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Antimicrobial Screening of Pulp Extracts of Cassia Fistula Linn

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ABSTRACT

The purpose of this study was to look into the antibacterial and antifungal properties of Cassia fistula Linn seeds. The study's goal is to determine the antibacterial activity of extracts and their zone of inhibition on bacterial and fungal strains. The microbiological activity of chloroform extracts of Cassia fistula Linn. (an ethno medicinal plant) seeds was tested for possible antibacterial activity against therapeutically significant bacterial and fungal strains in the current investigation. The antibacterial activity of the extracts was evaluated using the agar disc diffusion method. Cassia fistula crude extracts had moderate to strong antibacterial activity against the majority of microorganisms tested. The antibacterial activity of extracts was compared to that of several standards such as ampicilline, ciprofloxacin, norfloxacin, and chloramphenicol. The extracts had antibacterial activity against the organisms tested that was comparable to that of a standard. The results revealed a substantial suppression of bacterial growth against the organisms examined. The plants' phytochemical analyses were completed. The presence of numerous secondary metabolites contributed to the Cassia fistula's microbial activity. As a result, these plants can be exploited to uncover bioactive natural compounds that could lead to new pharmaceutical research activities.

Keywords: In vitro Antibacterial activity, antifungal activity, Cassia fistula, bacterial pathogens, secondary metabolites

INTRODUCTION

Antibiotics are one of our most essential weapons in the fight against bacterial illnesses, and they have had a significant impact on human health since their inception. However, in recent decades, many regularly used antibiotics have grown less and less efficient against certain infections, not only because many of them cause hazardous effects, but also because of the advent of drug-resistant bacteria. It's critical to look at novel drugs with lower resistance. Systematic examinations of numerous pharmacological compounds have demonstrated that any drug has the potential to perform a variety of activities, allowing it to be useful in a variety of medical fields. Natural-source drugs play an important role in the prevention and treatment of human diseases. Traditional medicine is one of the principal health-care systems in many impoverished countries. Herbs are frequently used in traditional medicine, and their curative properties have been thoroughly documented. [3] Natural products were used in about 61 percent of new medications created between 1981 and 2002, and they were quite successful, especially in the fields of infectious disease and cancer. [4] However, recent trends reveal that the pace of discovery of active new chemical entities is decreasing. [5] Natural compounds from higher plants could provide a new source of antimicrobials with potentially novel modes of action. [6, 7] A great number of researchers from all over the world have investigated the impact of plant extracts on bacteria. [8] In India, ethno medicinal plants have gotten a lot of attention. [9] In recent years, medicinal plant research has gotten a lot of attention around the world. A substantial body of evidence has developed to show the promising potential of medicinal plants employed in many traditional, complementary, and alternative methods of disease treatment. Secondary metabolites present in plants include tannins, terpenoids, alkaloids, flavonoids, glycosides, and others that have been shown to have antibacterial activities in vitro. 10 and 11]

Herbal medicines have been used by humans for millennia. Traditional medicine practitioners have highlighted the therapeutic usefulness of several indigenous plants for a variety of diseases. [12] Medicinal plants' antimicrobial capabilities are rapidly being reported from all over the world. Plant extracts or their active ingredients are estimated to be utilized as folk medicine by 80 percent of the world's population in traditional therapies, according to the World Health Organization. [13] Drugs can control harmful microbes, however this has resulted in the emergence of many drug-resistant bacteria, resulting in alarming clinical conditions in the treatment of illnesses. The pharmaceutical industry has developed a variety of novel antibiotics, yet bacterial resistance to these medications has increased. Bacteria have the genetic potential to transfer and acquire resistance to synthetic pharmaceuticals used as therapeutic agents in general. [14] As a result, steps must be done to mitigate the problem, such as reducing antibiotic use, developing resistance research among microorganisms, and continuing research to discover new medications, synthetic or natural, to control harmful microorganisms. Cassia fistula, a member of the Leguminosae family, was chosen in an effort to broaden the spectrum of antibacterial compounds derived from natural resources.

