial activity, *S. pyogenes* were more sensitive as compared to *S. aureus*, *E. coli* and *P. aeruginosa*, and for fungal activity *C. albicans* shows good result as compare to *A. niger* and *A. clavatus*. The growth inhibition zone measured ranged from 10–20 mm for all the sensitive bacteria, and ranged from 12–21 mm for fungal strains [Figure 1-5].

The results show that both the extracts of *C. fistula* were found to be more effective against all the microbes tested.

#### Discussion

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the words population. In this work, both the extracts obtained from *C. fistula* fruit pulp shows strong activity against most of the tested bacteria and fungal strains. The results were compared with standard antibiotic drugs. In this screening work, no extracts of *C. fistula* were found to be inactive against any organism, such as Gram positive, Gram negative and fungal strains were resistant to all the extracts of *C. fistula*.

This study shows the presence of different phytochemicals with the biological activity that can be of the valuable therapeutic index. The result of phytochemicals in the present investigation showed that the plant contains more

Table 1: Phytoche	emical scr	eening	of C	assi	a fistu	ıla	
fruit pulp							
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Functional groups	Metabolite (Hydro alcoholic extract of <i>Cassia fistula</i> )
Alkaloids	+
Tanins	+
Flavonoids	+
Saponins	+
Triterpenoids	+
Steroids	+
Glycosides	+
Coumarin	_
Anthraquinones	+
Reducing sugars	+
Carbohydrates	+
Gum and mucilage	+
Starch	_
Proteins	+
Amino acids	+
(+) present and (–) absent	

Table 2: Antibacterial activity of hydro alcoholic and chloroform extracts of *Cassia fistula* [zone of inhibition]

Microorganism	The second secon							)			
		Concentration in µg/mL									
		Hydroalcoholic					Chloroform				
		extracts (µg/ml) extracts (µg/ml)						ml)			
	5	25	50	100	250	5	25	50	100	250	
E. coli	-	16	17	17	19	-	14	16	18	20	
P. aeruginosa	-	13	14	17	18	-	13	15	17	18	
S. Pyogenes	-	12	13	15	17	-	10	15	16	20	
S. aureus	-	12	14	15	18	-	12	14	15	18	
= Nie war of inhibities											

- = No zone of inhibition

Drug	Concentration (µg/ml)	Zone of inhibition in mm							
		E. coli	P. aeruginosa	S. pyogenes	S. aureus				
Ampicilline	5	14	14	11	10				
	25	15	15	14	13				
	50	16	15	16	14				
	100	19	18	18	16				
	250	20	20	19	18				
Chloram-phenicol	5	14	14	10	12				
	25	17	17	13	14				
	50	23	18	19	19				
	100	23	19	20	20				
	250	23	21	20	21				
Ciprofloxacin	5	20	20	16	17				
	25	23	23	19	19				
	50	28	24	21	21				
	100	28	26	21	22				
	250	28	27	22	22				
Norfloxacin	5	22	18	18	19				
	25	25	19	19	22				
	50	26	21	20	25				
	100	27	23	21	26				
	250	29	23	21	28				

#### Table 4: Antifungal activity of hydro alcoholic and chloroform extracts of *Cassia fistula* [zone of inhibition]

Microorganism		Zone of inhibition in mm										
		Concentration in µg/ml										
		Hydroalcoholic					Chloroform					
		extracts (µg/ml)				extracts (µg/ml)						
	5	25	50	100	250	5	25	50	100	250		
A. niger	-	13	15	17	18	-	16	17	18	21		
A. clavatus	-	13	16	17	19	-	14	16	19	21		
C. albicans	-	16	17	18	20	-	12	16	17	20		

- = No zone of inhibition

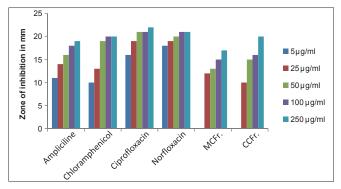


Figure 1: Antibacterial activity against S. pyogenes (MTCC 442) MCFr: Methanolic extract of *Cassia fistula* fruit pulp, CCFr: Chloroform extract of *Cassia fistula* fruit pulp

or less same components such as saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, gum, mucilage,

# Table 5: Antifungal activity of standard drugs [zone of inhibition]

Drug	Concentration	Zone of inhibition in mm						
	in (µg/ml)	A. niger	A. clavatus	C. albicans				
Greseofulvin	5	19	18	18				
	25	23	21	21				
	50	25	22	22				
	100	25	23	22				
	250	28	26	24				
Nystatin	5	18	19	18				
	25	19	21	21				
	50	24	24	24				
	100	29	26	25				
	250	29	27	26				

proteins and aminoacids. From the above results, the activity of hydroalcoholic extracts of *C. fistula* shows significant antibacterial and antifungal activity. The chloroform extracts, whose purity level is good as compared to hydroalcoholic extract and porosity is lower than hydroalcoholic extract, found to be more active against fungal strains. Results shows that this plant is rich in tannin and phenolic compounds have been shown to possess antimicrobial activities against a number of microorganisms.

