Original Article

Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: An ethnomedicinal plant

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Abstract

This study was carried out with an objective to investigate the antibacterial and antifungal potentials of leaves of *Cassia fistula* Linn*.* The aim of the study is to assess the antimicrobial activity and to determine the zone of inhibition of extracts on some bacterial and fungal strains. In the present study, the microbial activity of hydroalcohol extracts of leaves of *Cassia fistula* Linn. (an ethnomedicinal plant) was evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar disc diffusion method. The antibacterial and antifungal activities of extracts (5, 25, 50, 100, 250 μg/ml) of *Cassia fistula* were tested against two Gram-positive—*Staphylococcus aureus*, *Streptococcus pyogenes;* two Gram-negative—*Escherichia coli*, *Pseudomonas aeruginosa* human pathogenic bacteria; and three fungal strains—*Aspergillus niger*, *Aspergillus clavatus*, *Candida albicans*. Zone of inhibition of extracts were compared with that of different standards like ampicillin, ciprofloxacin, norfloxacin, and chloramphenicol for antibacterial activity and nystatin and griseofulvin for antifungal activity. The results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms. The phytochemical analyses of the plants were carried out. The microbial activity of the *Cassia fistula* was 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: *Cassia fistula*, *in vitro* antibacterial activity, antifungal activity, secondary metabolites

INTRODUCTION

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary healthcare systems.[1,2] Herbs are widely exploited in the traditional medicine and their curative potentials are well documented.[3] 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.[4] Recent trends, however, show that the discovery rate of active novel chemical entities is declining.[5] Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action.[6,7] The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world.[8] Much work has been done on ethnomedicinal plants in India.[9]

Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids,

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glycosides, etc., which have been found *in vitro* to have antimicrobial properties.[10,11]

Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine.[12] Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population.[13] The harmful microorganisms can be controlled with drugs and these results in the emergence of multiple drug-resistant bacteria and it has created alarming clinical situations in the treatment of infections. The pharmacological industries have produced a number of new antibiotics; resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents.[14]

In an effort to expand the spectrum of antibacterial agents from natural resources, *Cassia fistula* belonging to Leguminosae family has been selected. In the Indian literature, this plant has been described to be useful against skin diseases, liver troubles, tuberculoses glands and its use into the treatment of hematemesis, pruritus, leucoderma, and diabetes has been suggested.[15,16] 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.[17] Besides its pharmacological uses, the plant extract is also recommended as a pest and disease control agents in India.[18-20] This plant is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections.[21] The leaves are laxative, antiperiodic, depurative, anti-inflammatory, and are useful in skin diseases, boils, carbuncles, ulcers, intermittent fever, gouty arthritis, and rheumatalgia. *Cassia fistula* plant organs are known to be an important source of secondary metabolites, Indian people are using the leaves to treat inflammation; *Cassia fistula* plant organs are known to be an important source of secondary metabolites, notably phenolic compounds.[22]

Cassia fistula exhibited significant antimicrobial activity and showed properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents.[23] Thus, *Cassia fistula* is well anchored in its traditional uses has now found wide-spread acceptance across the world.

In the current investigation carried out, a screening of hydroalcoholic extracts of *Cassia fistula* leaves against pathogenic bacteria and fungi is done in order to detect new sources of antimicrobial agents.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh and healthy leaves of the plant *Cassia fistula* were collected between June and August, 2009 from various areas of Jamnagar district, Gujarat, India. The plant specimens were identified in department of Pharmacognosy I.P.G.T and R.A. (Institute of post graduate teaching and research in ayurveda), 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, a noted with the date of collection, locality, and their medicinal uses were recorded.

Preparation of Plant Extract

Extraction

The extraction of the *Cassia fistula* leaves was carried out using known standard procedures.[24] The plant materials were dried in shade and powdered in a mechanical grinder. The powder (25.0 g) of the plant materials were initially defatted with petroleum ether (60-80°C), followed by 900 ml of hydroalcohol by using a Soxhlet extractor for 72 hours at a temperature not exceeding the boiling point of the solvent. The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccator. The hydroalcoholic extract yields a dark greenish solid residue weighing 5.750 g (23.0% w/w). More yields of extracts were collected by this method of extractions. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml. The extract was preserved at 2- to 4°C. This crude extracts of hydroalcohol was used for further investigation for potential of antimicrobial properties.