This plant has been reported in Indian literature as beneficial to skin illnesses, liver problems, tuberculoses glands, and its usage in the treatment of haematemesis, pruritus, leucoderm, and diabetes.

15 and 16] The presence of fiber and mucilage in plant parts has led to the conclusion that they could be employed as a medicinal agent in the treatment of hypercholesterolemia. [17] The plant extract is also recommended as a pest and disease control agent in India, in addition to its medicinal benefits.

(18-20) Tribal people utilize this herb to treat a variety of diseases, including ringworm and other fungal skin infections. [21] Skin disorders are treated using seeds. Plant organs of Cassia fistula are recognized to be a rich source of secondary metabolites, particularly phenolic compounds. [22]
Cassia fistula showed antibacterial activity and qualities that support its traditional use as a broad-spectrum antimicrobial agent in the treatment of various ailments.
[23] As a result, C.fistula is well-established in its traditional usage and has gained widespread acceptance around the globe.

MATERIALS :

Plant Sample Collection :

The dried leaves of Cassia fistula, which are cultivated in Hyderabad, India, were employed in this investigation. These leaves were found near my home in Rampally village, Keesara mandal, Hyderabad, Telangana, India.

Washing was used to remove any clinging dirt, which was then broken into little pieces. The plant components were dried at room temperature, away from the sun. The powder was made by milling the dried pieces (2kg). In a glass jar capped by a glass cork, dry powdered leaves were steeped in ethanol (absolute alcohol) for 7 days with intermittent shaking and stirring. To get semisolid masses, the extract was filtered through cotton and filter paper and concentrated using a rotary evaporator at low pressure (below 50°C).
The leaves were ground into a fine powder and stored in an airtight container.

Fig 1 Fine dried leaf powder of cassia fistula.

Bacterial Sample :

Pathogenic strains of staphylococcus aureus and Lactobacillus were obtained from the Microbiology department from K.P .Labs Hyderabad , and were maintained on agar medium at 4 c for further experiments .

Antibacterial Screening

The ethanol extract (semisolid masses) were examined for their antibacterial potency by disc diffusion method against nine bacterial species (4 Gram-positive and 5 Gram-negative)

Culture Media And Chemicals :

Types of media was required for carrying out this study . Nutrient agar , peptone , sodium Chloride , beef extract .Also ethanol and methanol was used for extraction process and other Chemical etc…These chemical media and the solvent were used in the college (KGRITM)Hyderabad

Antibiotics & Non – Antibiotic Drugs :

Antibiotics used include : Streptomycin .

These were collected from the APOLLO PHARMACY near kushaiguda ECIL X ROADS.

TABLE 1 : ABOUT THE STANDARD DRUG

ANTIBIOTICS	ANTIBIOTICS POTENCY	MANUFACTURED BY
Streptomycin	250mg	Dr . Reddy's

METHOD :

Preparation of Hydroalcoholic Extracts (Maceration) :

Hydroalcoholic extract 50grams of leaves powder of CASSIA FISTULA was kept for maceration with 800ml of methanol , chloroform and petroleum ether separately for 5 days .
The extract was double filtered by using muslin cloth and Whatman no.1 filterpaper

Fig 2 Maceration with Methanol , Chloroform and Petroleum ether .

Drying of Extracts :

Extracts were dried separately . The chloroform , p.ether extracts were concentrated by evaporation on heating mantle at a temperature of 40^0 C . The extract was dried and used . The percentage yield of extract was found to be 45.89 grams . The methanolic extract was dried by evaporation until thick concentrated fluid was formed .