### Conclusion

Antimicrobial resistance is a global problem. Emergence of multidrug resistance has limited the therapeutic options. Hence,

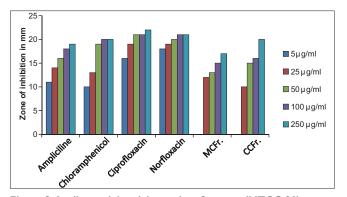


Figure 2: Antibacterial activity against S. aureus (MTCC 96) MCFr: Methanolic extract of Cassia fistula fruit pulp, CCFr: Chloroform extract of Cassia fistula fruit pulp

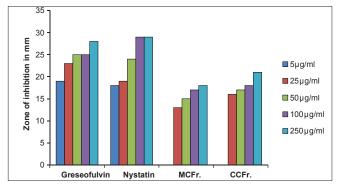


Figure 4: Antibacterial activity against P. aeruginosa (MTCC 424) MCFr: Methanolic Extract of Cassia fistula fruit pulp, CCFr: Chloroform extract of Cassia fistula fruit pulp

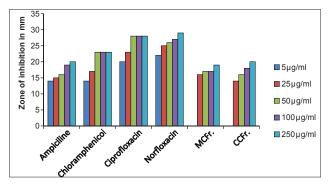


Figure 6: Antifungal activity against A. clavatus (MTCC 1323) MCFr: Methanolic Extract of Cassia fistula fruit pulp, CCFr: Chloroform extract of Cassia fistula fruit pulp

monitoring resistance is of paramount importance. Hence, this study was aimed to focus the antimicrobial properties of *C. fistula* on gram positive, gram negative, and fungal organisms. In the current investigations, the hydroalcoholic and chloroform extracts of *C. fistula* were found to be active on some isolated microorganism and fungi as compared to standard drugs. This study has justified the traditional use of fruit pulp in infectious conditions. However, before use in human being isolation of pure compound, toxicological study, and pharmacological activity should be carried out thereafter. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

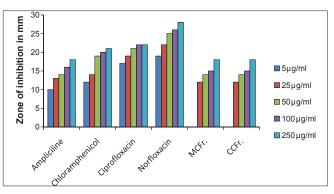


Figure 3: Antibacterial activity against E. coli (MTCC 443) MCFr: Methanolic extract of Cassia fistula fruit pulp, CCFr: Chloroform extract of Cassia fistula fruit pulp

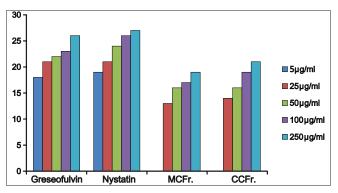


Figure 5: Antifungal activity against A.niger (MTCC 282) MCFr: Methanolic Extract of Cassia fistula fruit pulp, CCFr: Chloroform extract of Cassia fistula fruit pulp

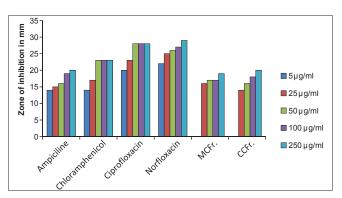


Figure 7: Antifungal activity against C. albicans (MTCC 227) MCFr: Methanolic Extract of Cassia fistula fruit pulp, CCFr: Chloroform extract of Cassia fistula fruit pulp

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# हिन्दी सारांश

# आरग्वध की फलमज्जा के विभिन्न सारों का इन–विट्रो प्रति सूक्ष्मजैविकीय एवं प्रतिकवकीय कर्मों का अध्ययन

भालोडिया एन. आर., नारिया पी. बी., आचार्य आर. एन., शुक्ला वी. जे.

इस अध्ययन का उद्देश्य औषधीय सार के प्रति सूक्ष्मजैविकीय कर्म का मूल्यांकन एवं कुछ सूक्ष्मजैविकीय व fungal strains पर 'जोन ऑफ इन्हीबिशन' का निरीक्षण करना है । प्रस्तुत अध्ययन में आरग्वधफलमज्जा के हाइड्रोअल्कोहोलिक सार एवं क्लोरोफोर्म सार का चिकित्सीय रूप से महत्वपूर्ण सूक्ष्मजीवाणुओं एवं कवकों (fungal strains) के प्रतिरोधी कर्म का अध्ययन किया गया । यह अध्ययन अगार डिस्क डिफ्युजन विधि द्वारा दो Gram +ve जीवाणु प्रजातियों *S.aureus, S.pyogenes,* दो Gram - ve जीवाणु प्रजातियों *E.coli, P.aeruginosa* तथा तीनों कवक प्रजातियों *A.niger, A.clavatus* एवं *C.albicans* में किया गया । दोनों प्रकार के सार मात्रानुरूप प्रभावी पाये गये । फाइटोकेमिकल विश्लेषण द्वारा उपापचयी तत्वों की उपस्थिति में प्रतिसूक्ष्म जैविकिय कर्म पाया गया है । इस प्रकार वनस्पतियां सक्रिय प्राकृतिक जैव उत्पाद शोध में कार्यरत नवीन फार्मास्यूटिकल शोध कार्यों के विकास में महत्वपूर्ण योगदान दे सकती हैं ।