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Antibacterial and Antifungal activity from Flower Extracts of *Cassia fistula* L. :An Ethnomedicinal Plant

Nayan R. Bhalodia*[1], Pankaj B. Nariya[1], V. J. Shukla[2]

*[1,1]Research Scholars in Phytochemistry,
[2]Head of the Department, Pharmaceutical Laboratory,
Department of Pharmaceutical chemistry, I.P.G.T&R.A,
Gujarat Ayurved University, Jamnagar – 361008 (G.U.J), India

*Corres. author: nayanbhalodia@gmail.com, Cell-(91)9925019682

Abstract: Since the advent of modern drug treatments, traditional medicine has greatly receded in occidental societies. Moreover, only a limited number of medicinal plants have received detailed scientific scrutiny; thereby prompting the World Health Organisation to recommend that this area be comprehensively investigated. 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 and chloroform extracts of flowers of *Cassia fistula* Linn. (an ethnomedicinal plant) were evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in both the extracts using agar disc diffusion method. Extracts were effective on tested microorganisms. The antibacterial and antifungal activities of solvent extracts(5, 25, 50, 100, 250µg/ml) of *Cassia fistula* were tested against 2 gram positive, 2 gram negative human pathogenic bacteria and 3 fungi respectively. The extracts showed broad spectrum of inhibition by showing antibacterial effect for both Gram positive and Gram negative human pathogen bacterial strains. Crude extracts of *Cassia fistula* exhibited moderate to strong activity against most of the bacteria tested. The tested bacterial strains were *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, and fungal strains were *A. niger*, *A. clavatus*, *C. albicans*. The antibacterial potential of the extracts were found to be dose dependent. The phytochemical analysis of the plants were carried out. The antibacterial activities of the various parts of *Cassia 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.

Keywords: Antibacterial activity, Antifungal activity, *Cassia fistula*, Bacterial pathogens, 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. Systematic studies among various pharmacological compounds have revealed that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine. 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 health care system[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].

In the recent years, research on medicinal plants have attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of Medicinal Plants used in various traditional, complementary and alternate 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[10,11]. Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by the physicians; several are already being tested in humans. Section A highlights the various Medicinal plants which have shown antibacterial, antifungal properties whereas, in Section B the main focus is on the different groups of phytochemicals exhibiting medicinal properties.

Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plant 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]. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases.

The harmful microorganisms can be controlled with drugs and this 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].

Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research of resistance, among microorganism 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, *Cassia fistula* belonging to Leguminosae family has been selected.

*Cassia fis*tula Linn.,(Leguminosae), a semi-wild Indian Labernum also known as the Golden Shower, is distributed invarious 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 pharmacopoiea[15].

C. fistula, a member of the Leguminosae family, 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[16]. and has been reported to treat many other intestinal disorders like healing ulcers[17,18]. The plant has a high therapeutic value and it exerts an antipyretic and analgesic effect[19]. In the Indian literature, this plant has been described to be useful against skin diseases, liver troubles, tuberculous glands and its use in to the treatment of haematemesis, pruritus, leucoderm and diabetes has been suggested[20,21]. *C. fistula* extract is used as an anti-periodic 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 hypercholesterolaemia partially due to their fibre and mucilage content[22]. Besides its pharmacological uses, the plant extract is also recommended as a pest and disease control agents in India[23,24,25]. This plant is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections[26] .It is used by Malaialis tribe in India to treat nasal infection[27]. The flowers are reported to have demulcent and lubricating effect, biiter, acrid, cooling, emollient and useful in skin diseases, pruritus, burning sensation, dry cough and bronchitis[28]. The whole plant is used to treat diarrhea; *Cassia fistula* plant organs are known to be an important source of secondary metabolites, notably phenolic compounds[29].

Cassia fistula exhibited significant antimicrobial activity and showed properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents[30]. Thus *C.fistula* is well anchored in its traditional uses and has now found widespread acceptance across the world.