Preparation of Plant Extracts And Drug Standard

Concentrations :

1gram of each drug ,hydroalcoholic extracts (each separately) was taken and the extracts were dissolved in 5ml of Di-Methyl Sulphoxide (DMSO) . Thus , 200mg/ml of stock was obtained as a standard concentration of alcoholic extracts and these were pasteurized for 15minutes at temperature 62^0C

Procedure :

28 grams dissolved in 1000 mL distilled water Bring to a boil to completely dissolve the medium. Dispense as desired and sanitize by autoclaving for 15 minutes at 15 lbs pressure (121 0C). Before pouring, make sure everything is well combined.
The antibacterial activity of the extracts was investigated using the agar cup method (ACM).
[18] Briefly, bacteria cultures from culture plates were scooped with a wire loop and mixed individually with normal saline before being stirred with a vortex mixer. By streaking with a sterile swab, a loop full was extracted and uniformly spread on the surface of the agar plate. A sterile borer was used to create wells on the surface of the solid medium that were approximately 6 mm in diameter and 2.5 mm deep. The plates were inverted and the wells were marked with a marker. By dissolving the extracts in dimethyl sulfoxide, they were reconstituted (DMSO). A test sample was placed in each well. The negative control was sterile DMSO, while the positive controls were gentamicin and ciprofloxacin. The plates were incubated for 24 hours at 37°C. The plates were removed after 24 hours and the zones of inhibition were assessed using the Himedia antibiotic scale, with the findings summarized. Positive extracts had zones of inhibition bigger than or equal to 8 mm in diameter. The antibacterial activity of the extracts was assessed using the mean SD of the inhibitory zone.

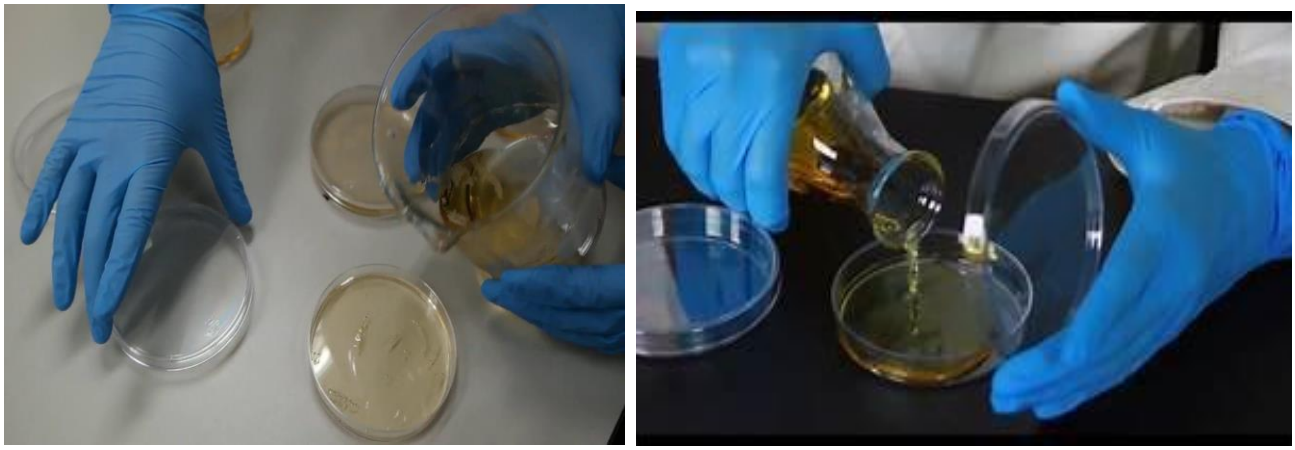


Fig 3 Pouring of Agar medium in to petri plates .

RESULTS :

Sterility Testing Of Plant Extracts:

After 1 week of incubation at room temperature, Methanolic, Chloroform, and Petroleum ether extracts of CASSIA FISTULA were confirmed to be free of bacterium and fungal contamination when streaked on Nutrient agar plates.
Chloroform, petroleum ether, methanol, distilled water, and ethanol solvents extracted 15.2, 24.5, 43.8, 68.2, and 158.6 mg of crude material per gram of powdered C. fistula leaf, respectively. When phytochemicals such as alkaloids, flavonoids, carbohydrates, glycosides, protein and amino acids, saponins, and triterpenoids were analyzed, polar extracts such as ethanol, methanol, and aqueous extracts revealed the presence of the majority of the constituents when compared to nonpolar extracts (petroleum ether and chloroform). Flavonoids, proteins and amino acids, tannins, and phenols, on the other hand, were found to be present in all of the extracts.

