



Short Communication

Screening of the *Cassia fistula* Flowers Extract for the Anti-Acne Activity

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Abstract

Extract of *Cassia fistula* flowers were screened for their *in vitro* antimicrobial activity using agar disc diffusion method. The antimicrobial activity of methanolic extract of *Cassia fistula* flowers were studied against acne causing bacteria, namely *Staphylococcus epidermidis*. Methanolic extract of *Cassia fistula* flowers possessed highest antibacterial activity against *S. epidermidis*. Minimum inhibitory concentration (0.42 mg/mL) and antimicrobial screening (10.01 ± 0.04 mg/ml) against *S. epidermidis* were also observed for methanolic extract of *Cassia fistula* flowers. Phytochemical screening revealed the presence of alkaloids, tannins, saponins, anthraquinones, anthocyanides flavonoids, glycosides and terpenoids which indicates that these phytoconstituents may be responsible for their anti-acne activity.

Keywords: Antimicrobial activity, acne, cassia fistula, disc diffusion method.

Introduction

Infectious diseases are disorders caused by pathogenic microorganisms like bacteria, viruses, fungi, protozoa and multi-cellular parasites. These diseases are also called as communicable or transmissible diseases since they can be transmitted from one person to another via a vector or replicating agent. Infectious diseases account for about half of the deaths in tropical countries¹. Bacteria diseases are a type of infectious diseases caused by pathogenic bacteria. It is notable that majority of bacteria are non pathogenic and are not harmful to human health. Some bacteria are even helpful and necessary for the good health. Millions of bacteria normally live in the intestine, on the skin and the genitalia. Bacterial diseases results when the harmful bacteria get into a body area, multiply their and thrash the body's defensive mechanism. Pathogenic bacteria can invade in the body through various routes like inhalation into nose and lungs, ingestion in food or through sexual contact. Once bacteria enter the body, the immune system of the body recognizes the bacteria as foreign intruder and tries to kill or stop them from multiplying. However, even a healthy immune system is not always able to stop the bacteria from reproducing and spreading. As a result bacteria thrive in the body and emit toxins which damage cells and tissues that consequently results in the symptoms of bacterial disease. The extract of *Cassia fistula* flowers has antibacterial activity against Gram-positive organisms².

Material and Methods

Collection of materials: The plant materials belonging to the genus *Cassia fistula* (Leguminosae) were collected from Kerala. The plant materials were authenticated when they were collected. The specimens were used for the extraction.

Staphylococcus epidermidis were obtained from the Kerala Research Centre, Kerala.

Method of Extraction: The flowers of *Cassia fistula* plant were dried under shade and finely powdered. The powdered material was used as a source for the extraction of secondary metabolites in plants. The shade dried finely powdered flowers (1kg) of *Cassia fistula* was extracted by Soxhlet apparatus with methanol as solvent. The extract was concentrated under reduced pressure in freeze drier and this extract was used for the anti-acne investigation³.

Antimicrobial screening: Fresh cultures of the isolates of bacteria were suspended in nutrient broth and reinforced clostridium medium, *S. epidermis* cultures were incubated for 24 at 37°C. *P. acnes* culture was incubated in an anaerobic chamber at 37°C consisting of 15% CO₂, 15% H₂ and 70% N₂ for 24 hrs. Antibacterial activity of extracts was tested using agar disc diffusion method⁴. Fresh culture suspension (100 µl) of test bacteria was evenly spread on nutrient agar and reinforced clostridial agar plates. The concentration of cultures was 5×10^5 CFU/ml. For screening, 6 mm diam. filter paper disc, impregnated with 15 µl of extract solution equivalent to 0.5 mg of extract was placed on the surface of inoculated media agar plates. Incubation was done at 37°C or 30°C for 24 .Clear zones of inhibition were measured in mm, including the diameter of disc. Zone measuring 15 mm or more was considered as effective against test organisms. Clindamycin (15 µg/disc) was used as positive control and the respective solvents, which were used for extraction, served as negative control.

Minimum inhibitory concentrations using micro dilution: Minimum inhibitory concentration (MIC) of active methanolic

extracts (AME) was studied by using broth micro dilution method with slight modify cations⁵. Extracts were dissolved in (DMSO) dimethyl sulfoxide (15% of total volume) and two fold dilutions were done using pre-sterilized culture broth to give final concentrations ranging from 3-0.21 mg/ml. 100 µl of each dilution was distributed in 96 well plates. Sterility control (sterilized nutrient broth) and growth control (culture broth with DMSO) were also set up. Each test and growth control well was inoculated with 2 µl of a bacterial suspension (5x10⁵ CFU/ml). All experiments were performed in triplicate and the micro dilution plates were incubated under optimum conditions. Bacterial growth was detected after the addition of 15 µl of 85% alcoholic solution of INT (0.3 mg/ml) into each well followed by incubation for 30 minutes. Colour change from yellow to purple indicated the presence of microbial growth.

Results and Discussion

In present investigation, percentage yield of extract indicated that extract showed anti-acne activity. In vitro antimicrobial screening using clindamycin phosphate as a positive control clearly indicated that the extract showed promising antimicrobial activity against the organisms (table 1). It was observed that the extract of *Cassia fistula* flowers showed significant antimicrobial activity against test organisms. Zone of inhibition 10.01± 0.04 mm was observed for *Cassia fistula* flowers extract against *S. epidermidis*. The results, after preliminary antimicrobial screening, revealed that active methanolic extracts have potential in the treatment of Acne, hence they were further evaluated for their MICs. The MICs against *S. epidermidis* (0.42 mg/ml) were recorded (table 2). The flavonoid, kaempferol are the three most active compounds having antibacterial activity and effective against *P. acnes*⁷.

Table-1

Antimicrobial screening of *Cassia fistula* flowers extract against *S. Epidermidis* Using disc diffusion method, zone of inhibition in mm

	<i>Staphylococcus epidermidis</i>
<i>Cassia fistula</i> flowers extract	10.01±0.04
Clindamycin phosphate	20.11±1.02

Table-2

Minimum inhibitory concentration of *Cassia fistula* flowers extract and Clindamycin

Methanolic Extract	<i>Staphylococcus epidermidis</i> MIC (mg/ml)
<i>Cassia fistula</i> flowers extract	0.42
Clindamycin phosphate	0.18

Conclusion

The results clearly indicates that the methanolic extracts of *Cassia fistula* flowers possessing broad-spectrum activity could

be utilized in treating acne vulgaris and formulating anti-acne herbal products.

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