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Short Communication

Ascertaining antibacterial potential of *Cassia fistula* against *Escherichia coli*

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The emergence of antibiotic resistance has forced scientists to use natural products to fight against microbes. *Cassia fistula* L. has been used in traditional medicine to treat various diseases such as diarrhoea, skin diseases, fever, abdominal pain, leprosy, etc. The present study focussed on the growth inhibitory activity of *C. fistula* leaf ethanol extract against *Escherichia coli* DH5α strain. The antibacterial activity of three different concentrations (2, 1, and 0.5%) of *C. fistula* leaf extract was investigated. The results showed that the extract inhibited the growth of *E. coli* significantly. The density of *E. coli* in the control group was 1.97×10^9 cells/mL. The number of cells in the treatment at 2% extract concentration was significantly reduced; after 24 hours, 1.04×10^8 cells/mL were detected. In the treatments at 1% and 0.5% concentrations, there were noticeably fewer cells than in the control (6.48×10^8 and 7.04×10^8 cells/mL, respectively). It was found that the extracts' antibacterial effectiveness varied in a dose-dependent manner. Several secondary metabolites were present in *C. fistula*, which contributed to its antibacterial action.

Keywords: Antibacterial activity, *Cassia fistula*, *Escherichia coli*

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Introduction

One of the most effective ways to combat microbial infections is using antibiotics. Although antibiotics have extended life expectancy for millions of people, the emergence of multi-drug resistance (MDR) bacteria has put a serious challenge against antibiotics' therapeutic[1]. Indiscriminate and irrational use of antibiotics causes the development of genes resistant to antibiotics in microbes, leading to loss of antibiotic efficacy and resistance development[2]. The fast alteration of resistant bacteria's genetic makeup may lessen antibiotic efficacy in as little as five years[3]. In addition to microbial resistance, antibiotics often induce hypersensitivity, immunological suppression, and allergic reactions[4]. A report by the World Health Organization (WHO) found that resistance is more prevalent in cases of bacteria that cause meningitis, TB, respiratory tract infections, and diarrhoea, the majority of the world's lethal infections[5]. Developing new and safe antimicrobial medications that can be used against microbes is, therefore, desperately needed[6,7].

Natural products, especially medicinal plants, are receiving more attention as essential antibacterial agents as they have low mammalian toxicity and compatibility with human physiology[8]. A great deal of studies have been done on the use of several medicinal plants to treat microbial infectious diseases globally. Nearly 119 chemical substances derived from higher plants have been employed in medicine worldwide[9]. Of 45000 plant species in India, 3000 plants are scientifically documented with medicinal potential[10].

Cassia fistula L. (Fabaceae Family), commonly known as "golden shower tree," "Amaltas", and "Indian Laburnum"[11] is native to India, the Amazon, and Sri Lanka, and also found in many other nations, including Mexico, Mauritius, China, South Africa, and West Africa[12]. The plant is well-known for its therapeutic qualities and has the potential to be used in alternative medicine[13].

It is well known that different plant parts of *C. fistula* constitute a significant source of secondary metabolites that demonstrate notable antimicrobial activity and help treat various disorders. Proanthocyanidins are abundantly present in the leaves, flowers, and pods of *C. fistula*. Phenolic compounds present in the leaves of *C. fistula* are about 7.8%[14]. Sennosides B, Oxalic acids, tannins, oxyanthraquinones, and anthraquinones are present in both the leaves and the fruits of *C. fistula*[15]. These compounds constitute antimicrobial activity[16,17]. Various other secondary metabolites, such as hextriacontanoic, triacontanoic, nonacosanoic, and heptacosanoic acids, were reported in the cuticular wax of leaves[18]. Roots of *C. fistula* have β-sitosterol, stigmasterol, β- sitosterol-3-O-β-glucopyanoside, lupeol, betulinic acid, fistucacidin[18,19], and rhamnetin 3-O-gentiobioside[20]. In stem bark, polyphenolic compounds like anthraquinones,

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proanthocyanidin, xanthones, alkaloids, and flavonols are abundantly present[14,21].

Various types of essential oils are present in the flower extract, like (E) –nerolidol and 2-hexadecane[18,22,23]. Major fatty acids found in the seeds of *C. fistula* are linoleic acid, oleic acid, stearic acid, and palmitic acid; Caprylic acid and Myristic acid are in minor amounts[18]. Various types of compounds like triterpene, lupeol, emodin, physcion, citreorosein, rhein, ziganein, coumarins, scopoletin, chromones, isovanillic acid, and vanillic acid are present in the aril of seeds of *C. fistula*.

Scientific reports have indicated that extract from *C. fistula* has a range of pharmacological properties, including anti-inflammatory[24], antioxidant[25], antimicrobial[26], wound healing[27], anticancer[28], hepatoprotective[29,30], antipyretic[31], analgesic[31], hypoglycemic, and antiviral properties[32,33]. Tribal people utilise *C. fistula* extensively to cure a wide range of conditions, such as ringworm and other fungal skin infections[34]. In addition to treating skin conditions, boils, carbuncles, ulcers, intermittent fever, gouty arthritis, and rheumatalgia may be cured[35]. In *E. coli*, the extract from the leaves decreased mutagenicity. Numerous studies have demonstrated the antibacterial and antioxidant properties acquired by certain *Cassia* species. The current work aimed to investigate the growth inhibitory effects of a leaf extract from *C. fistula*, prepared by a sequential extraction method, against *E. coli* DH5α.

