



Antidiarrhoeal Studies of Methanol Leaf Extract of *Cassia sieberiana* Dc in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author EOO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author JN managed the analyses of the study. Author IBA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was aimed at evaluating the antidiarrhoeal effect methanol leaf extract of *Cassia sieberiana*.

Methodology: The leaf extract of *Cassia sieberiana* was obtained by extraction using methanol and subjected to phytochemical screening using standard methods. The anti-diarrhoeal effect of methanol leaf extract of *Cassia sieberiana* DC was investigated in Wistar Albino rats.

Results: The result of phytochemical screening revealed the presence of saponins, tannins, phenols, terpenoids, cardiac glycosides, steroids, flavonoids and anthraquinones. The methanol extract significantly ($P < .05$) reduced the number of unformed faeces in castor oil induced diarrhoea in the rats. It also significantly ($P < .05$) reduced the gastrointestinal transit of activated charcoal as well as enteropooling in the rats used for the experiment was significantly reduced ($P < .05$) in the groups treated with 500 mg/kg and 750 mg/kg.

Conclusion: Based on the findings of this research, the methanol leaf extract of *Cassia sieberiana* possess anti-diarrheal activity and therefore validates its use in traditional medicine for the treatment of diarrhoea.

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1. INTRODUCTION

Diarrhoea is a condition in which there is the passage of three or more loose or liquid stools per day or more frequent passage than is normal for an individual [1]. It is usually characterised by frequent passage of watery faeces, involving increased peristaltic movement of the gastrointestinal tract, and an acute reduction in the absorption of fluids, leading to great loss of water, nutrients and electrolyte loss [2]. When a subject has diarrhoea, there is an imbalance in the absorption and secretory mechanisms in the intestinal mucosal, which results in an increase in fluid and electrolyte loss into the gut lumen, leading to the production of unformed liquid faeces and severe dehydration. This can become life threatening especially in malnourished individual [1]. In some severe cases, the stool may also contain mucus, pus, blood or excessive amount of fat. Diarrhoea can be accompanied by painful abdominal cramps, nausea, fever, bloating and generalized weakness. Diarrhoea is usually a symptom of gastrointestinal infection, which can be caused by a variety of bacterial, viral and parasitic organisms. Infection is spread through contaminated food or drinking water or from person to person due to poor hygiene [3].

Every year, on average, nearly 1.7 billion cases of diarrhoea are reported among children under five year and it is the third leading cause of infant and child mortality in developing countries leading to about the death of 1.8 million children die per year [4]. The prevalence of diarrhoea in children Nigeria is 18.8% with 26% of cases treated with oral rehydration salts (ORS) solution. Antibiotics are the major remedy of diarrhoea; however, significant increase in antibiotics resistance has been observed in common human pathogens worldwide [5].

Medicinal plants in traditional and modern medicine form the basis and source of its effectiveness and efficacy. Medicinal plants play a prominent role in disease management in developed as well as in developing countries and form basis for the treatment of many ailments which has been in existence for thousands of years [6]. Traditional medicine is gaining prominence in developing and developed countries owing to its natural origin and less side effects. According to the World Health Organization (WHO), about 80% of the world's

population depend on traditional medicine for their primary healthcare needs [6,7] and their therapeutic value cannot be overemphasised. In fact, it is estimated that two-third of the world population rely on traditional medical remedies due to the toxicity, limited availability and affordability of pharmaceutical products [8]. As such, a lot of people have embarked on the use of indigenous plants. Therefore the search for the safe and more effective agents from plant origin has continued to be of great interest.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh leaves of *Cassia sieberiana* were collected from Gwandagwaji Area in Birnin Kebbi Local Government Area of Kebbi State Nigeria. The leaves were identified and authenticated by a Taxonomist from the Department of Plant Science and Biotechnology, Kebbi State University of Science and Technology, Aliero, with a voucher specimen number VSN96 which was deposited in the Herbarium of the same department.

2.2 Experimental Animals

The Wistar albino rats were purchased from Animal House, Usmanu Danfodiyo University Sokoto. Twenty five (25) healthy Wister albino rats of both sexes weighing 120 – 180 g were used for this study. All protocols were carried out in compliance with NIH guidelines for care and use of Laboratory Animals (Pub. No. 85-23, Revised 1985).

2.3 Qualitative Phytochemical Screening

The phytochemicals present were screened using standard methods [9,10,11,12].

2.4 Antidiarrhoeal Studies

2.4.1 Castor oil induced diarrhoea

Twenty five (25) rats weighing 150-200 g were used for this study. The rats were deprived of feed for 18 hours before the commencement of the experiment but were allowed free access to water. They were divided into 5 groups of five rats each. The first group received 1ml of distilled water. The second group received 5 mg/kg body weight of standard anti diarrhoea drug,

Loperamide. Groups 3 – 5 received 250 mg/kg, 500 mg/kg, and 750 mg/kg doses of the plant extract respectively. One hour after extract treatment, the rats were each given 1ml of castor oil orally, to induce diarrhoea. The rats were observed for 6 hours for watery (wet) or unformed faeces.

