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Research Article

Antidiarrheal and Antibacterial Actions of Methanol Extract of *Cassia fistula* (Linn) Leaves

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Abstract

Background and Objective: *Cassia fistula* (Linn) is a member of Leguminosae family which is widely known for its medicinal properties. The methanol extract of *Cassia fistula* (Linn) leaves was investigated for antidiarrheal and *in vitro* antibacterial potentials. The study revealed that *Cassia fistula* exhibited significant efficacy against diarrhoea and microorganisms. **Materials and Methods:** The antidiarrheal activity of the extract was evaluated using castor oil and the sulphate-induced diarrhoea method in the albino rats models at the dose of 50, 100, 250, 500, 1000 and 2000 mg kg⁻¹ b.wt., orally, while the antibacterial test was carried out using the agar well method against standard bacteria: *Escherichia coli* (Gram -ve), *Staphylococcus aureus*, (Gram +ve), *Acinetobacter baumannii* (Gram -ve), *Exiguobacterium aquaticum* (Gram+ve) and *Klebsiella pneumoniae* (Gram +ve). **Results:** The result of antidiarrheal activity showed that methanol extract of *Cassia fistula* (Linn) leaves significantly ($p < 0.001$) decreased the frequency of defecation and wet stools in a dose-dependent manner after receiving magnesium sulphate (2 g kg⁻¹ b.wt.) and castor oil (0.4 mL kg⁻¹ b.wt.). The extract also showed significant growth inhibition on the selected bacteria in a concentration-dependent manner, with the lowest inhibition: 7.32 ± 0.04 , 6.26 ± 0.14 , 5.47 ± 0.13 , 8.45 ± 0.14 and 7.50 ± 0.22 mm for the bacteria, respectively observed at 50 $\mu\text{g mL}^{-1}$, while highest inhibition: 12.59 ± 0.18 , 11.86 ± 0.08 , 10.59 ± 0.12 , 15.58 ± 0.09 , 15.60 ± 0.10 mm, respectively was observed at 1000 $\mu\text{g mL}^{-1}$ compared to that of the control (chloramphenicol) between 18.00 ± 1.11 - 21.10 ± 0.22 mm. In all cases, the effects elicited by the extract are comparable to those of loperamide and chloramphenicol, the respective standard antidiarrheal and antibacterial drugs. These findings, therefore, justify the use of *Cassia fistula* (Linn) leaves as an effective antidiarrheal and antibacterial agent.

Key words: *Cassia fistula* (Linn), methanolic extract, antidiarrheal, antibacterial, *in vitro*

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The search for new drugs globally has become essential due to so many health challenges bobbing up from resistant species of diseases causative agents and discovery of different species and trends of microorganism (bacteria and viruses). These have therefore enticed the attention of researchers into sourcing for plants with bioactive compounds that can remedy health challenges. Thus, plants are considered as the key resources with which the well-being and health of humans can be actualized¹.

These various plants on which human and animal life depend for food contain thousands of phytochemicals and allelochemicals which constitute anti-nutritional or beneficial factors to the consumer. It was therefore observed from the outcome of research works that the medicinal value of these plants/medicinal plants lies in some chemical substances that produce a definite physiological action in the human body².

Many of the world's population are versed in the use of available plants and herbs in their environment in the treatment of different diseases. The use of medicinal plants in world traditional medicine has been in practice for a long time and the practice is now becoming increasingly popular, especially as an alternative or as a complement to modern medicines³.

The use of traditional medicine at the primary health care level is widespread and plant-based therapies are being endorsed by trade-medical practitioners worldwide for combating sicknesses and diseases. The phytochemicals present in the plant parts, fruits and vegetables are gaining attention day-by-day for their active role in the treatment of various human diseases. More than 400 plant species have been reported to have hypoglycaemic activity⁴. However, the search for new antidiarrheal and antibacterial drugs from natural plants is still attractive.

