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ANTIMICROBIAL ACTIVITY OF *CASSIA FISTULA* LINN. LEGUMES

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ABSTRACT
Cassia fistula Linn. (Leguminoseae), commonly known as the Golden Shower, Indian Laburnum. Cassia fistula trees as leguminous plants are popularly grown in Thailand. It is native to India, the Amazon and Sri Lanka and diffused in various countries including Mexico, China, Mauritius, South Africa, East Africa, and West Indies. The antibacterial activities of the petroleum ether, chloroform, ethyle acetate, methanolic and 50% (v/v) hydro alcoholic successive extracts of *Cassia fistula* (L) fruits were studied. The extracts of *Cassia fistula* were tested in vitro against 4 bacterial species by the disc diffusion method. Staphylococcus aureus, *Streptococcus epidermidis,* Escherchia coli and Klebsiella Pneumoniae were used in this investigation. Only methanolic extract exhibited fair antibacterial activity against all the test bacteria whereas other extract were not observed to inhibit the growth of any of the test bacteria under study. *Aragvadha* (*Cassia fistula* Linn.) is a well known, commonly used plant in various disorders in Ayurvedic system of medicine. In traditional medicine, it is used in the treatment of hematemesis, pruritis, intestinal disorders, leucoderma, diabetes, & as antipyretic, analgesic & laxative.

KEYWORDS: Antimicrobial, Hepatoprotective, Antidiabetic, Antioxidant, *Cassia fistula*, Anthraquinone glycosides.

INTRODUCTION

Cassia fistula Linn. (family-caesalpinaceae) commonly known as the Golden Shower Indian Laburnum.[1] It is an Indian medicinal plant. It is native to India, the Amazon and Sri Lanka and diffused in various countries including Mexico, Mauritius, South Africa, East Africa, West Indies, and China.3 Medicinally it has been various pharmacological activities like antimicrobial, antifungal, antipyretic, analgesic, larvicidal, anti-inflammatory, antioxidant, anti-tumor, hepatoprotective and hypoglycemic activities. *Cassia fistula* is a moderate sized deciduous tree, distributed throughout India. It is 8-15m to 24m in height, with greenish grey smooth bark when young & rough, dark brown when mature. Leaflets 8 to 12 pair, flowers yellow and long drooping racemes. Pod cylindrical & pulpy. Seeds light brown, hard & shiny. Ayurvedic medicine recognizes the seeds as antibilious aperitif, carminative and laxative.[4, 5]

Plant description

A tropical ornamental tree with a trunks consisting of hard reddish wood, growing up to 40 feet tall. The wood is hard and heavy. It has showy racemes, up to 2" long, with bright, yellow, fragrant flowers. These flowers are attractive to bees and butterflies. The fruits are dark-brown cylindrical pods, also 2' long, which also hold the flattish, brown seeds (up to 100 in one pod) these seeds are in cells, each containing a single seed. Cana fistula is a fast-growing, medium-sized, deciduous tree which grows to about 9 meters in height. Leaves are compound, with 4-8 pairs of opposite leaflets. It produces flowers which are golden yellow and hang in showering bunches of up to 40 cm long earning its common name of "golden shower tree." The ensuing pods are one inch thick, and can reach lengths of 24 inches. *Cassia fistula* is semi-deciduous after flowering.[7] It is an upright, rather narrow tree with an open top, and slightly drooping branches. *Cassia fistula* is a moderate sized deciduous tree, distributed throughout India. It is 8-15m to 24m in height, with greenish grey smooth bark when young & rough, dark brown when mature. Leaflets 8-12 pairs, flowers yellow, with long drooping racemes. Pod cylindrical & pulpy. Seeds light brown, hard & shiny.[8]

According to Ayurveda, it is astringent to bowels; useful in treatment of biliousness, ulcers, erysipelas, vomiting, vaginal complains, fever, inflammations, leprosy. According to Unani system of medicine, its latex is aphrodisiac, tonic, vulnerary, maturant, lessens inflammations; useful in piles, nose diseases, gonorrhoea etc. The aerial root is styptic, useful in syphilis, biliousness, dysentery, inflammation of liver etc.[9]

There are few reports on systematic studies pertaining to antibacterial evaluation of *Cassia fistula.* Hence, considering its therapeutic potential, it was essential to prove it for its exact rational use as medicine by scientific means. Therefore, the present investigation was undertaken to evaluate antibacterial activity of *Cassia fistula* dried fruits against some pathogenic bacteria.[10]

MATERIAL AND METHODS

Collection of plant material

C. fistula L. fruit was purchased from local market of Jaipur, India. The fruits were collected locally, and then air dried. The dried plant material was homogenized to coarse powder and stored in airtight container.