Materials and Methods

Plant sample collection and identification

In the present research work, *C. fistula* leaves were used for extraction. The leaves were collected in March 2023 from Botanical Garden, Deshbandhu College campus, Kalkaji, New Delhi, India (22°31′03.28″N and 88°20′42.74″E). Only leaves in good condition were used for extraction. The leaves were closely inspected, and any contaminated leaves were disposed off to prevent infection.

Plant sample preparation and extraction

The leaves of *C. fistula* were shade-dried for two weeks at room temperature after washing in running water. The dried leaves were ground into a fine powder. A Soxhlet extraction apparatus was used for extraction. Twenty-five grams of ground leaf powder was extracted in 250 mL of ethanol at 42°C for 24 h. The extract was filtered through Whatman filter paper No. 1 and concentrated using a Rotavapor vacuum evaporator (Buchi) at 40°C. The extract was stored at -20°C as a crude extract. Three concentrations of *C. fistula* leaf ethanol extract (*Cf*-LEE), i.e. 2, 1, and 0.5%, were used for the experimental purpose. Dimethyl Sulfoxide (DMSO) was used as a solvent to prepare desired concentrations. In the control condition, *E. coli* was treated with DMSO.

Antibacterial activity

E. coli DH5α pure bacterial strain was employed as a model organism to assess the antibacterial activity of *Cf*-LEE. An axenic culture of *E. coli* was maintained in 1.5% LB broth at 37°C in the BOD incubator. The bacteria were routinely sub-cultured and preserved in nutrient agar slants at 4 and 20°C. Each experimental tube contained 4 mL LB broth, I mL *Cf*-LEE, and 20 µL of inoculum. In control, 1 mL of DMSO was added in place of *Cf*-LEE. The tubes were kept in a shaker set at 37°C for 24 h. The bacterial growth in each tube was determined by measuring the absorbance at 600 nm wavelength using a spectrophotometer (Carry 60 UV-Vis Agilent technologies). The optical density (OD) was converted to cell density with the help of Agilent Genomics: Tool-Bio Calculators[36]. All the experiments were replicated three times. The results were presented as Mean±Standard Error (SE).

Results and Discussion

The results of the growth inhibitory effect of *Cf*-LEE on *E. coli* are presented in Fig. 1. The results indicate that *Cf*-LEE has an adverse impact on the growth of *E. coli* DH5α. It is evident from the results

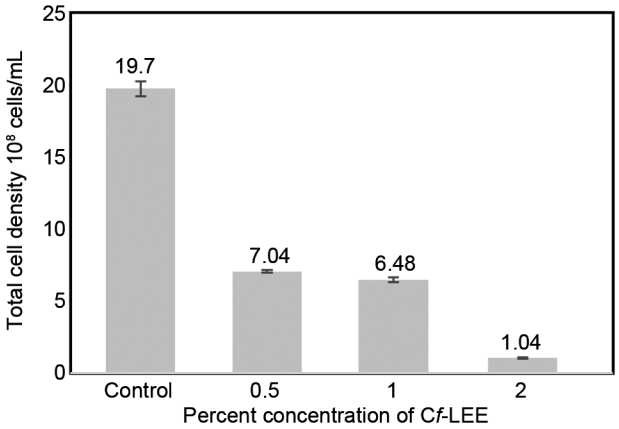

Fig. 1 — Dose-dependent growth inhibition of treated Gram-negative bacteria, *E. coli* DH5α, in response to *C. fistula* leaf ethanol extract.

that bacterial growth in the culture was inhibited in a dose-dependent manner. In control, the density of *E. coli* after 24 h of incubation was 1.97×10^9 cells/mL. There was a sharp reduction in the number of cells in the treatment at 2% extract concentration; the number of cells recorded after 24 h was 1.04×10^8 cells/mL. Similarly, in the treatments at 1 and 0.5% concentration, the number of cells was 6.48×10^8 and 7.04×10^8 cells/mL, respectively, significantly less than the control.

Antibacterial activities of many plant extracts have been documented against many bacteria[37]. *Ocimum sanctum* extract's antibacterial activity was investigated against a range of gram-positive and gram-negative bacteria, such as *Aggregatibacter actinomycetemcomitans*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *E. coli*[38-40]. *C. fistula* fruit pulphydroalcoholic and chloroform extract showed antibacterial activities against *Streptococcus pyogenes*, *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa*[41].

The essential oil extracted from *Mentha viridis* had significant bactericidal activity against *Listeria monocytogenes* and *S. aureus*[42]. *Murraya koenigii* leaves, also known as Indian curry leaves, had the lowest minimum inhibitory concentration (MIC) value of 15.63 µg/mL when tested against *S. aureus*, *E. coli*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, and *Yersinia enterocolitica*[37]. Takó *et al.*[43] and Khameneh *et al.*[44] reported that coumarin extracted from plant extract decreased the MIC value against *Salmonella spp*., *Enterobacter spp*., *Staphylococcus spp*., *Bacillus spp*., and *Klebsiella*, with a range of 0.625–1024 µg/mL.