Cassia fistula Linn (Fabaceae) is an evergreen plant. It is a wild native species of plant from Africa and a small medium-sized tree (of about 4-10 m high) with white bark. It has medium leaves and long brown fruits about 30-50 cm. The plant has been used in traditional folk medicine for the treatment of piles and headaches and to increase appetite. As a member of the Leguminosae family, it is widely used for its diverse medicinal properties, its main property being that of a mild laxative suitable for children and pregnant women and was reported to contain a substantial number of phytochemicals⁵. *Cassia fistula* has been alleged of medicinal properties such as antitumor, antifungal, antimicrobial, antiviral, laxative, antioxidant, anti-

inflammatory, wound healing, antipyretics, antidiabetic, antiparasitic, hepatoprotective and antifertility activity⁶. Secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides were reported to present in the ethanol, methanol and aqueous extracts of the plant⁷. The present study was carried out to evaluate the antidiarrheal and *in vitro* antibacterial activities of methanol extract of *Cassia fistula* (Linn) leaves.

MATERIALS AND METHODS

Collection, identification and extraction of plant material:

The whole plant of *Cassia fistula* (Linn) was collected in June, 2020 from the local area of Michika and was identified by a botanist at the Department of Botany, University of Mubi. The plant was washed with running water and the leaves were separated and dried in a shady place for 3 weeks. The leaves after drying were ground into a coarse powder with the grinding machine. The ground materials (powder) were kept in airtight bottles until further use. After that, 1000 g of the ground leaves were subjected to maceration for the process of extraction using methanol as a solvent. The powdered sample was soaked for 14 days with constant stirring and then filtered with a filter paper. The extract was concentrated in a rotary evaporator and the dried extract was then refrigerated at 5°C until usage.

Drugs, chemicals and instruments: Magnesium sulphate and loperamide were purchased from Sigma Aldrich, Steinheim, Germany. Other chemicals and equipment used in this study were gotten from standard suppliers.

Test microorganism: *Escherichia coli* (Gram -ve), *Staphylococcus aureus*, (Gram +ve), *Acinetobacter baumannii* (Gram -ve), *Exiguobacterium aquaticum* (Gram+ve) and *Klebsiella Pneumoniae* (Gram +ve) were used for antibacterial studies.

Test animals: Male and female albino rats having a normal weight range from 150-250 g were procured from the National Institute VOM-Jos Plateau and employed in this study. The animals were kept in plastic cages in the animal house under standard environmental conditions (23-25°C). The night before the experiment, the animals were fasted overnight and treated according to the rules and protocols, Scientific Procedures Issue I involving the use of animals as sanctioned by official bodies (Ethical Committee) of the University.

Acute toxicity study: Organization for Economic Co-operation and Development (OECD) guideline 423 was used for determining the acute toxicity study with some modifications⁸. For instance, the different doses of the extracts (50, 100, 250, 500, 1000 and 2000 mg kg⁻¹ b.wt., were employed for the animals. The animals were distributed into seven groups consisting of five animals each. Group I was the control, groups II, III, IV, V, VI and VII received a single dose of methanol extract of *Cassia fistula* (Linn) 50, 100, 250, 500, 1000 and 2000 mg kg⁻¹ b.wt., respectively by oral route. The animals were then examined for 4 hours, after 3 days within the first 4 hrs and then two times daily for 14 days for probable toxic effects mortality. Behavioural changes and other parameters such as body weight, urination, water intake, respiration, food intake, tremors, convulsions, temperature, constipation and changes to the skin and eye colours were also monitored.

Dose selection: Six different doses of methanol extract of *Cassia fistula* were selected according to 1/10th of the maximum toxic dose and were diluted in normal saline.