Preliminary Phytochemical Screening

The extracts were subjected to preliminary phytochemical testing to detect for 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, coumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids, as described in literatures.[25-27]

Test Microorganisms and Growth Media

The following microorganisms

Staphylococcus aureus (MTCC 96), *Streptococcus pyogenes* (MTCC 442), *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) and fungal strains *Aspergillus niger* (MTCC 282), *Aspergillus clavatus* (MTCC 1323), *Candida albicans* (MTCC 227) were chosen based on their clinical and pharmacological importance.[28] The bacterial strains obtained from Institute of Microbial Technology, Chandigarh, were used for evaluating antimicrobial activity. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) medium (Microcare laboratory, Surat, India), respectively, 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 Sabouraud dextrose agar and 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 activities were examined for hydroalcohol extracts. Antibacterial and antifungal activities of plant part extracts against four pathogenic bacteria (two Gram-positive and negative) and three pathogenic fungi were investigated by the agar disk diffusion method.[29-31] Antimicrobial activity testing was carried out by using agar cup method. Each purified extracts were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. For the determination of zone of inhibition, pure Gram-positive, Gram-negative, and 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 *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes* and the fungi *Candida albicans*, *Aspergillus niger*, and *Aspergillus clavatus*. The sets of five dilutions (5, 25, 50, 100, and 250 μg/ml) of *Cassia fistula* extract and standard drugs were prepared in double-distilled water using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (10^8 cfu) and allowed to stay at 37°C for 3 hours. Control experiments were carried out under similar condition by using ampicillin, chloramphenicol, ciprofloxacin, and norfloxacin for antibacterial activity and nystatin and griseofulvin for antifungal activity as standard drugs. The zones of growth inhibition around the disks were measured after 18 to 24 hours of in incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivities 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 AND DISCUSSION

Results

Preliminary phytochemical screening

It was found that hydroalcoholic extracts of *Cassia fistula* leaves contained tannins, flavonoids, saponins, triterpenoids, steroids, glycosides, anthraquinones, reducing sugars, carbohydrates, proteins, and amino acids.

Microbial activity

The antimicrobial activity of the extracts of *Cassia fistula* were studied in different concentrations (5, 25, 50, 100, and 250 μg/ml) against four pathogenic bacterial strains, two Gram-positive (*Staphylococcus aureus* MTCC 96, *Streptococcus pyogenes* MTCC 442) and two Gram-negative (*Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 424), and three fungal strains (*Aspergillus niger* MTCC 282, *Aspergillus clavatus* MTCC 1323, *Candida albicans* MTCC 227). These strains have been selected for the basis of its application purpose of further formulation study.

Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial and antifungal activities are presented in Tables 1 to 4.

The antibacterial and antifungal activities of the extracts increased linearly with increase in concentration of extracts (μg/ml). As compared with standard drugs, the results revealed that in the extracts for bacterial activity, *S. pyogenes and S. aureus* were more sensitive as compared with *E. coli* and *P. aeruginosa*, and for fungal activity, *C. albicans* shows good result as compare with *A. niger* and *A. clavatus*. The growth inhibition zone measured ranged from 11 to 20 mm for all the sensitive bacteria, and ranged from 14 to 20 mm for fungal strains [Figures 1-7].

The results show that the extracts of *Cassia 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 world's population. In the present work, the extracts obtained from *Cassia fistula* show strong activity against most of the tested bacterial and fungal strains. The results were compared with standard antibiotic drugs. In this screening work, extracts of *Cassia fistula* were found to be not inactive against any organism, such as Gram-positive, Gram-negative, and fungal strains were resistant to all the extracts of *Cassia fistula*.

Table 1: Antibacterial activities of hydroalcoholic extracts of leaves of *Cassia fistula* against bacterial test organism

Antibacterial activity (Zone of inhibition)					
Microorganism	*Cassia fistula* - Zone of inhibition in mm				
	Concentration in μg/ml				
	Hydroalcohol extracts (μg/ml)				
	5	25	50	100	250
E. coli	-	15	16	17	20
P. aeruginosa	-	13	15	16	20
S. pyogenes	-	11	13	15	18
S. aureus	-	13	15	16	18

Values are mean ± SD of three parallel measurements - = No zone of inhibition

Table 2: Antibacterial activity of standard drugs against bacterial test organism