Cup Plate Method :

After extracting the extract components from the solvent, nutrient agar media was produced, sterilised, and inoculated within 24 hours, culture of the test organism was performed, and plates were made using a sterile rubber borer.
The chemical concentrate was applied to the cup using a micropipette and allowed to diffuse before being incubated at 37°C for 24 hours to detect the inhibition zones in the plates.
The efficiency of a test and a variety of drugs against Staphylococcus aureus was determined,.

An inoculum bacterium was put to melted, chilled agar in this approach. After that, the agar inoculums mixture is placed into petriplates.
Isolated cells are confined within the agar media as it solidifies. These cells produce isolated pure bacterium colonies. A single sample was obtained from an individual petri plate, and the activity was compared using the minimal inhibitory concentration and zone of inhibition investigations.

Evaluation Of Activity :

Inhibitory Concentration Minimum:
In microbiology, the minimum inhibitory concentration (MIC) is the lowest antimicrobial concentration that would limit observable microbe growth following an overnight incubation period.

ZONE OF INHIBITION (Kirby –Bauer Test) :
The size of the inhibition zone represents the degree of sensitivity of bacteria to a medication in this case. A larger area of germ-free media surrounding an antibiotic disk indicates that the bacteria are more sensitive to the medicine contained on the disk.

Activity Against Staphylococcus Aureus :

As shown in the table below the different zone of inhibition of chloroform , petroleum ether and methanolic extract , streptomycin was given . The zone of inhibition appeared as

Table 6 : Evaluation Activity Against Staphylococcus Aureus .

S.NO	Concentration	Zone of inhibition (mm)
Methanolic extract	300µg /ml	28
Chloroform extract	300µg /ml	23
Petroleumether extract	300µg /ml	21
Streptomycin	300µg /ml	24

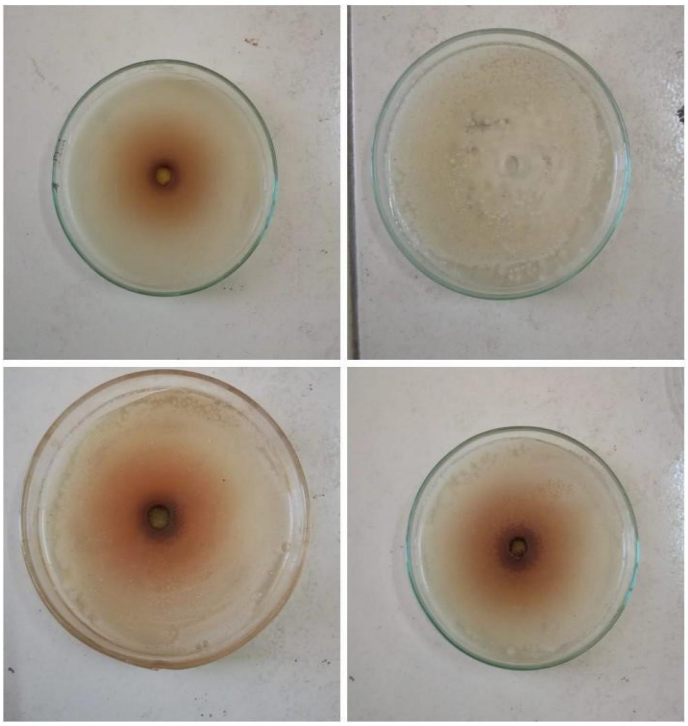


Fig 4 Activity against staphylococcus aureus .

Preliminary phytochemical screening:

To determine the presence of different chemical groups of chemicals, the extracts were submitted to preliminary phytochemical testing. Saponins, tannins, alkaloids, flavonoids, triterpenoids, steroids, glycosides, anthraquinones, cumarin, saponins, gum, mucilage, carbohydrates, reducing sugars, starch, protein, and amino acids were examined in air-dried and powdered plant materials.