Experimental design

Castor oil-induced diarrhoea: The antidiarrheal activity was performed on the albino rats using castor oil-induced diarrhoea with some modifications in the protocols⁹. Animals were randomly assigned into seven groups of five animals each. Group I was the control and received normal saline, while group II was the standard that received loperamide at a dose rate of 5 mg kg⁻¹ b.wt. Groups III, IV, V, VI, VII and VIII were the tested groups that received the methanol extract at a dose rate of 50, 100, 250, 500, 1000 and 2000 mg kg⁻¹ b.wt., respectively. After the treatment, each animal was put in separate cages laid with papers for collecting the faecal mass. Diarrhoea was induced by oral administration of castor oil (0.4 mL kg⁻¹ b. wt.). The methanol extract and loperamide were given 1h before the oral administration of the castor oil, the time is taken for the first faecal discharge and the total number of faecal outputs within 6 hrs of administration was recorded. The inhibition of defecation was calculated using the following Eq.:

$$\text{Inhibition (\%)} = \frac{M_o - M}{M_o} \times 100 \quad (1)$$

Where:

M_o = Mean defecation of control

M = Mean defecation of the experimental group

The employed standard drug, loperamide, retards the motility of the intestine and revamps the movement of water and electrolyte in the bowel. It is an effective agonist of opiate receptor that impedes the secretion of prostaglandins and acetylcholine in the wall of the gut, which consequently leads to a reduction in peristalsis and increase of transit time of the intestinal. The drug has also been reported to increase the activity of the anal sphincter¹⁰. It thus antagonizes the diarrheal activity induced with castor oil.

Magnesium sulphate-induced diarrhoea: The effect of *Cassia fistula* on magnesium sulphate-induced diarrhoea was also determined on the albino rats¹¹. After overnight fasting, the animals were distributed into seven groups of 5 animals each. Group I, the control, received only normal saline. Group II, the standard, received loperamide at a dose of 20 mg kg⁻¹ b.wt., while groups III to VIII, which were the test groups, received a methanol extract of *Cassia fistula* at a dose of (50, 100, 250, 500, 1000 and 2000 mg kg⁻¹ b.wt.). Sixty minutes after-treatment of the respective drug, all the animal groups were treated orally with magnesium sulphate at a dose of 2 g kg⁻¹ b.wt. The frequency of defecation and the faecal material was again noted for up to 4 hrs. The rats were in transparent (clear) cages with pre-weighed plastic dishes for faeces collection at the bottom of all cages. The plastic dishes for tested rats and control were weighed and recorded before and after defecation and then compared.

Test microorganisms and growth conditions

Five standard bacterial strains: *Escherichia coli* (Gram -ve), *Staphylococcus aureus*, (Gram +ve), *Acinetobacter baumannii* (Gram -ve), *Exiguobacterium aquaticum* (Gram +ve) and *Klebsiella pneumoniae* (Gram +ve) were used for antibacterial studies. The agar nutrient medium was used for the growth of bacterial strains and was allowed to stand for a period of 24 hrs at 37°C. Nutrient agar was poured into a conical flask containing distilled water in the right proportion and mixed properly. The aqueous solution was made by adding 20 mg of agar nutrient in 1000 mL of distilled water with constant shaking for 6 min. The solution was then sterilized in an autoclave and transferred to Petri dishes for the inoculation of bacterial strains.

Well diffusion method: The antibacterial activity of *Cassia fistula* was determined individually by the agar well diffusion method¹². Twenty millilitres of molten nutrient agar was poured into each of the Petri dishes and allowed to solidify. The 0.5 McFarland standardized bacterial broth, was

spread on the dry nutrient agar with the aid of a spreader pre-sterilized in ethanol and flame overnight. With the aid of a sterile cork-borer, eight holes of 6 mm depth each and about 5 cm apart, were made in the nutrient agar. Six of the wells were filled with 200 μ L of the *Cassia fistula* plant extract dissolved in sterile distilled water, one well with the water only (the negative control) and the last with 1% standard antibiotic, chloramphenicol.

The positive control was dispensed into the wells in triplicates. After incubating for 24 at 37°C, the antibacterial activities were determined by measuring the diameter of the inhibition zone. The zones of inhibition observed with the extract were compared with that of the standard antibiotic, chloramphenicol. The experiment was carried out in three sets. The measured chloramphenicol inhibition zones' diameters were subsequently matched with the respective standard zones' diameters for *Escherichia coli* (Gram -ve), *Staphylococcus aureus*, (Gram +ve), *Acinetobacter baumannii* (Gram -ve), *Exiguobacterium aquaticum* (Gram +ve) and *Klebsiella Pneumoniae* (Gram +ve). The *Cassia fistula* zone of inhibition from 9-14 mm in diameter was taken as a positive antibacterial activity based on the growth inhibition standard as reported by Mothana *et al.*¹³ and Karima *et al.*¹⁴.