The total number of diarrhoeal faeces expelled in the groups were counted at the end of the experiment and a group mean obtained. The total number of diarrhoeal faeces expelled in the test groups was expressed as percentage inhibition of diarrhoea [13].

Calculation:

$$\% \text{ inhibition of faeces in rat} = \frac{(M_o - M)}{M_o} \times 100$$

Where

Mo = Mean number defecation of control

M = mean number defecation of test groups

2.4.2 Castor oil induced enteropooling

Castor oil-induced enteropooling test helps to determine the prevention of fluid accumulation ability of the plant extract. Intraluminal fluid accumulation was determined by the method of Islam [14]. Animals and treatment was similar to castor oil induced diarrhoea model. After one hour, each rat was administered 1 ml of castor oil orally. One hour after the castor oil treatment, the rats were anaesthetized by inhalation of chloroform. The small intestine from the pylorus to the caecum was dissected out and weighed with its content [15]. The contents were then expelled into a graduated cylinder to measure the volume of the intestinal content. Each intestine was reweighed and the difference between the full and the empty intestines was calculated [13].

The percentage inhibition was determined using the formula;

$$\text{Percentage Inhibition (\%)} = \frac{MWICC - MWICT}{MWICC} \times 100$$

Where

MWICC – Mean weight of the intestinal content of the control group

MWICT - Mean weight of the intestinal content of the test group

2.4.3 Gastrointestinal motility test

This test was carried out according to the method of [16]. Seventy five (75) rats were fasted for 18 hours and after which they were divided to 5 groups of 5 rats each. Animals and treatment was similar to castor oil induced diarrhoea model. Then 1 ml of castor oil was given orally to every rat in each group to induce diarrhoea after one hour. After one hour following charcoal meal administration, all the animals were sacrificed and the small intestine was dissected out from the pylorus to the caecum. Thereafter, the distance travelled by the charcoal meal in the intestine from the pylorus to the caecum was measured and was expressed as percentage of distance travelled along the total length of the intestine [17].

$$\text{Distance travelled by charcoal meal (\%)} = \frac{D - D_1}{D} \times 100$$

D = Dist. travelled by charcoal meal in control group

D = Distance travelled by charcoal meal in test groups

2.5 Statistical Analysis

All data were reported as Means ± Standard Error of Mean (SEM). The values were analysed using Statistical Package for Social Sciences (SPSS) 20.0, Duncan Post Hoc. Comparison test were used to check the differences between the individual groups. Test of significance between means were carried out using one-way analysis of variance (ANOVA). Differences in mean was considered significant if P<.05.

3. RESULTS

3.1 Phytochemical Screening

Table 1. Qualitative phytochemical composition of methanol leaves extract of *Cassia sieberiana*

Tests	Inference
Saponins	+
Flavonoids	+
Steroids	+
Phenols	+
Tannins	+
Terpenoids	+
Cardiac glycosides	+
Alkaloid	-
Anthraquinones	+
Xanthoproteins	-

Key: - = Not detected; + = Present

3.2 Antidiarrhoeal Studies

3.2.1 Castor oil induced diarrhoea

The Methanol extract of the leaves of *Cassia sieberiana* (250-750 mg/kg per body weight) and loperamide 5 mg/kg body weight significantly ($P<.05$) protected rats against castor oil induced diarrhoea in a dose dependent manner when compared with the control group. The extract and loperamide provided 79.25 – 97.56 percent protection to the animals against diarrhoea induced by castor oil (Table 2).

3.2.2 Castor oil induced enteropooling

The result of the effect of *Cassia sieberiana* methanol leaf extract on castor oil induced enteropooling in rats is presented in Table 3.

Treatments with 250, 500 and 750 mg/kg body weight of extract and loperamide, the standard antidiarrhoeal drug (5 mg/kg) significantly ($P<.05$) decreased the accumulated fluid when compared with the control group.

3.2.3 Gastro-intestinal motility test

The methanol leaf extract of *Cassia sieberiana* (250-750 mg/kg) given orally significantly ($P<.05$) reduced the gastrointestinal distance travelled by the charcoal meal in rats compared with control. The extract produced a dose dependent decrease of gastrointestinal transit in rats. The gastrointestinal transit of charcoal meal produced by Loperamide (5 mg/kg) was not significantly different ($P>.05$) to that of the group treated with 750 mg/kg of the extract.

