ANTIMICROBIAL ACTIVITY OF METHANOL SOLVENT EXTRACTS OF CASSIA FISTULA

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ABSTRACT

*Cassia fistula* Linn. (*Caesalpiniaceae*) is a very common plant and is widely known for its medicinal properties. Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various illnesses. It has attracted increasing public attention over the past 20 years as this type of medicine is easily accessible in some regions. Methanol extracts of various plant parts were screened *in vitro* for their antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, *Micrococcus luteus*, *Bacillus megaterium*, and *Pseudomonas aeruginosa* were studied. Maximum activity was observed in *B. megaterium* followed by *S. typhi*, *M. luteus*. The lower activity was observed in *P.aeruginosa* and *E.coli*.


INTRODUCTION

*Cassia fistula* Linn. (*Caesalpiniaceae*) is a very common plant and is widely known for its medicinal properties. 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 rheumatism, hematemesis, pruritus, leucoderma, and diabetes (Prashanth *et al*., 2006; Ballabh and Chaurasia, 2007). Besides, it has been found to exhibit anti-inflammatory and hypoglycemic activity and widely used as a mild laxative suitable for children and pregnant women (Humber *et al*., 2002). Several reports are present on hepato protective, (Agarwal *et al*., 2005). Antifertility (Danish *et al*. 2011) and antioxidant properties of *C. fistula*. (Gupta *et al*., 2010). It is widely used for its medicinal properties, its main property being that of a mild laxative suitable for children and pregnant women. It is also a purgative due to the wax aloin
and a tonic. (Raja et al., 2000). It has been reported to treat many other intestinal disorders like healing ulcers (Duraipandiyan and Ignacimuthu, 2007; Morimoto et al., 1988). The plant has a high therapeutic value and it exerts an antipyretic and analgesic effect (Prashanth et al., 2006). The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments (Ballabh and Chaurasia, 2007). Traditional and alternative medicine is extensively practiced in the prevention, diagnosis, and treatment of various illnesses. It has attracted increasing public attention over the past 20 years as this type of medicine is easily accessible in some regions (Humber et al., 2002).

In the recent years, researches on medicinal plants have attracted a lot of attention globally. Evidences have been accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary, and alternative systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides etc. which have been found in vitro to have antimicrobial properties (Dahanukar et al., 1986; Cowan et al., 1999). Herbal medicines have been known to man since centuries. Therapeutic efficacy of many indigenous plants for several disorders have been described by practitioners of traditional medicine (Ramasamy et al., 2009). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. Traditional medicine continues to be a valuable source of remedies that have been used by millions of people around the world to secure their health (Shaik et al., 1994). The pharmaceutical industries have produced a number of new antibiotics, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs that are utilized as therapeutic agents. (Towers et al., 2001) Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics and to continue studies to develop new drugs, either synthetic or natural to control pathogenic microorganism. (Asolkar et al., 1992; Jaipal et al., 1983). In an effort to expand the spectrum of antibacterial agents from natural resources, C. fistula belongs to family Caesalpiniaceae, has been attempted in this study.

**MATERIALS AND METHODS**

**Plant sample collection**

Fresh plant parts of C. fistula including fruit pulp, leaves and seed were collected randomly from the Bapalal Garden of Bioscience Department at Veer Narmad South Gujarat University, Surat, Gujarat, India. The taxonomic identity of this plant was confirmed by Prof. M. H. Parabia, Department of Biosciences, South Gujarat University, Surat (Gujarat). Fresh
plant material including leaves, fruit pulp and seed were collected and washed under running tap water. From pods seed were removed and then all materials dried in oven at 30°C and seeds were dried in oven at 50°C.

**Preparation of plant powder**

After air drying 100gm of each leaves, fruit pulp and seed of *C. fistula* were taken homogenized in grinder, thus 88gm, 85gm, 84gm fine powder are obtained respectively.