Test microorganisms and growth media:

The following microorganisms: S. aureus (MTCC 96) was chosen.

Method for determining the zone of inhibition Hydro alcoholic and chloroform extracts were tested in vitro for antibacterial activity. The agar disk diffusion method was used to test the antibacterial activity of plant extracts against four pathogenic bacteria (two gram positive and two gram negative) and three pathogenic fungi. The Agar cup method was used to determine antimicrobial activity. Purified extracts were dissolved in DMSO, filtered through a sintered glass filter, and kept at 4°C. Two gram positive, two gram negative, and three fungal strains were used as a standard antibiotic for comparison of the results for determining the zone of inhibition (ZOI). The antibacterial and antifungal properties of all the extracts were tested against E. coli, P. aeruginosa, S. aureus, S. pyogenes, and the fungus Candida albicans, Aspergillus niger, and Aspergillus clavatus. Using nutrient agar tubes, sets of five dilutions (5, 25, 50, 100, and 250 g/mL) of C. fistula extract and standard medicines were produced in double distilled water. The indicator bacterial strains (108 cfu) were planted on Muller Hinton sterile agar plates and incubated at 37°C for 3 hours. After 18 to 24 hours of incubation at 37°C for bacteria, the zones of growth inhibition around the disks were assessed. The sensitivity of the microorganism species to the plant extracts was assessed by measuring the diameters of inhibitory zones on the agar surface around the disks (including the diameter of the disk) and their values.

Antibacterial activity of hydro alcoholic and chloroform extracts of Cassia fistula [zone of inhibition]

Microorganism	Zone of inhibition (mm) Concentration in µg/mL									
	Hydroalcoholic extracts (µg/ml)					Chloroform extracts (µg/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

- = No zone of inhibition

TABLE 7 Cellular Toxicity by Mtt Assay for the Ethanol, Methanol, Chloroform Extracts of Cassia Fistula Leaves

S.No	Concentration (Ug)	% Cytotoxicity		
		Ethanol	Methanol	Chloroform
1	20	30.48	15.20	12.12
2	40	37.34	20.29	12.66
3	60	54.33	22.49	26.85
4	80	55.45	29.45	28.67
5	100	58.52	36.56	31.45
6	120	58.62	39.15	40.45
7	140	58.65	48.56	53.21
8	160	61.92	61.92	61.24

TABLE 8

S.No	Concentration (ug)	% Inhibition			
		Ethanol	Methanol	P.Ether	Chloroform
1	5	51.31	40.17	22.65	4.20
2	25	13.26	41.30	51.18	16.36
3	50	60.70	43.42	25.03	15.14
4	100	20.15	44.30	11.90	35.66
5	250	25.03	31.06	35.16	10.63

% Inhibition of DPPH free radical scavenging activity for different extracts of Cassia fistula leaves in e.coli