We carried out a screening of hydroalcoholic and chloroform extracts of *Cassia fistula* against pathogenic bacteria and fungi in order to detect new sources of antimicrobial agents. In this paper, we report the results of the antibacterial and antifungal activity of hydroalcoholic extracts of *Cassia fistula*. We also carried out a screening of chloroform extract of *Cassia fistula* against pathogenic bacterial and fungal strains. We have separated a chloroform extracts after successive separation from hydroalcoholic extracts and which was used for further isolation study; So, We have checked microbial activity of this pure chloroform extracts to check the better results against bacterial and fungal strains, In this paper, we report the results of the antibacterial and antifungal activity of hydroalcohol and chloroform extracts of flowers.

MATERIALS AND METHODS

Collection of plant materials

The fresh and healthy flowers of *Cassia fistula* were collected in April-June 2009 from various areas of Jamnagar district, Gujarat, India. The plant specimens were identified in department of Pharmacognosy I.P.G.T & R.A. Jamnagar. Plant parts were collected on the basis of the information provided in the ethanobotanical survey of India. Each specimen/plant material was labeled, numbered, annoted with the date of collection, locality and their medicinal uses were recorded.

The flowers were washed thoroughly with tap water followed with sterilized distilled water for the removal of dust and sand particles. The flowers were shade dried in the dark at room temperature for few days and then homogenized to fine powder by a mechanical grinder, The powdered materials were passed through sieve number 40, and stored in airtight bottles and quality control tests were carried out for plants as per WHO guidelines and quality samples were selected for extraction. This was used as new material for the extraction of antimicrobial compounds against the microbes.

Extraction

The extraction of the *Cassia fistuala* flowers were carried out using known standard procedures[31]. The powdered *Cassia fistula* flowers were successively extracted by soxhlet extraction with solvents of increasing polarity biginning with petroleum ether (60°C-80°C) and dilluted methanol. The extracts were filtered while hot and concentrated in vacuum under reduced pressure using rotary flask evaporator and dried in a desiccators, and further hydroalcoholic extract was used to separate chloroform soluble portions used as chloroform extratcs, 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 on a hot water bath. The extracts were cooled and filtered. The concentrated extracts were further subjected for its antimicrobial studies. The residence was dissolved with dimethyl sulfoxide (DMSO) with different concentrations and checked it for antimicrobial activity.

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, cumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein and amino acids as described in literatures[32,33,34].

Test microorganisms and growth media

The following microorganisms:

S. aureus (MTCC 96), *S. pyogenes* (MTCC 442), *E. coli* (MTCC 443), *P. aeruginosa* (MTCC 424) & fungal strains *A. niger* (MTCC 282), *A. clavatus* (MTCC 1323), *C. albicians* (MTCC 227) were chosen based on their clinical and pharmacological importance[35]. 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 24h at 37°C on Nutrient Agar and Potato Dextrose Agar medium (Microcare lab., 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 (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 hydroalcohol and chloroform extracts. Antibacterial and antifungal activities of plant parts extracts against 4 pathogenic bacteria(2 Gram positive and 2 Gram negative) and 3 pathogenic fungi were investigated by the Agar disk diffusion method[36,37,38]. Antimicrobial activity testing was carried out by using Agar cup method. Each purified Extracts were dissolved in dimethyl sulfoxide (DMSO), sterilized by filtration using sintered glass filter and stored at 4°C. For the determination of ZOI, 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 *E. coli*, *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 *Cassia fistula* extract and Standard drugs (5, 25, 50, 100 and 250 µg/ml) were prepared in double distilled water using nutrient agar tubes. Muller Hinton sterile agar plates were seeded with indicator bacterial strains (10^8cfu) and allowed to stay at 37°C for 3 h. Control experiments were carried out under similar condition by using Ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin for antibacterial activity and Nystatin and Greseofulvin for antifungal activity as standard drugs. All of the plates were incubated at 37°C for 18 to 24 h for bacteria and at 28°C for 48 to 96 h for fungi. 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 was 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.