Antibacterial activity (Zone of inhibition)					
Drug	Concentration (μg/ml)	Zone of inhibition in mm			
		E. coli	*P. aeruginosa*	*S. pyogenes*	*S. aureus*
Ampicillin	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
Chloramphenicol	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 3: Antibacterial activities of hydroalcoholic extracts of leaves of *Cassia fistula* against fungal test organism

Antifungal activity (Zone of inhibition)					
Microorganism	*Cassia fistula* - Zone of inhibition in mm				
	Concentration in μg/ml				
	Hydroalcohol extracts (μg/ml)				
	5	25	50	100	250
A. niger	-	14	16	18	20
A. clavatus	-	15	17	19	20
C. albicans	-	17	18	19	20

Values are mean ± SD of three parallel measurements; - = No zone of inhibition

Table 4: Antifungal activity of standard drugs against fungal test organism

Antifungal activity (Zone of inhibition)				
Drug	Concentration in (μg/ml)	Zone of inhibition in mm		
		A. niger	*A. clavatus*	*C. albicans*
Griseofulvin	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

CML = Hydroalcoholic extract of *Cassia fistula* leaves (5 - 250 μg/ml), μg/ml = microgram/ml 5, 25, 50, 100, 250 = various concentration

The above results show that the activity of hydroalcohol extracts of *Cassia fistula* shows significant antibacterial and antifungal activities. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The result of phytochemicals in the present investigation showed that the plant contains more or less same components like saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. Results show that plant rich in tannin and phenolic compounds have been shown to posses antimicrobial activities against a number of microorganisms.

CONCLUSION

In the current investigation, the hydroalcohol extract in the ratio of 8 : 2 has been selected after study of such a selected plant with water extracts and methanol extracts, hydroalcohol extract gave higher yield of chemical constituents expected for this research work; the originality of this work is that good results have been found with hydroalcohol ratio, and it will be helpful to carry out other data with MIC and other formulation study, because in comparison of methanol or water extracts, hydroalcohol is more suitable for clinical study. The hydroalcoholic extracts of *Cassia fistula* were found to be active on most of the clinically isolated microorganism and fungi, as compare with standard drugs. The present study justified the claimed uses of leaves in the traditional system of medicine

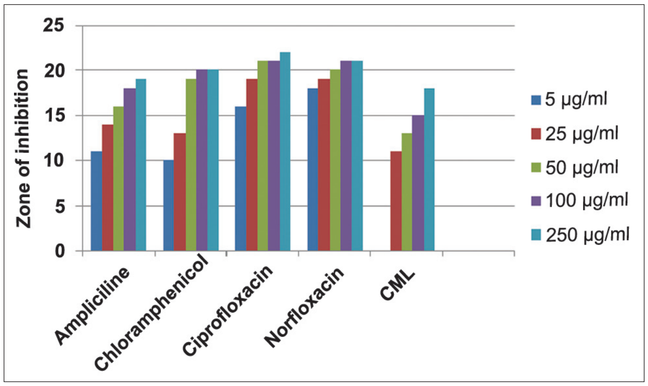

Figure 1: Antibacterial activity against *S. pyogenes* (MTCC 442)

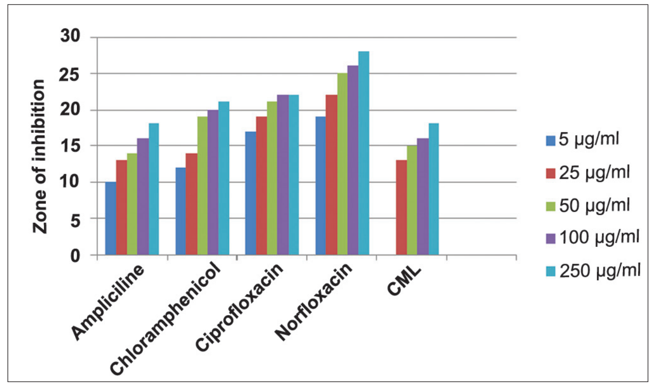

Figure 2: Antibacterial activity against *S. aureus* (MTCC 96)