Extraction of plant material

The powdered cassia fistula legumes were successively extracted by Soxhlet extraction with solvents of increasing polarity beginning with Pet. ether, chloroform, ethyl-acetate, methanol and hydro-alcoholic. The solvents were removed under reduced pressure in a rotary evaporator until they became completely dry. The percentage yield for each extract was determined. All the crude extracts were subjected to antimicrobial assay.[11, 12]

ANTIBACTERIAL ACTIVITY

Preparation of inoculums

The suspension of all organisms were prepared by inoculating one colony of the strain in 20 ml of nutrient broth in conical flask and incubated at 37^{0}C for 24 hours to activate the strain.

Preparation of dilutions

The dried plant extracts of *Cassia fistula* and antibiotic Kanamycin were weighed and dissolved in sterile distilled water to prepare appropriate dilution to get required concentration of 200mg/ml. The inoculum of *Escherichia coli* and *Staphylococcus aureus*, were prepared in nutrient broth medium and kept incubation at 37˚C for 24 hours. After growth was observed, the cultures are stored in the refrigerator at 2-8˚C for analysis.

Micro-organism used

Gram positive bacteria
Streptococcus aureus
Streptococcus epidermidis

Gram negative bacteria
Escherichia coli
Klebsiella pneumonia

Procedure for performing the Disc Diffusion test

The required amount of Mueller-Hinton plates is prepared as per manufacturer instructions, (Himedia) and autoclaved at 121˚C for 15

minutes. And they were allowed to cool under Laminar air flow (Class 100). Aseptically transfer about 25 ml of media into each sterile Petri dishes and allowed to solidify. A readily prepared sterile cotton swab (Himedia) was dipped into the turbid culture suspension. The dried surface of Muller-Hinton agar plate was inoculated by streaking two more times rotating the plate approximately 60˚ each time. The lid may be left aside for 3-5 minutes to dry the excess surface moisture content. The readily prepared sterile discs (Himedia) were loaded with concentration of 200mg/ml of plant extracts of Cassia *fistula* and antibiotic *kanamycin* into each separate disc of about 200µl. The discs were placed on the medium suitably apart and the plate were incubated at 5˚C for 1 hour to permit good diffusion and then transferred to an incubator at 37˚C for 24 hours. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader.[13]

RESULTS AND DISCUSSION

The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial agents with significant potential activity against infective microorganisms. Recently, much attention has been directed toward isolation of active compounds with biological response available in plant. Many reports of natural antimicrobial agent of plant origin have been published and their importance in health, food and preventive medicine has been well documented.

The existing antibiotics are become resistant to the infectious diseases and urgent need is to discover new antimicrobial compounds with diverse chemical structure and novel mechanism of action for newly diagnosed and re-emerging infectious diseases.

The present study deals with extraction, and antibacterial activity of different extracts of *cassia fistula.* The percent extractability was highest for aqueous and lowest for petroleum ether.

The methanolic extract was found to be effective against all the pathogenic bacteria under test by disc diffusion assay. The other extract failed to exhibit any zone of inhibition by disc diffusion assay. Microbial activity was performed in two gram positive and two gram negative bacteria. The results were compared with those of Kanamycin as a standard antibiotic. Of the four extracts, only methanolic extract showed activity against gram- negative *E.coli.* The methanolic extract was more effective than the standard drug (Kanamycin) against *E. coli.* Bacteria when studied by disc diffusion assay.

The methanolic fruit extract of *Cassia fistula* had impressive antibacterial and could lead to the discovery of new antibiotics. This becomes more relevant as the current antibiotics in use are fast loosing effectiveness due to emergence of resistant microorganisms.

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Table-1: Amount and Physical appearance of different extracts of *Cassia fistula* legume:

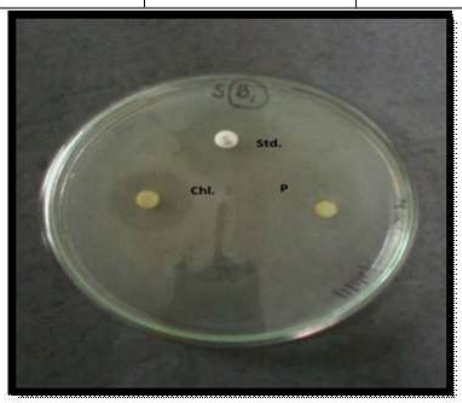
S. No.	Solvent extract	% yield of dry extract	Color	Odour	Consistency
1	**Pet. Ether**	0.81%	Yellowish	Characteristics	Sticky
2	**Chloroform**	1.18%	Blackish	Characteristics	Hard
3	**Ethyl acetate**	1.56%	Blackish, sticky	Characteristics	Sticky
4	**Methanol**	31.20%	Dark, brownish	Fruity	Sticky
5	**Hydro-alcoholic**	32.352%	Dark, Blackish	Characteristics	Sticky