Table 2. Effect of methanol leaf extract of *Cassia sieberiana* on castor oil induced diarrhoea in wistar albino rats

Treatment (mg/kg)	Mean number of unformed faeces	Percent Protection (%)
Distilled water + castor oil	4.00±0.37 ^d	0.00
250mg/kg + castor oil	0.83±0.31 ^c	79.25
500mg/kg + castor oil	0.50±0.42 ^b	87.50
750mg/kg + castor oil	0.40±0.59 ^{ab}	92.12
Loperamide (5mg/kg) + castor oil	0.20±0.44 ^a	97.56

Values are expressed as mean ± standard error of mean, n = 5. Mean values having the different superscript letters in a column are significantly different ($P<.05$) (one-way ANOVA followed by Duncan's multiple range test)

Table 3. Effect of methanol leaf extract of *Cassia sieberiana* on castor oil induced enteropooling

Treatment (mg/kg)	Weight of intestine + content (gram)	Weight of empty intestine (gram)	Weight of accumulated fluid (gram)	% of fluid accumulation
Distilled Water + Castor oil	8.00±1.50 ^a	4.88±1.00 ^a	4.06±0.59 ^b	50.75
Loperamide + Castor oil	8.91±0.84 ^b	7.55±0.73 ^b	1.37±0.21 ^a	15.38
250 + Castor oil	8.25±0.89 ^b	5.95±0.71 ^a	2.30 ±0.38 ^{ab}	27.88
500 + Castor oil	8.23±0.19 ^b	6.58±0.37 ^{ab}	1.65±0.23 ^a	2.05
750 + Castor oil	9.45±0.46 ^b	7.44±0.53 ^b	2.01±0.32 ^{ab}	21.27

Values are expressed as mean ± standard error of mean, n = 5. Mean values having the different superscript letters in a column are significantly different ($P<.05$) (one-way ANOVA followed by Duncan's multiple range test)

Table 4. Effect of methanol leaf extract of *Cassia sieberiana* on gastrointestinal transit (motility) of activated charcoal

Treatment (mg/kg)	Total length of Intestine (cm)	Total movement of charcoal (cm)	Percentage distance travelled by charcoal meal (%)
Distilled Water	108.00±3.42 ^a	104.60±2.01 ^c	96.85
250 mg/kg b. wt	109.72±1.65 ^a	79.70±2.81 ^b	72.64
500 mg/kg b. wt	116.40±2.94 ^a	66.12±3.42 ^{ab}	56.80
750 mg/kg b. wt	124.26±1.45 ^b	58.64±3.63 ^a	47.19
Loperamide (5 mg/kg b.wt)	123.40±4.42 ^b	55.22±3.70 ^a	44.75

Values are expressed as mean ± standard error of mean, n = 5. Mean values having the different superscript letters in a column are significantly different ($P<.05$) (one-way ANOVA followed by Duncan's multiple range test)

4. DISCUSSION

Cassia sieberiana is a tropical deciduous small tree and one plant that is widely used for its therapeutic value. Medicinal plants like *Cassia sieberiana* are rich source of novel drugs that form the basic constituents of traditional and orthodox medicine [18]. Scientific studies have shown that the use of plant extracts as therapeutic agents is due to the presence of active chemical compounds in the extract [19]. Flavonoids, saponins and tannins have been reported to have antispasmodic and antidiarrhoea activity [20].

Ricinoleic acid, an unsaturated omega-9 fatty acid and a hydroxyl acid, one of the active components in castor oil, confers it the ability to induce diarrhoea [21]. The ricinoleic acid is poorly absorbed in the small intestines resulting in irritation and inflammation of the intestinal mucosa [22]. This leads to the release of autocoids and prostaglandins which stimulate intestinal motility thereby altering the electrolyte permeability of the intestinal mucosa leading to hypersecretions and diarrhea [23]. In this study, the methanol leaf extract of *Cassia sieberiana* significantly inhibited castor oil induced diarrhoea. It was evident by reduction in total number of wet faeces produced on administration of methanol leaves extract of *Cassia sieberiana*. The antidiarrhoeal activity of methanol Leaves extract of *Cassia sieberiana* is likely due to the active secondary metabolites found in the plant extract like saponins, flavonoids, steroids, phenols, tannins, terpenoids, cardiac glycosides and anthraquinones. Previous studies have shown that antidysenteric and antidiarrhoeal properties of medicinal plants were due to alkaloids, flavonoids, tannins, sterol, saponins, titerpenes and reducing sugars [24,25,26,27]. Flavonoids have been shown to exert antidiarrheal effect by inhibiting intestinal motility and hydro-electrolytic secretion [24].

Since the extract has the ability to inhibit the castor oil induced diarrhoea, the mechanism of antidiarrhoea effect exerted by the extract may be decreased gastrointestinal secretion and/or inhibition of gastrointestinal motility [25]. This could be due to the extract's ability to inhibit electrolyte permeability to the intestine due to castor oil and through the inhibition of prostaglandin release. Suppression of the intestinal fluid accumulation by the extract might also suggest inhibition of gastrointestinal

functions. The stimulated fluid, Na⁺ and K⁺ secretion induced by the castor oil was inhibited by the extract in a dose dependent manner [28]. From this study, it is possible that the extract may mediate its effect through similar mechanism.

5. CONCLUSION

The results indicate that methanol leaves extract of *Cassia sieberiana* possesses significant anti-diarrhoeal activity due to its inhibitory effect both on gastrointestinal propulsion and fluid secretion. In conclusion, the findings in this research work have justified the use of this plant in ethnomedical treatment of diarrhoeal diseases.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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