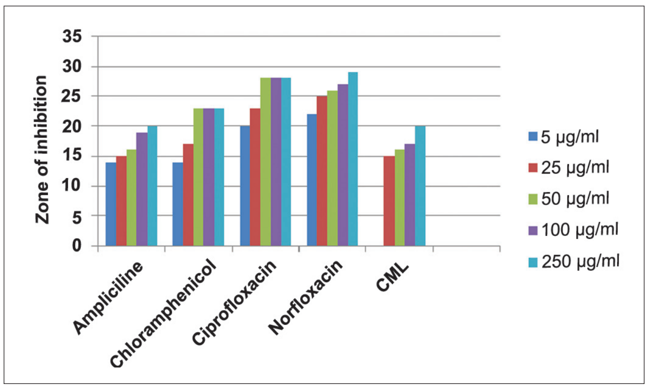

Figure 3: Antibacterial activity against *E. coli* (MTCC 443)

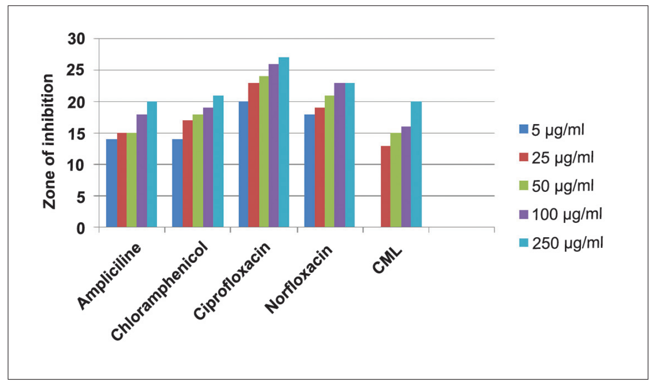

Figure 4: Antibacterial activity against *P. aeruginosa* (MTCC 424)

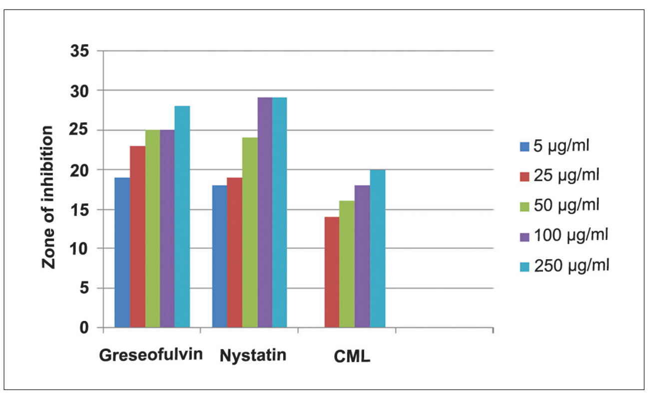

Figure 5: Antifungal activity against *A. niger* (MTCC 282)

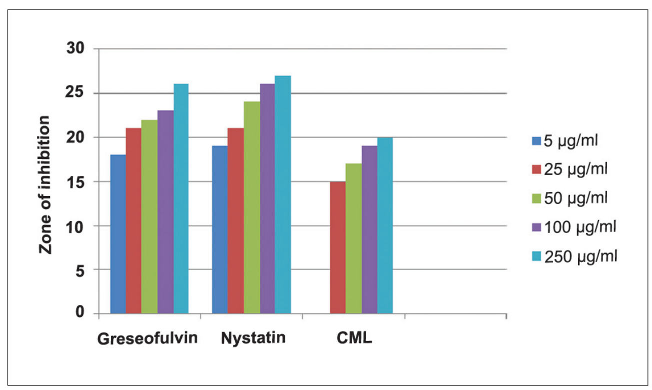

Figure 6: Antifungal activity against *A. clavatus* (MTCC 1323)

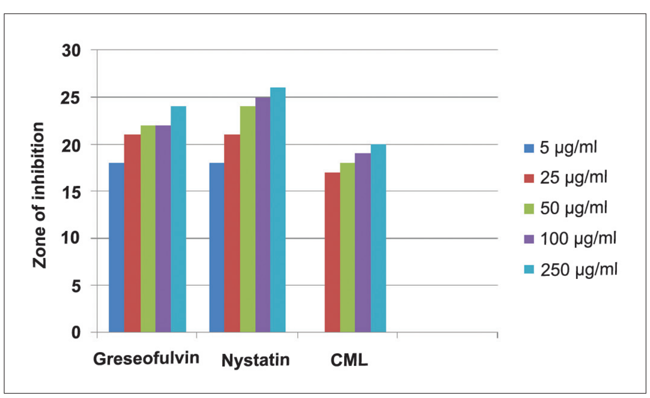

Figure 7: Antifungal activity against *C. albicans* (MTCC 227); CML - Hydroalcoholic extract of leaves

to treat various infectious disease caused by the microbes. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

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