



THE POTENTIAL OF AQUEOUS AND ISOLATED FRACTION FROM LEAVES OF *CASSIA FISTULA* LINN AS ANTIBACTERIAL AGENT

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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. In the present study, the alcoholic extract of leaves of *C. fistula* showed antimicrobial activity against *S. aureus*, *P. aeruginosa*, *E. coli* and Group A. strep, some common human pathogens. The bioassay guided isolated compound showed MBC values ranged from 4 mg/mL-8 mg/mL and MIC values ranged from 1 mg/mL-2 mg/mL.

Key words : *Cassia fistula*, Antibacterial.

INTRODUCTION

Cassia fistula Linn., a semi-wild Indian Labernum also known as the Golden Shower, Native to India, the Amazon and Sri Lanka has become extensively diffused in various countries including Mauritius, South Africa, Mexico, China, West Indies, East Africa and Brazil as an ornamental tree for its beautiful bunches of yellow flowers. Recognized by the British Pharmacopoeia¹, *C. fistula*, is a member of the Leguminosae family, The plant has a high therapeutic value and it exerts an antipyretic and analgesic

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effect². Besides, it has been found to exhibit anti-inflammatory and hypoglycaemic activity and it is widely used as a mild laxative suitable for children and pregnant women^{3,4}. 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, leucoderm and diabetes has been suggested^{5,6} and the leaf extract is also indicated for its anti-tussive and wound healing properties⁷. *C. fistula* plant organs are known to be an important source of secondary metabolites, notably phenolic compounds⁸.

EXPERIMENTAL

Materials and methods

Plant material

Plant was collected randomly from tropical -humid regions of Kerala, India, under the guidance of an expert from Indian herbs. The specimens were identified; voucher specimen was prepared and stored for future use. Plant materials were dried in the dark at room temperature and quality control tests were carried out for plants as per WHO-guidelines and quality samples were selected for extraction.

Extraction and isolation

The powdered cassia fistula leaves were successively extracted by Soxhlet extraction with solvents of increasing polarity beginning with pet. ether, methanol and water. 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, and the methanol extract was found to be very active⁹. Therefore, the water extract was applied on silica gel column chromatography and successfully eluted with stepwise gradient of chloroform, methanol (2 : 1 and 1 : 1). The elution was monitored by thin layer chromatography and antimicrobial assay. Similar fractions were pooled together concentrated and dried under vacuum.

Determination of antimicrobial action

Microorganisms used

The test organisms used were *Staphylococcus aureus* (ATCC29737), *Escherichia coli* (ATCC2068), *Pseudomonas aeruginosa* (ATCC9027) and *Group. A Streptococcus*.

Agar diffusion assay

The modified agar well diffusion method⁹ was employed. Mueller-Hinton agar plates

were inoculated by streaking the swab over the entire sterile agar surface. Each extract was checked for antimicrobial activity by introducing 100 μL of 4000 $\mu\text{g}/\text{mL}$ concentration into triplicate wells. Simultaneously, gentamicin sulfate was used as positive controls at a concentration of 1.0 $\mu\text{g}/\text{mL}$ and the dilution medium for the positive controls was sterile distilled water, ethanol and petroleum ether. The plates were allowed to stand at room temperature for 1 hr for extract to diffuse into the agar and then they were incubated at $35 \pm 2^\circ\text{C}$ for 24 h.

Determination of Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The MIC was determined by micro-broth dilution method¹⁰. The reconstituted drug was serially diluted 2-fold in Mueller-Hinton broth (Oxoid) medium. Duplicate tubes of dilution ranging from 0.025 mg/mL to 25.6 mg/mL, were inoculated with 5×10^5 cells (cfu) of the test bacterial strain and cultures incubated at 37°C for 18 h. MIC was taken as the highest dilution (least concentration) of drug showing no detectable growth. MBC was determined by sub-culturing the test dilution on to a fresh drug-free solid medium and incubating further for 18-24 h. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC

RESULTS AND DISCUSSION

The crude methanol extract of the leaves of *C. fistula* showed significant antimicrobial activity against tested microorganisms (Table 1). The aqueous extract is liquid-liquid partition between chloroform-methanol in 1:1 and 2:1. The medium polar 4th and 5th fraction eluted with 1 : 1 chloroform – methanol showed highest antimicrobial activity against selected organisms. This fraction again purified with chloroform-methanol (1 : 1) 5th fraction gave orange oil with highest bioassay result and single component on TLC. The results of microbial assay guided purification of *C. fistula* extract and MIC and MBC values of purified compound are summarised in Table 1

Our study showed that the isolate from the methanol extract inhibited the gram-positive bacteria better than gram-negative. generally, plant extracts are usually more active against gram-positive bacteria than gram-negative bacteria¹¹. The results of the zone of inhibition demonstrated that the isolate had very high growth inhibitory effects on all the microorganisms. The findings were consistent with those of Mahesh et al.¹², who observed that *Cassia* species containing anthraquinone, flavonoids and polysaccharides showed considerable activity against gram-positive microorganisms. They also agreed with the findings of Abo et al.¹³ that extracts from the leaves and pods of *C. fistula*, showed

significant antimicrobial activity. The antimicrobial activity of the active constituent are reported in the present study. The results of the study justified the use of the extract of the leaves of *C. fistula* in the treatment of diseases of microbial origin in herbal medicine. MBC values of both the extracts were 1-2 times greater than their MIC values. This suggests that the bioactive compound in the extracts was bacteriostatic rather than bactericidal as reported previously. It is well known that anthraquinone containing phenolic group, which is soluble in water, alcohol and chloroform but gives precipitates with protein¹⁴. The similarity in the antimicrobial activity of both the methanol extracts and alcohol-chloroform fraction suggest that these extracts may have high total content¹⁵. The antimicrobial activity seems to depend on the contents of phenolic group in the plant extracts. High amounts of phenolic group present in the leaves of *Cassia fistula*¹⁵ implied that anthraquinone may be the active compound, which may be responsible for the antibacterial activity. In conclusion, the extracts of the leaves of *Cassia fistula* have high potential as antibacterial agent. This finding provides an insight into the usage of the leaves of *Cassia fistula* in traditional treatment of wounds or burns associated with bacterial infections.

Table 1

Bacterial strains	MIC (mg/mL) \pm SD	MBC (mg/mL) \pm SD
S. aureus	0.1 \pm 0.001	0.4 \pm 0.00
P. aeruginosa	0.2 \pm 0.00	0.8 \pm 0.002
E. coli	0.2 \pm 0.003	0.4 \pm 0.002
Group A strep.	0.1 \pm 0.001	0.4 \pm 0.001

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