According to Levison and Jawetz, *P. aeruginosa* infections are among the hardest to cure with traditional antibiotics. The effect of three extracts was tested against *P. aeruginosa.* Out of three extracts, two inhibited its growth moderately, while the *Dracaena cinnabari* extract inhibited it completely. Thus, these plants may serve as a source of potential drug molecules that could enhance the management of illnesses brought on by this bacterium[45]. Tests were conducted on the flower extracts of *C. fistula* (hexane, chloroform, ethyl acetate, methanol, and water) against bacteria and fungi. The minimum inhibitory concentrations (MIC) for all of the extracts against Gram-positive pathogens were reported between 0.078 and 2.5 mg/mL. Only *P. aeruginosa* showed susceptibility to the extracts among the Gram-negative bacteria. *C. fistula's* aqueous extract had notable effectiveness against *S. aureus* in the disc diffusion method. More inhibition against *S. aureus* was demonstrated by the alcoholic extract than by the aqueous extract[46,47].

Lectins (CSL-1, CSL-2 and CSL-3) purified from *C. fistula* seeds were screened for their antibacterial activity against *Bacillus subtilis*, *Bacillus megaterium*, *Streptococcus haemolyticus*, *S. aureus*, *Sarcina lutea*, *Shigella sonnei*, *E. coli*, *Klebsiella sp., Shigella shiga, Shigella boydii, Shigella flexneri, Shigella dysenteriae, Salmonella typhi* and *P. aeruginosa*. All bacterial strains were susceptible to CSL-3's action, which was particularly potent against *Bacillus megaterium, S. haemolyticus,* and *S. boydii*. Only *S. haemolyticus* exhibits high activity against CSL-2, which had weak activity against most bacterial strains. All bacterial strains except *S. lutea* and *S. haemolyticus* were inactive against CSL-1[46,47].

The effect of *C. fistula* leaf extract (aqueous, methanol and chloroform) was tested for screening antimicrobial activity against *Bacillus cereus, S. aureus*, *E. coli, Klebsiella pneumoniae, P. aeruginosa* and *Proteus mirabilis*. Results revealed that the methanol extract from leaves exhibits potent antimicrobial activity against the entire tested organism. *B. cereus, S. aureus, E. coli*, *K. pneumoniae, P. aeruginosa,* and *P. mirabilis* were all effectively inhibited by the chloroform extract. Lastly, the aqueous extracts demonstrated moderate efficacy against *P. mirabilis, K. pneumoniae, B. cereus,* and *S. aureus*[48]. According to Perumal Samy *et al.*[49], *C. fistula* has moderate antibacterial activity against a variety of bacteria, including *Proteus vulgaris, Bacillus mycoides*, *E. coli, Mycobacterium smegmatis*, *B. subtilis, Klebsiella aerogenes,* and *P. aerogenes*.

Using the broth dilution method, Valsaraj *et al.*[50] investigated the antibacterial activity of *C. fistula* seeds. *E. coli* and *Bacillus subtilis* were seen to be inhibited at a concentration of 12.5 mg/mL, whilst *S. aureus* was inhibited at a concentration of 6.25 mg/mL. Using the agar dilution streak method, Kumar *et al.*[51] observed the fruit of *C. fistula* to have antibacterial activity. *B. subtilis* and *Staphylococcus epidermidis* showed no inhibition, whereas only *E. coli* exhibited moderate level of inhibition.

Seyyednejad *et al.*[52] reported that gram-negative bacteria were more sensitive to ethanolic and methanolic extracts of *C. fistula,* as compared to

Gram-positive bacteria. The extract may also affect the outer membrane of Gram-negative bacteria or protein synthesis mechanisms that can lead to suppressing the bacterial cell density[52].

The majority of *C. fistula's* antibacterial properties are attributed to its constituent parts and secondary metabolites, such as phenolic chemicals[53,13]. *C. fistula* is a rich source of various types of secondary metabolites such as saponin, triterpenoids, glycosides, anthraquinone, steroids, and flavonoids that are also responsible for inhibiting the growth of bacterial strain. It was noted by Rizvi *et al.* that *Cassia* species exhibited noteworthy action against Gram-positive bacteria. They asserted that some chemicals, such as polysaccharides and flavonoids, are mainly involved in reducing bacterial growth[54].

Conclusion

GC-MS analysis of *C. fistula* ethanol extract, as reported in the literature, reveals the presence of several groups of phytochemicals with antimicrobial action, like saponin, triterpenoids, glycosides, anthraquinone, steroids, flavonoids, β-sitosterol, rhein, etc. The extract of *C. fistula* has the potential to suppress *E. coli* growth because of its unique chemical composition or the combined actions of its constituents. According to this study, *C. fistula* may be employed to find phytocompounds of pharmacological significance that could treat a range of bacterial diseases.

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Conflicts of interest

The author/s declares no conflicts of interest regarding the publication of this paper.

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