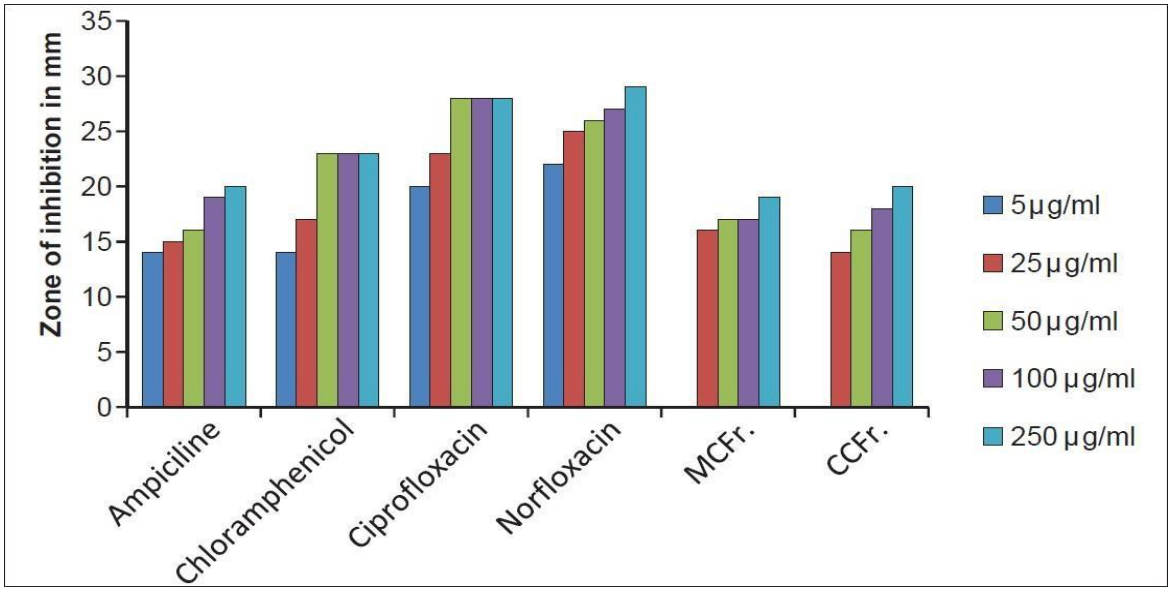


FIG: 5

Antibacterial activity against P. aeruginosa (MTCC 424) MCF: Methanolic extract of Cassia fistula leaves, CCF: Chloroform extract of *Cassia fistula*

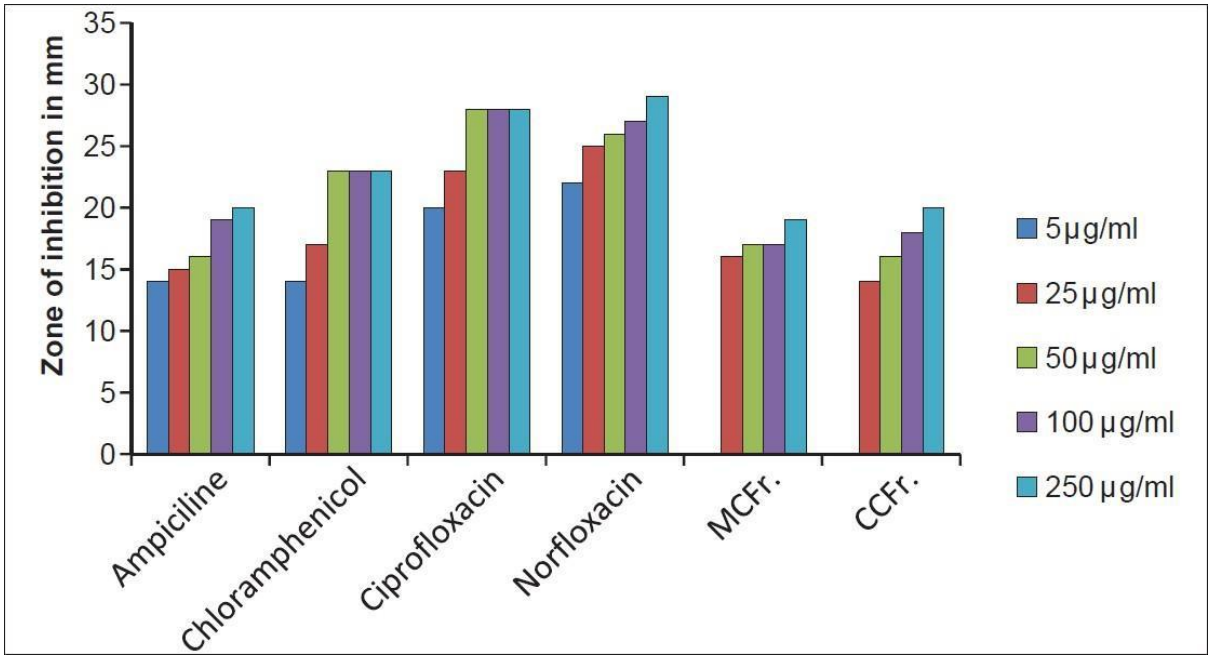


FIG : 6

Antibacterial activity against pyrogens MCF: Methanolic extract of Cassia fistula leaves, CCF: Chloroform extract of Cassia fistula