Table-2: Phytochemical Screening of each extracts (legumes of *Cassia fistula*):

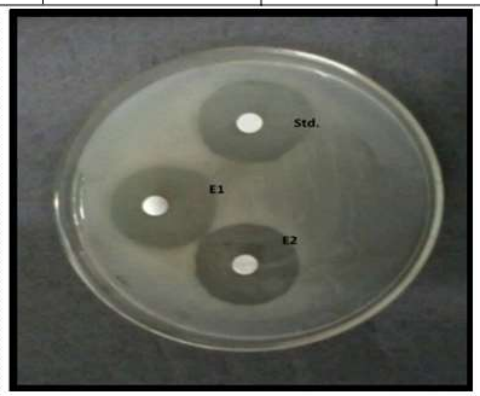
S.No.	Name of the test	Pet. Ether	Chloroform	Ethyl acetate	Methanol	Hydro-alcoholic
1	Alkaloids	-	-	-	-	-
2	Carbohydrates	-	-	-	+	+
3	Glycosides	-	-	+	+	+
4	Tannins	-	-	-	+	+
5	Protein and amino acid	-	-	-	-	+
6	Flavonoids	-	-	+	+	+
7	Saponins	-	-	+	+	+
8	Fatty acids	+	+	-	+	+
9	Terpenoids	+	+	+	+	+

Table-3: Zone of inhibition of different extracts of fruit of plant *Cassia fistula* and reference drug against different bacteria

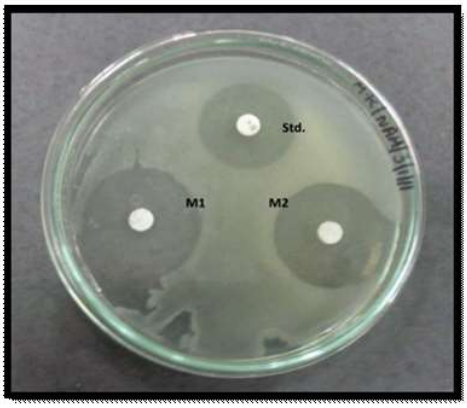
Name of bacteria	Zone of inhibition (Radius in mm)					Standard drug
	Pet. Ether	Chloroform	Ethyl acetate	Methanolic	Hydro-alcoholic	*Kanamycin*
Gram positive						
Staphylococcus aureus	–	–	–	–	–	10 mm
Streptococcus epidermidis	–	–	–	–	–	10 mm
Gram negative						
Escherichia coli	–	8 mm	9 mm	11 mm	10 mm	10 mm
Klebsiella pneumonia	–	–	–	–	–	10 mm



A. Pet. Ether and chloroform extract with Std.

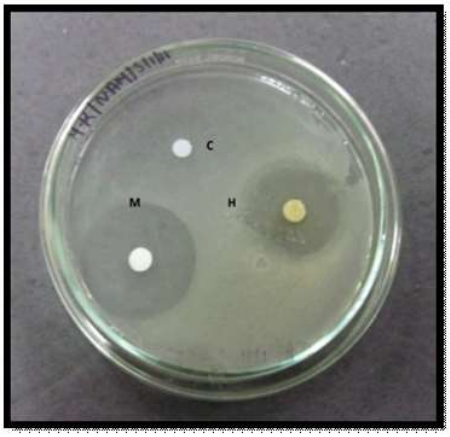


B. Ethyl acetate extract with Std.

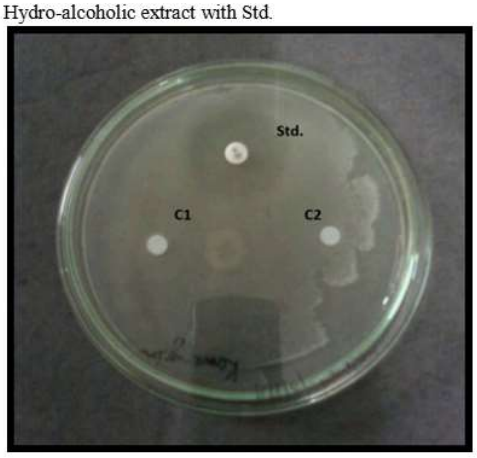


C. Methanolic extract with Std.

D. Hydro-alcoholic extract with Std.



F. Comparison between C and D



E. Control with Std.

Figure- 5: Antibacterial activity of different extracts of *Cassia fistula* legumes

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