RESULTS

Effect of *Cassia fistula* extracts on behavioural and general appearances of rats: The effect of *Cassia fistula* extract on behavioural and general appearances is displayed in Table 1. The result showed that no mortality or toxic effects were observed after the administration of *Cassia fistula* extract at 50, 100, 250, 500, 1000 and 2000 mg kg⁻¹ b.wt., to the albino rats, indicating that the extract is safe at the administered dose range.

Effect of *Cassia fistula* extracts on castor oil-induced diarrhoea: The antidiarrheal activities of the methanol extract of *Cassia fistula* and standard drug in castor oil-induced diarrhoea are given in Table 2. A significant ($p < 0.001$) decrease in the mean number of defecations was noted in the methanol extract and loperamide-receiving groups when compared to the control. The attenuation in the mean number of defecations with *Cassia fistula* at a dose rate of 50 mg kg⁻¹ b.wt., was 7.56 ± 1.68 mg kg⁻¹. Similarly, the attenuation in the mean number of defecations in the animal group that received a *Cassia fistula* extract at the dose rate of 1000 and 2000 mg kg⁻¹ b.wt., were 5.98 ± 1.21 and 4.78 ± 1.47 ,

respectively which seemed to be more significant ($p < 0.01$) as a match to the control group. However, with 5 mg kg⁻¹ b.wt., the dose of loperamide, the recorded mean of defecations was more significant ($p < 0.001$) at 3.78 ± 0.67 . The results revealed that the percentage of defecations attenuation by the extracts at both lowest and highest doses were 15.91% and 46.83% respectively. Additionally, the percentage of attenuation in the mean number of defecations with loperamide was 57.95%. The latent period for *Cassia fistula* increased significantly ($p < 0.001$) when compared with the control.

Effect of *Cassia fistula* extracts on magnesium sulphate-induced diarrhoea: The antidiarrheal activity of the *Cassia fistula* Linn extract was also evaluated with the magnesium sulphate-induced diarrhoea method. The results presented in Table 3 show that the groups that received *Cassia fistula* Linn extract at the dose rates of 50, 100, 200, 250, 500, 1000 and 2000 mg kg⁻¹ b.wt., exhibited a significant ($p < 0.01$) as well as a dose-dependent reduction in the total number of both stools and wet stools when compared to the control. The mean number of defecations with the *Cassia fistula* Linn extract at a dose rate of 50 mg kg⁻¹ b.wt., which is the lowest was 8.17 ± 1.34 , while the mean numbers of defecations at 1000 and 2000 mg kg⁻¹ b.wt., were 5.36 ± 1.21 and 5.18 ± 1.47 which were significant ($p < 0.001$) compared to the control. The mean number of defecations produced by the standard drug was 4.78 ± 0.67 . The percentage of inhibition of defecations with the methanol extract at doses 50, 1000 and 2000 mg kg⁻¹ b.wt., were 25.66%, 51.23% and 52.87%, respectively, while that of the loperamide standard, at dose 5 mg kg⁻¹ b.wt., was 56.51%.

Antibacterial activity of *Cassia fistula* extracts: The antimicrobial activity *Cassia fistula* Linn leaves extract was determined as a zone of inhibition against *Escherichia coli* (Gram -ve), *Staphylococcus aureus*, (Gram +ve), *Acinetobacter baumannii* (Gram -ve), *Exiguobacterium aquaticum* (Gram +ve) and *Klebsiella pneumoniae* (Gram +ve) growth. This was compared with chloramphenicol as a positive standard in which *Escherichia coli* (Gram -ve), *Staphylococcus aureus*, (Gram +ve), *Acinetobacter baumannii* (Gram -ve), *Exiguobacterium aquaticum* (Gram +ve) and *Klebsiella pneumoniae* (Gram +ve) were susceptible to chloramphenicol with zones of inhibition (mm) that were 12.59 ± 0.18 , 11.86 ± 0.08 , 11.59 ± 0.12 , 16.58 ± 0.09 and 15.60 ± 0.10 , respectively in Table 4. The result revealed that