TABLE 8

S.No	Concentration (ug)	% Inhibition			
		Ethanol	Methanol	P.Ether	Chloroform
1	5	50.44	42.17	22.67	4.25
2	25	12.30	42.32	52.15	16.36
3	50	60.72	44.36	25.06	15.24
4	100	22.45	45.66	12.09	35.96
5	250	27.34	31.06	36.87	10.62

In-vitro antibacterial activity of (2S)-7-hydroxy-5-hydroxypropyl-2- (2-hydroxypropyl)chromone (compound 1) and Ampicillin

TABLE 9

Text organism	**Zone of Inhibition (diameter in mm)**		
	Compound 1 (5ug/disc)	**Compound 1 (10ug/disc)**	**Ampicillin (2ug/disc)**
	Gram-positive bacteria		
Staphylococcus aureus	6.86 +/-0.02	11.73+/-0.04	16.56+/-0.01
	Gram-Negative bacteria		
Escherichia coli	6.65 +/- 0.03	11.53 +/- 0.04	17.10 +/- 0.02
Pseudomonas aeruginosa	5.56 +/- 0.02	11.93 +/- 0.04	17.13 +/- 0.01

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

The extracts' antibacterial and antifungal properties rose linearly as the concentration of extracts (g/ml) increased. In comparison to standard medications, the results demonstrated that S. pyogenes and S. aureus were more sensitive to the extracts for bacterial activity than E. coli and P. aeruginosa, and C. albicans showed good results for fungal activity.

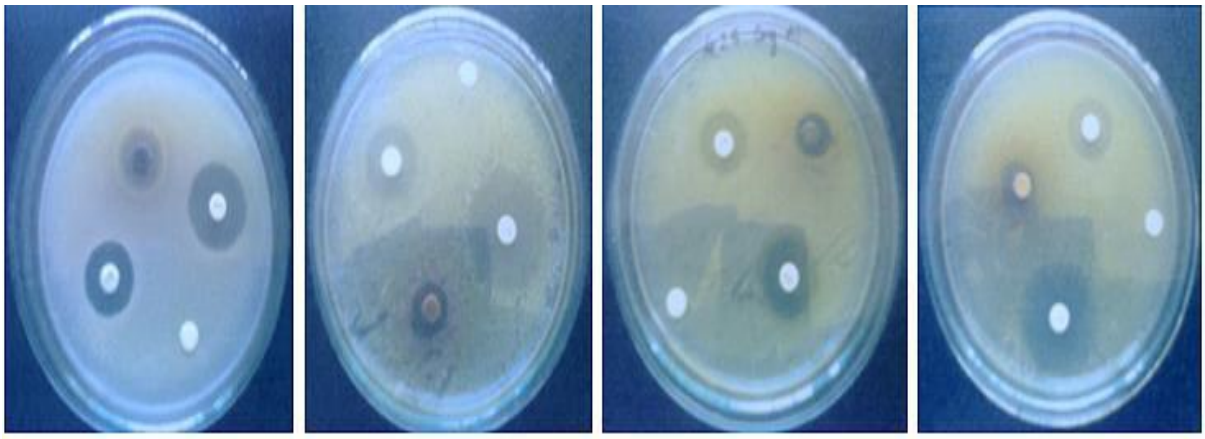
Compared to Aspergillus niger and Aspergillus clavatus. All of the sensitive bacteria had a growth inhibition zone of 11 to 20 mm, while fungal strains had a zone of 14 to 20 mm.

The findings suggest that Cassia fistula extracts are more efficient against all of the microorganisms tested.