**Preparation of crude plant extract**

The 10gm of fine powder of leaves, fruit pulp, and seed of *C. fistula* were extracted in 250ml methanol using soxhlate apparatus at 70°C for four cycles with yield 6.48%, 11.39%, 6.24% respectively. Solvent was evaporated in oven to make a final concentration 40mg/ml. this stock solution were stored at 4°C in air tight bottles for further studies.

**Organisms**

Five isolates of *Escherichia coli*, *Salmonella typhi*, *Micrococcus luteus*, *Bacillus megaterium*, and *Pseudomonas aeruginosa* were studied; they were obtained from the Department of Biotechnology, Veer Narmad South Gujarat University, Surat (Gujarat). These isolates were maintained on Sabouraud’s dextrose agar SDA (HIMEDIA Laboratories, Mumbai-India) at 4°C.

**Determination of zone of inhibition**

*In vitro* antibacterial activity were examined for methanol extracts. Antibacterial activities of plant extracts against five pathogenic bacteria (three gram positive and two gram negative) were investigated by the agar cup diffusion method Barry, (1980). Antimicrobial activity testing was carried out by using the Agar cup method. Each purified extracts were dissolved in DMSO, sterilized by filtration using sintered glass filter and stored at 4°C. For the determination of zone of inhibition (ZOI), three gram positive and two gram negative were taken as a standard antibiotic for comparison of the results. All the extracts were screened for their antibacterial activities against the *E. coli*, *S. typhi*, *M. luteus*, *B. megaterium*, and *P. aeruginosa*. The sets of five dilutions (5, 25, 50, 100, and 250μg/mL) of *C. fistula* extract and standard drugs were prepared in double distilled water using nutrient agar tubes. Muller Hinton sterile agar plates were seeded with indicator bacterial strains (10⁸ CFU) and allowed to stay at 37°C for 3 hours. The zones of growth inhibition around the disks were measured after 18 to 24 hours of in incubation at 37°C. The sensitivity of the microorganism species to
the plant extracts were determined by measuring the sizes of inhibitory zones (including the
diameter of cup) on the agar surface around the cup, and values <8 mm were considered as
not active against microorganisms.

**Preparation of media**
The many media available, Mueller -hintone agar is considered to be the best for routine
susceptibility testing of bacteria. Components and their amount are shown in Table 1.

**Table 1: Muller-Hinton(PH 7.4)**

<table>
<thead>
<tr>
<th>Components</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef extract</td>
<td>30.0g</td>
</tr>
<tr>
<td>Casein</td>
<td>17.5g</td>
</tr>
<tr>
<td>Starch</td>
<td>1.5g</td>
</tr>
<tr>
<td>Agar</td>
<td>17.0g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000.0 ml</td>
</tr>
</tbody>
</table>

**Preparation of Mueller-Hinton agar as following steps:**
1. Mueller-hinton agar medium was prepared as
2. Autoclave Mueller-hinton agar medium at 15psi for 20 min
3. Immediately after autoclaving, allow it to cool at 45-50°C temperature.
4. Pour medium into sterile glass petri dishes and allow it to solidify at room temperature.

**Turbidity standard for inoculums preparation**
To standardize the inoculums density for a susceptibility test a 0.5 McFarland standard were
prepared. Density 0.5 McFarland is equal to 10⁸ cells/ml of bacteria. Thus, the activated
microbial cultures were compared with 0.5 McFarland standards to get idea about load of
microorganism because antimicrobial activity has to be performed at 10⁸ cells/ml of bacterial
load. If the density of activated microbial cultures is higher than the 0.5 McFarland standards
then it has to be diluted to get equivalent density of 0.5 McFarland standard.

**Procedure for performing the well diffusion method**
**Inoculums preparation**
Two to three well- isolated colonies of the same morphological type of each test
microorganism were selected from an agar plate culture and transferred into a tube containing
10 ml of nutrient- broth medium separately. The broth culture was incubated at 35°C until it
achieved the turbidity of the 0.5 McFarland standards (usually 5 to 6 hours). The turbidity of
the actively growing broth culture were adjusted with sterile saline or broth to obtain turbidity
optically comparable to that of the 0.5 McFarland standards. This resulted in a suspension containing approximately $1 \times 10^8$ CFU/ml. This step was performed in the presence of adequate light to compare the inoculum tubes and the 0.5 McFarland standard.