Table 1. Antibacterial activity of hydroalcohol and chloroform extracts 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)					Chloroform extracts (µg/ml)				
	5	**25**	**50**	**100**	**250**	**5**	**25**	**50**	**100**	**250**
E. coli	-	14	15	17	19	-	15	16	19	21
P. aeruginosa	-	12	15	17	19	-	12	15	17	19
S. Pyogenes	-	12	14	17	19	-	12	15	16	18
S. aureus	-	12	13	15	17	-	12	13	15	16

- = 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***
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

RESULTS AND DISCUSSION

RESULTS

Preliminary phytochemical screening

It was found that hydroalcoholic extracts of *Cassia fistula* flowers contained tannins, flavonoids, saponins, triterpenoids, steroids, glycosides, anthraquinones, reducing sugars, carbohydrates, protein and amino acids, and chloroform extracts contained glycosides, phenolic compounds, tannins, and anthraquinones type compounds in higher amount.

Microbial activity

The antimicrobial activity of both the extracts of *Cassia fistula* were studied in different concentrations (5µg/ml, 25µg/ml, 50µg/ml, 100µg/ml, 250µg/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 Table 1-4.

The antibacterial and antifungal activity of the extracts increased linearly with increase in concentration of extracts (µg/ml). As Comapred with Standard Drugs, the results revealed that in both the extracts for 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 from12-21 mm for fungal strains.

Table 3. Antifungal activity of hydroalcohol and chloroform extracts 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)					Chloroform extracts (µg/ml)				
	5	**25**	**50**	**100**	**250**	**5**	**25**	**50**	**100**	**250**
A. niger	-	15	17	19	20	-	14	17	22	22
A. clavatus	-	14	17	19	20	-	13	16	20	22
C. albicans	-	16	17	18	19	-	13	16	18	20

- = 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***
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

Figure 1. Antibacterial activity against S.Pyogenes(MTCC 442)

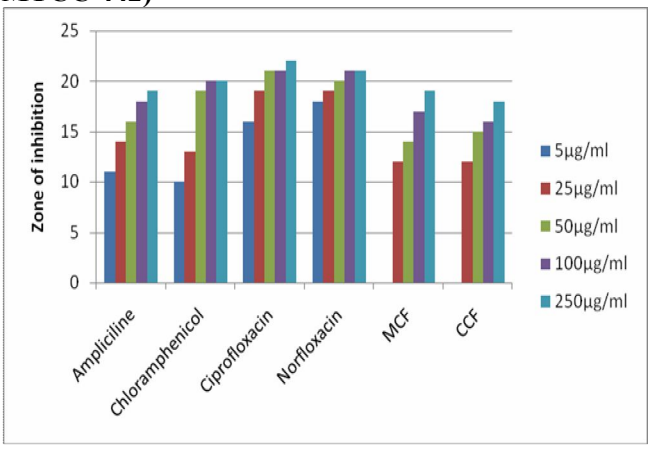


Figure4.Antibacterial activity against P.Aeruginosa (MTCC 424)

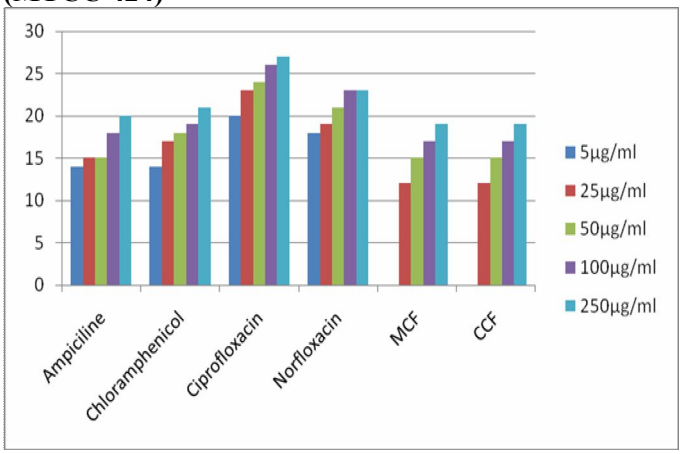


Figure 2. Antibacterial activity against S.Aureus(MTCC 96)