Medicinal plants' antimicrobial capabilities are rapidly being reported from all over the world. Plant extracts or active ingredients are estimated to be utilized as folk medicine by 80 percent of the world's population in traditional therapies, according to the World Health Organization. The extracts produced from Cassia fistula exhibit high efficacy against the majority of the investigated bacterial and fungal strains in this study. The findings were matched to those of regular antibiotics. Extracts of Cassia fistula were found to be active against all organisms tested, including Gram-positive, Gram-negative, and fungal strains, which were all resistant to all Cassia fistula extracts.

The following findings reveal that hydro alcohol extracts of Cassia fistula have potent antibacterial and antifungal properties. This research also reveals the presence of many phytochemicals with biological activity that may have therapeutic value. The phytochemical results in this study revealed that the plant includes similar components such as saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. Plants high in tannin and phenolic compounds have been demonstrated to have antibacterial properties against a variety of microbes, according to the findings.

Methanolic extract of cassia fistula against gram positve and gram negetive bacteria



SUMMARY AND CONCLUSION:

- Anti-microbial agent : Any chemical or biological agent that either destroys or inhibits the growth of micro organisms is known as anti-microbial agent .
- It consists of dried leaves of CASSIA FISTULA belonging to family Leguminosae .
- C. fistula has been shown to possess anti oxidant , antidiabetic activity , hypolilidemic activity , hepatoprotective activity , antipyretic activity , antitussive activity , anti- inflammatory activity , antitumor activity , antiulcer, wound healing activity .
- The extraction of cassia fistula leaves was done by Maceration for methanol, chloroform, petroleum ether extract .
- To all above mentioned extracts phytochemical tests are performed .
- Nutrient agar medium was prepared and growth of pathogenic strains of staphylococcus aureus was induced by pour plate method .
- By cup plate method the effectiveness of test and a range of antibiotics was determined against staphylococcus aureus by MIC and zone of inhibition .
- The activity was observed along with the standard drug like Streptomycin .
- The zone of inhibition was appeared as methanolic extract 28mm , chloroform extract 23mm , petroleum ether extract 21mm . The test methanolic extract of Cassia fistula leaves shows maximum zone of inhibition then standard drugs .
- Antimicrobial resistance is a global problem. Emergence of multidrug resistance has limited the therapeutic options. Hence, 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.

References

1. Farnsworth NR. Ethno pharmacology and future drug development: the North American experience. Journal of Ethnopharmacol. 1993; 38:145-152.
2. Houghton PJ. The role of plants in traditional medicine and current therapy. Journal of Alter and Complement Med. 1995; 1:131-143.
3. Dubey NK, Kumar R, Tripathi P. Global promotion of herbal medicines: India's opportunity. Current Science. 2004; 86: 37-41.
4. Cragg GM, Newman DJ. Biodiversity: a continuing source of novel drug leads. Pure Appl Chem. 2005; 77: 7-24.
5. Lam KS. New aspects of natural products in drug discovery. Trends Microbiol. 2007; 15: 279-289.
6. Runyoro D, Matee M, Olipa N, Joseph C, Mbwambo H. Screening of Tanzanian medicinal plants for anti-Candida activity. BMC Complement Altern Med. 2006; 6(11).
7. Shahidi BH. Evaluation of antimicrobial properties of Iranian medicinal plants against Micrococcus luteus, Serratia marcescens, Klebsiella pneumonia and Bordetella bronchoseptica. Asian J Plant Sci. 2004; 3: 82-86.

8. Reddy PS, Jamil K, Madhusudhan P. Antibacterial activity of isolates from Piper longum and Taxus baccata. Pharmaceutical Biol. 2001; 39: 236-238.
9. Maheshwari JK, Singh KK, Saha S. Ethno botany of tribals of Mirzapur District, Uttar Pradesh. Economic Botany Information Service, NBRI, Lucknow. 1986.
10. Dahanukar SA, Kulkarni RA, Rege NN. Pharmacology of Medicinal Plants and Natural Products.