Table 1: Effects of *Cassia fistula* extracts on behavioural and general appearances of albino rats

Observations	Effects of <i>Cassia fistula</i> extracts mg kg ⁻¹ b.wt.						
	Control	50	100	250	500	1000	2000
Digestion	N/O	N/O	N/O	N/O	N/O	N/O	N/O
Body weight	N	N	N	N	N	N/C	N/C
Itching	N	N	N	N	N	O	O
Food intake	N	N	N	N	N	N	N
Skin	N/E	N/E	N/E	N/E	N/E	N/E	N/E
Laziness	N/P	N/P	N/P	N/P	N/P	P	P
Sedation	N/E	N/E	N/E	N/E	N/E	O	O
Diarrhea	N/P	N/P	N/P	N/P	N/P	N/P	N/P
G. physique	N	N	N	N	N	Lethargy	Lethargy
Coma	N/P	N/P	N/P	N/P	N/P	N/P	N/P
Eye colour	N/E	N/E	N/E	N/E	N/E	N/E	N/E
Death	N/O	N/O	N/O	N/O	N/O	N/O	N/O

N/O: Not observed, O: Observed, N/P: Not present, N/E: No effect, N: Normal, N.C: No change and P: Present, b.wt.: Body weight and G.P: General physique

Table 2: Effect of *Cassia fistula* Linn extract on castor oil-induced diarrhoea

Groups	Dose (mg kg ⁻¹ b.wt.)	Mean ± SE		
		Latency	Defecation	Percentage of inhibition
Normal control	10 mL kg ⁻¹	0.48 ± 0.32	8.99 ± 0.87	-
Standard (Loperamide)	5	3.67 ± 0.76	3.78 ± 0.67***	57.95
<i>Cassia fistula</i> Linn extract	50	0.84 ± 0.57	7.56 ± 1.68	15.91
<i>Cassia fistula</i> Linn extract	100	1.25 ± 0.34	7.12 ± 0.98*	20.80
<i>Cassia fistula</i> Linn extract	200	1.69 ± 0.56	6.98 ± 1.33*	22.36
<i>Cassia fistula</i> Linn extract	250	1.89 ± 0.36	6.73 ± 1.27**	25.14
<i>Cassia fistula</i> Linn extract	500	2.11 ± 1.10	6.45 ± 0.88**	28.25
<i>Cassia fistula</i> Linn extract	1000	3.23 ± 0.98	5.98 ± 1.21**	33.48
<i>Cassia fistula</i> Linn extract	2000	4.38 ± 0.66	4.78 ± 1.47***	46.83

Data are represented as Mean ± SEM, N: 05, * p<0.05, ** p<0.01 and *** p<0.001, significant when compared with control, the statistical test employed was a one-way ANOVA test followed by Tukey's multiple comparisons

Table 3: Effect of methanol extract of *Cassia fistula* Linn on magnesium sulphate-induced diarrhoea

Groups	Dose (mg kg ⁻¹ b.wt.)	Mean ± SEM		
		Latency	Defecation	Percentage of inhibition
Normal control	10 mL kg ⁻¹	0.88 ± 0.12	10.99 ± 0.87***	-
Standard (Loperamide)	5	3.55 ± 0.54	4.78 ± 0.67	56.51
<i>Cassia fistula</i> Linn extract	50	0.97 ± 0.66	8.17 ± 1.34*	25.66
<i>Cassia fistula</i> Linn extract	100	1.65 ± 0.34	7.22 ± 0.67*	34.30
<i>Cassia fistula</i> Linn extract	200	1.98 ± 0.56	6.78 ± 1.33*	38.31
<i>Cassia fistula</i> Linn extract	250	2.09 ± 0.36	6.45 ± 1.27**	41.31
<i>Cassia fistula</i> Linn extract	500	2.41 ± 1.10	6.23 ± 0.88**	43.31
<i>Cassia fistula</i> Linn extract	1000	3.63 ± 0.98	5.36 ± 1.21***	51.23
<i>Cassia fistula</i> Linn extract	2000	4.18 ± 0.66	5.18 ± 1.47***	52.87