Antimicrobial activity was carried out by Agar well diffusion method. Fresh bacterial cultures of 0.1ml having $10^8$ CFU were spread onto MHA plate using sterile cotton swab. The wells were punched off into MHA medium with sterile well borer. Each well filled with 100μl of each plant extracts (leaf, fruit pulp and seed) by using micro pipette in aseptic condition. Repeat the all step for each organism. The plates were then kept in a refrigerator to allow pre-diffusion of extract for 30 minutes. Then further incubated in an incubator at 37°C for 24 hours. The antibacterial activity was evaluated by measuring the zone of inhibition.

RESULTS AND DISCUSSIONS

The zones of inhibition (ZOI) of extract in methanol 40 mg/ml concentrations against the test organisms are shown Table 2. Provides the measured ZOI of the extract of various parts (leaves, fruit pulp and seed) of C. fistula with methanol against five bacteria.

Table 2: Result of determination of zone of inhibition bacteria

<table>
<thead>
<tr>
<th>Sr no</th>
<th>Extract</th>
<th>Concentration 40 mg/mL</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100μl</td>
<td>EC</td>
</tr>
<tr>
<td>1</td>
<td>Leaves</td>
<td>100μl</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>Fruit pulp</td>
<td>100μl</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>Seeds</td>
<td>100μl</td>
<td>NI</td>
</tr>
<tr>
<td>4</td>
<td>Tetracycline</td>
<td>50μl</td>
<td>46</td>
</tr>
</tbody>
</table>


![Fig. 1: Antimicrobial activity of C. fistula leaves in methanol extract](image)
Fig. 2: Antimicrobial activity of *C. fistula* fruit pulp in methanol extract

![Graph showing Antimicrobial Activity](image)

Fig. 3: Antimicrobial activity of *C. fistula* seed in methanol extract

![Graph showing Antimicrobial Activity](image)

Fig. 4: A: ZOI of *E. coli*; B: ZOI of *S. typhi*; C: ZOI of *P. aeruginosa*; D: ZOI of *M. luteus*
Methanolic extracts of *C. fistula* exhibited antibacterial activity against all the tested bacterial strain. Results were compared with *tetracycline* as a standard antibiotic. However, the activity was significantly lower than that of *tetracyclin*. The well diffusion assay result of *C. fistula* (seed, leaves, fruit pulp) extract with the inhibition zones formed by well diffusion, showed in Table 2. Maximum activity was observed in *B. megaterium* (24mm ± 1mm) followed by *Salmonella typhi* (22mm ± 1mm), *Micrococcus luteus* (20mm ± 1mm). The lower activity was observed in *P.aeruginosa* (12 mm ± 1mm) and *E.coli* (14 ± 1mm). In contrast, the zone of inhibition of methanol (negative control) was zero so that there were no effect of methanol against all of the tested microorganisms, however the antibiotic 40 mg/ml of *tetracyclin* (positive control) were more effective than the fruit pulp extract of *C. fistula* with the diameter of zone inhibition ranging between 24mm ± 1mm. Methanol extract of *cassia fistula* was very less effective against gram negative bacteria.

**CONCLUSION**

It is concluded based on the findings of the present study that the aerial parts of *C. fistula* shows higher antibacterial activity against bacterial such as *E. coli, S. typhi, M. luteus, B. megaterium*, and *P. aeruginosa*. The extract of *C. fistula* showed maximum zone of inhibition at the concentration of 40mg/ml for antibacterial activity against bacterial pathogens. Methanol extracts of *C. fistula* exhibited antibacterial activity against all the tested bacterial strain. Results were compared with *tetracycline* as a standard antibiotic. However, the activity was significantly lower than that of *tetracycin*. Maximum activity was observed in *B. megaterium* followed by *S. typhi, M. luteus*. The lower activity was observed in *P.aeruginosa* and *E.coli*.

**REFERENCES**