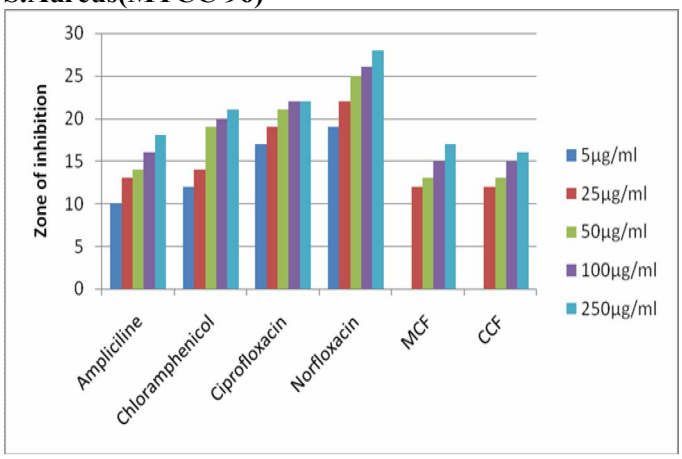


Figure 5. Antifungal activity against A.niger (MTCC 282)

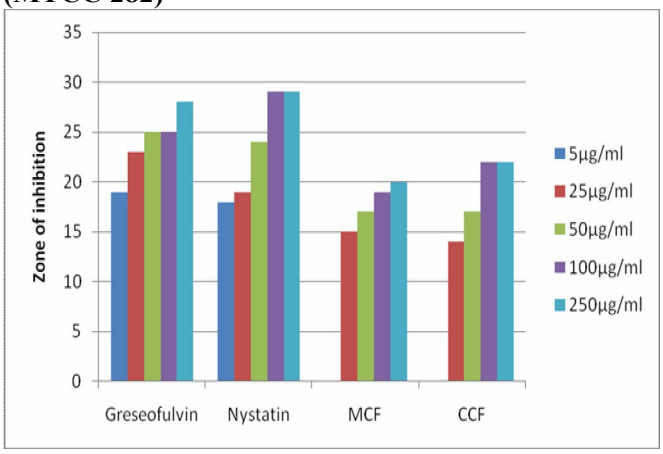


Figure 3. Antibacterial activity against E.coli (MTCC 443)

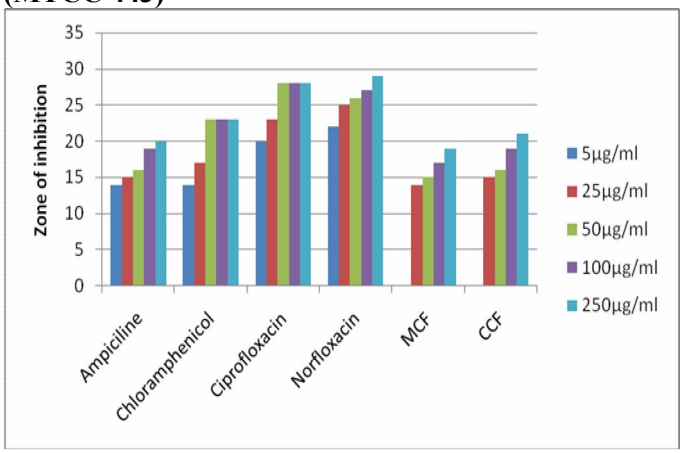


Figure 6. Antifungal activity against A.Clavatus (MTCC 1323)