Data are expressed as Mean ± SEM, N: 05, * p<0.05, ** p<0.01 and *** p<0.001, significant as matched to the control, the statistical analyses were carried out using a one-way ANOVA test and Turkey's multiple comparison test

Table 4: Effect of methanol leaves extract of *Cassia fistula* Linn on bacteria

Extract (µg mL ⁻¹)	<i>Escherichia coli</i> (Gram -ve) inhibition	<i>Staphylococcus aureus</i> (Gram +ve) inhibition	<i>Acinetobacter baumannii</i> (Gram -ve) inhibition	<i>Exiguobacterium aquaticum</i> (Gram +ve) inhibition	<i>Klebsiella pneumoniae</i> (Gram +ve) inhibition
	zone (mm)	zone (mm)	zone (mm)	zone (mm)	zone (mm)
Chloramphenicol	18.00 ± 1.11	19.00 ± 1.05	19.52 ± 0.03	21.10 ± 0.22	20.49 ± 0.23
25	7.32 ± 0.04*	6.26 ± 0.14*	5.47 ± 0.13*	8.45 ± 0.14*	7.50 ± 0.22*
50	8.33 ± 0.34*	9.43 ± 1.13*	7.47 ± 0.18*	9.37 ± 0.05*	10.49 ± 0.12*
100	10.43 ± 0.07*	9.47 ± 0.17*	8.44 ± 0.16*	11.53 ± 0.21*	11.57 ± 0.16*
250	11.49 ± 0.19**	10.53 ± 0.15**	8.50 ± 0.17*	12.49 ± 0.19*	13.50 ± 0.12*
500	11.57 ± 0.13**	11.55 ± 0.14**	9.55 ± 0.10**	14.57 ± 0.15**	14.58 ± 0.06#
1000	12.59 ± 0.18	11.86 ± 0.08#	11.59 ± 0.12#	16.58 ± 0.09	15.60 ± 0.10

Result is Mean ± SD, N: 3, * and #Significant activity when compared to the standard (p<0.05) and concentration of standard is 30 µg mL⁻¹ of chloramphenicol

1. Effect on behaviour and general appearance
2. *Cassia fistula* on castor oil-induced
3. Effect of methanol extract of *Cassia fistula* Linn on magnesium sulphate-induced
4. Effect of methanol leaves extract of *Cassia fistula* Linn on bacteria

Methanol crude extract

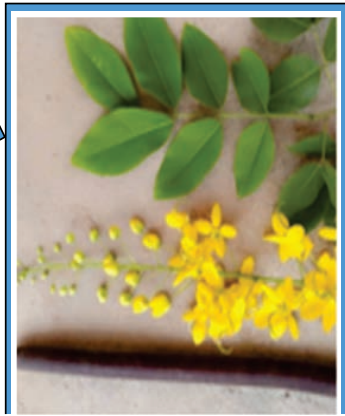


Table 1: Effects of *Cassia fistula* extracts on behavioural and general appearances of albino rats

Observation	Effects of <i>Cassia fistula</i> extracts mg kg ⁻¹ b.wt.									
	Control	50	100	250	500	1000	2000			
Digestion	N/O	N/O	N/O	N/O	N/O	N/O	N/O			
Body weight	N	N	N	N	N	N	N			
Itching	N	N	N	N	N	N	N			
Food intake	N	N	N	N	N	N	N			
Skin	N/E	N/E	N/E	N/E	N/E	N/E	N/E			
Laziness	N/P	N/P	N/P	N/P	N/P	N/P	N/P			
Sedation	N/E	N/E	N/E	N/E	N/E	N/E	N/E			
Diarrhoea	N/P	N/P	N/P	N/P	N/P	N/P	N/P			
G. physique	N	N	N	N	N	N	N			
Coma	N/P	N/P	N/P	N/P	N/P	N/P	N/P			
Eye colour	N/E	N/E	N/E	N/E	N/E	N/E	N/E			
Death	N/O	N/O	N/O	N/O	N/O	N/O	N/O			

Effects of *Cassia fistula* extracts on behavioural and general appearances of albino rats

Groups	Dose (mg kg ⁻¹ b.wt.)	Mean±SE		Percentage of inhibition
		Latency	Defecation	
Normal control	10 mL kg ⁻¹	0.48±0.32	8.99±0.87	-
Standard (Loperamide)	5	3.67±0.76	3.78±0.67***	57.95
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Effect of *Cassia fistula* Linn extract on castor oil-induced diarrhoea

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Effect of methanol extract of *Cassia fistula* Linn on magnesium sulphate-induced diarrhoea

Extract (µg mL ⁻¹)	Chloramphenicol	Escherichia coli (Gram -ve) inhibition zone (mm)	Staphylococcus aureus (Gram +ve) inhibition zone (mm)	Acinetobacter baumannii (Gram -ve) inhibition zone (mm)	Exiguobacterium ignis (Gram +ve) inhibition zone (mm)	Klebsiella pneumoniae (Gram +ve) inhibition zone (mm)
25	18.00±1.11	19.00±1.05	19.52±0.03	21.10±0.22	20.49±0.23	7.50±0.22*
50	7.32±0.04*	6.26±0.14*	5.47±0.13*	8.45±0.14*	9.37±0.05*	10.49±0.12*
100	8.33±0.34*	9.43±1.13*	7.47±0.18*	11.53±0.21*	11.57±0.16*	11.57±0.16*
250	10.43±0.07*	9.47±0.17*	8.44±0.16*	12.49±0.19*	14.57±0.15**	13.50±0.12*
500	11.49±0.19**	10.53±0.15**	8.50±0.17*	14.57±0.15**	14.58±0.06*	14.58±0.06*
1000	11.57±0.13**	11.55±0.14**	9.55±0.10**	16.58±0.09	15.60±0.10	15.60±0.10

Effect of methanol leaves extract of *Cassia fistula* Linn on bacteria

Fig. 1: Antidiarrheal and *in vitro* antibacterial actions of methanol extract of *Cassia fistula* (Linn) leaves in view

the methanol extract has a significant antibacterial potential against all bacterial strains and the zones of inhibition (mm) were observed at $1000 \mu\text{g mL}^{-1}$. In all bacterial species, the range of 9.55 ± 0.10 - 15.60 ± 0.10 mm is considered susceptible.

The summary of the antidiarrheal and antibacterial actions of methanol extract of *Cassia fistula* (Linn) leaves as well as the effects of *Cassia fistula* extracts on behavioural and general appearances of albino rats is as shown in Fig. 1.

DISCUSSION

Table 1 presented the effect of methanol extract of *Cassia fistula* (Linn) leaves on behavioural and general appearances of the albino rats showed no mortality or toxic effects upon administration of *Cassia fistula* extract at (50, 100, 250, 500, 1000 and 2000 mg kg^{-1} b.wt.). Consequently, the extract is said to be safe at the administered dose range.

Herein, we evaluated the antidiarrheal activity and *in vitro* antibacterial potentials of methanol extract of *Cassia fistula* (Linn) leaves using recognized and validated criteria and measurement scales as summarized in Fig. 1. The results revealed dose-dependent antidiarrheal effects in the castor oil (Table 2) and the magnesium sulphate induced diarrhoea methods (Table 3) and this effect was comparable to that of loperamide which is normally used as antidiarrheal medication. The result of the present study aligns with that of Rahman *et al.*¹⁵, who demonstrated that methanol extract of *Cassia fistula* Linn shows a significant inhibition on the accumulation of intestinal fluids and contents with 57.67% diarrhoea inhibition. It also validates the ethnobotanical survey of One hundred and eighty-two plants carried out by Tetali *et al.*¹⁶ at Parinche valley, Pune district, Maharashtra, India in which *Cassia fistula* was among the plants documented to be efficacious against diarrhoea. It further agrees with the survey of Gairola *et al.*¹⁷, who revealed that *Cassia fistula* was among the most preferred species for the treatment of dysentery and diarrhoea by the Bhoja community of district Dehradun, Uttarakhand, India. This observed antidiarrheal activity may be consequent to the essential chemical constituents reported to be present in the plant. Anthraquinone glucoside, sennosides A and B, glycosides, rhein, barbaloin, aloin, formic acid, butyric acid and their ethyl esters, oxalic acid, pectin and tannin were documented to be present in leave extract^{18,19}. Meanwhile, the antidiarrheal activity of plants extract has been linked to the presence of tannins that form protein tannate to denature