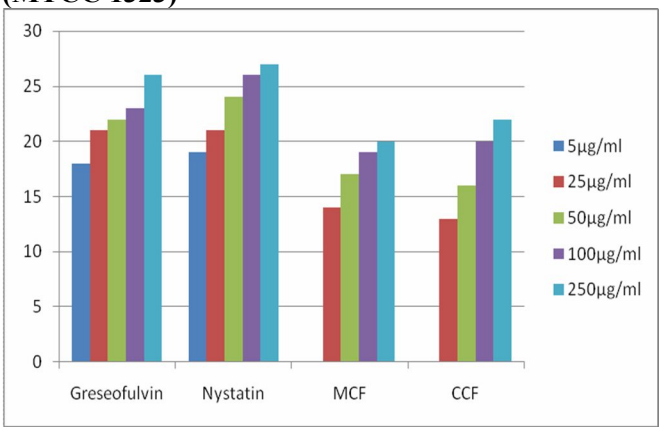
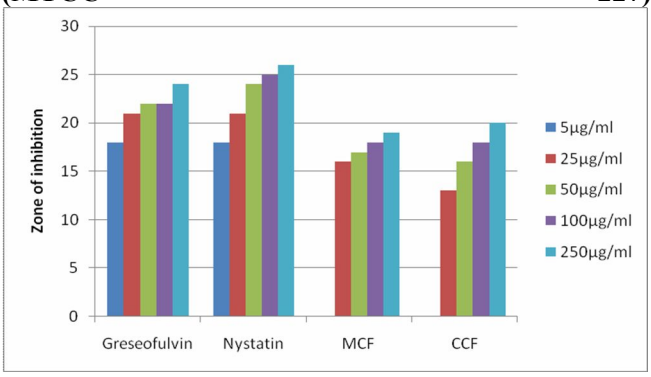


Figure 7.Antifungal activity against C.Albicans (MTCC 227)



The inhibitory effect of *Cassia fistula* flowers hydroalcohol extracts showed at 25, 50, 100, 250 µg/ml were (12, 13, 15, 17mm) for *S. aureus*, (12, 14, 17, 19mm) for *S. pyogenes*, (14, 15, 17, 19mm) for *E. coli* and (12, 15, 17, 19mm) for *P. aeruginosa* for bacterial strains, and were (15, 17, 19, 20mm) for *A. niger*, (14, 17, 19, 20mm) for *A. clavatus*, (16, 17, 18, 19mm) for *C. albicans* for fungal strains respevtively.
The inhibitory effect of *Cassia fistula* flowers chloroform extracts showed at 25, 50, 100, 250 µg/ml) were (12, 13, 15, 16mm) for *S. aureus*, (12, 15, 16, 18mm) for *S. pyogenes*, (15, 16, 19, 21mm) for *E. coli* and (12, 15, 17, 19mm) for *P. aeruginosa* for bacterial strains and were (14, 17, 22, 22mm) for *A. niger*, (13, 16, 20, 22mm) for *A. clavatus*, (13, 16, 18, 20mm) for *C. albicans* for fungal strains respevtively.
The results shows that *Cassia fistula* extracts 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 the present work, both the extracts obtained from *Cassia fistula* flowers 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 *Cassia 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 *Cassia fistula.*
From the above results the activities of hydroalcohol extracts of *Cassia fistula* shows significant antibacterial and antifungal activity. also we have used chloroform extracts, which purity level is good as compared to hydroalcoholic extract and, it's much purified, it's porosity is lower than hydroalcoholic extract, and it was further used for the isolation of pure phenolic compounds which are more active against fungal strains , So we got good results in zone of inhibition. 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 contain more or less same components like saponin, triterpenoids, steriods, glycosides, anthraquinone, flavonoids, Proteins and amino acids. Results shows, plant rich in tannin and phenolic compounds have been shown to posseses antimicrobial activities against a number of microorganisms. The present study justifies the claimed uses of flowers in the traditional system of medicine to treat various infectious disease caused by the microbes. Therefore, It may be concluded from the above results, that the crude extracts obtained from the flowers of *Cassia fistula* may be used enough as drug to treat disease caused by those bacteria, which are sensitive to the above mentioned samples. But before use in human being isolation of pure compound, toxicological study, and clinical trial in animal model should be carried out thereafter. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

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