proteins. It creates resistance in the intestinal mucosa and consequently reduces secretion²⁰. Rode *et al.*²¹ and Yacob *et al.*²² both reported that the antidiarrheal activity of plants' extracts is conferred on them by their anti-electrolyte permeability actions. The use of *Cassia fistula* Linn extracts as an antidiarrheal agent has been linked to its ability to counteract the increase in the electrolyte secretion perhaps due to the anti-electrolyte permeability property.

Furthermore, the result of the evaluation of the antibacterial potentials of methanol extract of *Cassia fistula* (Table 4) showed significant growth inhibition of *Escherichia coli* (Gram -ve), *Staphylococcus aureus*, (Gram +ve), *Acinetobacter baumannii* (Gram -ve), *Exiguobacterium aquaticum* (Gram +ve) and *Klebsiella pneumoniae* (Gram +ve). Lower inhibition was observed at $25 \mu\text{g mL}^{-1}$ with 7.32 ± 0.04 , 6.26 ± 0.14 , 5.47 ± 0.13 , 8.45 ± 0.14 and 7.50 ± 0.22 mm, respectively, while the highest inhibition was observed at $1000 \mu\text{g/mL}$ with growth inhibition of 12.59 ± 0.18 , 11.86 ± 0.08 , 11.59 ± 0.12 , 16.58 ± 0.09 and 15.60 ± 0.10 , respectively for all the selected bacterial strains. It is therefore rational to deduce that the extracts showed a substantial antibacterial activity which is comparable to that of chloramphenicol, a standard drug. This result is in trend with the study of Seyyednejad *et al.*²³, who demonstrated the microbial inhibitory effect of methanolic and ethanolic extracts of *Cassia fistula* against 3 Gram-positive: *Bacillus cereus*, *Staphylococcus aureus* and *S. epidermidis* and 5 Gram-negative bacteria. They observed that the plant exhibited substantial antimicrobial activity, especially against *B. cereus*, *S. aureus*, *S. epidermidis*, *E. coli* and *K. pneumoniae*. Dutta and Madharia²⁴ also documented similar results against Gram-positive and Gram-negative pathogenic bacteria. The current result is also in tandem with that of Bhalodia and Shukla²⁵, who demonstrated that extracts of *Cassia fistula* possess remarkable inhibition against bacterial growth. Mohanta *et al.*²⁶ also revealed that biogenic-synthesized silver nanoparticles (AgNPs) derived from an extract of *Cassia fistula* leaf exhibited antimicrobial action. Moreover, in agreement with the result herein, Hetta *et al.*²⁷ and Bhalodia *et al.*⁷ reported similar results on the antimicrobial activity of the plant.

CONCLUSION

The results of this observation suggest that the tested methanol leaves extracts of *Cassia fistula* is an effective candidate for the treatment of diarrhoea as well as

combating bacterial infections. Therefore, *Cassia fistula* leaves extract should be considered as a potential antidiarrheal and antimicrobial agent.

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SIGNIFICANCE STATEMENT

This study discovered the antidiarrheal and antibacterial actions of methanol leaves extracts of *Cassia fistula* that can be beneficial and harnessed for the management of diarrhea and bacteria. This study will help the researchers to uncover the critical aspect of the plant that many researchers were not able to explore. Thus a new theory on the therapeutic action of the plant may be arrived at.

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