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Toxicity Profile and Antidiarrhoeal Efficacy of *Cassia singueana* (Del.) Methanol Stem Bark Extract in Rats

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ABSTRACT

Cassia singueana is a medicinal plant used in Northern Nigeria for the treatment and management of ailments which include ulcer, as diuretic, acute malaria, sooth stomach spasm, cure abdominal pain etc. This research study aimed to analyse the phytochemical content and evaluate the acute and antidiarrhoeal activity of the leaf of *Cassia singueana.* Fresh stem bark of the plant was air-dried, extracted using methanol and screened for phytochemicals. Acute toxicity of the crude methanol extract was evaluated using Lorke's method, while the antidiarrhoeal study was carried out using castor oil-induced diarrhoea, gastrointestinal meal transit of charcoal meal and castor oil-induced enteropooling. The crude methanol stem bark extract revealed the presence of carbohydrate, cardiac glycoside, steroid, saponins, flavonoids and terpenes. The extract had oral and *i.p*LD_{50} of ≥ 5000 mg/kg and 2150mg/kg respectively. It alsoproduced a marked anti-diarrhoeal effect in rats. Extract doses at 200 mg/kg, 400 mg/kg, 800 mg/kg, exhibited % protection of 28, 53.30, 69.33against faeces produced upon administration of castor oil; it also slowed down the propulsion of charcoal meal through the gastrointestinal tract with % inhibition of 37.81, 60.09 and 76.60; as well as the extract significantly decreased intestinal fluid with % protection of 48.50, 38.80 and 22.30 volume in rats, when compared to standard antidiarrhoeal drug, loperamide (94.60 %) at 3 mg/kg, atropine (92.50 %) at 3 mg/kg and loperamide (15.90 %) at 3 mg/kg respectively. Thus, the methanol extracts of *Cassia singueana* exhibit anti-diarrhoeal properties, which may be due to the presence of phytochemicals. This study justifies its ethno-medicinal use in the treatment of diarrhoea.

Keywords: *Cassia singueana*; Phytochemicals; Loperamide; Extract; Anti-diarrhoeal

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INTRODUCTION

Diarrhoea is defined by the World Health Organization as having three or more loose or liquid stools per day, or as having more stools than is normal for that person [1]. Diarrhoea has caused several millions of deaths in the world annually [2]. In developing countries, they are the most common causes of morbidity and mortality [3]. Entherotoxigenic *Escherichia coli* (ETEC) are responsible for approximately 380,000 deaths annually [4]. Other bacteria which produce enterotoxins are *Salmonella typhimurium, Clostridium difficile, Clostridium freundii, Aeromonas hydrophyla, Yersinia enterocolitica, Campylobacter jejuni and Vibrio cholerae.* Enterotoxins have their effect on the enterocyte functions by stimulating the secretion of transpithelial electrolytes, thus increasing the osmotic flux of water and ions to the intestinal lumen [5] parasites that cause diarrhea [6]. *E. coli* is also a significant cause of disease among travelers [7].

Developing countries face numerous resource constraints, so that it is necessary to focus on particular interventions that are very expensive and likely to reduce the burden of disease attributable to specific risk factor. Evaluating the risk of diarrhoeal diseases requires knowledge of the complex interactions between biological, socio-economic, behavioral, and environmental factors over the time [8]. Many risk factors have been analyzed, most of them have been done retrospectively and only few of them have been able to associate the risk factors with subsequent incidence of diarrhoea. In view of this problem, the World Health Organization has initiated the Diarrhoea Disease Control Program, which includes studies oftraditional medicinal practices together with the evaluation of health education and prevention approaches [9]. In spite of the importance of diarrhoea as a problem of public health, itis counted by relatively reduced number of drugs for its treatment. Most of them produce undesirable side effects in man [4].

Diarrhoea is an important health problem whole over the world especially in developing countries. Every year, around more than 5-8 million deaths in infant and children under 5 years [10]. Several folklore medicines collected from plant are used to treat infectious disease such as diarrhoea, urinary tract infection, cutaneous abscesses, bronchitis and parasitic diseases [11]. According to the World Health Organization (WHO) about 80% people use plant as traditional medicine [12]. The death caused by diarrhea is highly visible in tropical and subtropical countries [13]. Local people usually relay on various herb source to diarrhea and other infectious diseases. The world Health Organization has encouraged the study for the treatment and prevention diarrhoeal diseases [14].

Cassia Singueana is a leguminous plant which has many medicinal uses throughout Africa15. The leaf of the plant is noted for being effective in the treatment of different ulcer cases by the Fulani and Hausa herbal medicine Practitioners of Northern Nigeria [16]. In Ethiopia, the inner bark of the plant is chewed fresh to sooth stomach spasm and smoke from its wood and bark is used for smoke baths [16]. It is also used in northern Nigeria for the treatment of acute malaria attack [17].

Recent statistics have shown that 75-90% of the rural population in the world rely on herbal medicine for their main healthcare delivery. Different research works have been carried out on different medicinal plants, yet, to the best of our knowledge no research work has been carried out on anti-diarrhoeal activity of *Cassia singueana.* Since the stem bark has been claimed for the treatment of diarrhoea in traditional medicine, this work intends to investigate these attributes with a view to confirming them scientifically.

MATERIALS AND METHODS

Plant Collection and Identification

Fresh stem bark of *Cassia singueana* was collected from Dass Local Government Area (L.G.A) Bauchi State, Nigeria. It was identified and authenticated by a Plant Taxonomist at Biological Science Department, University of Maiduguri, Maiduguri. It was prepared and deposited in the Postgraduate Research Laboratory of Chemistry Department, University of Maiduguri. It was cleaned, air-dried under shade for several days and

pulverized to powder using a mortar and pestle and used for the research work.

Plant Extraction

The air-dried powdered stem bark of *Cassia singueana* (1500 g) was extracted by soxhlet apparatus exhaustively with 95% methanol. The crude extract obtained was concentrated to dryness on a water bath at 45^{0}C.

Experimental Animals

All experiment performed on laboratory animals in this study followed the standard procedure for the treatment of animals. All the animals were handled according to the International Guiding Principles for Biomedical Research Involving Animals [18].

One hundred and ten (110) Wistar strain albino rats of both sexes weighing (120-200g) were obtained from the Zoo, Maiduguri, Borno state for this study. They were kept in well ventilated plastic cages under standard conditions of temperature (25 ^{0}C) and light approximately 12/12 hr (light/day cycle), humidity 65 ± 5% in the Pharmacology, Physiology and Biochemistry Laboratory, Faculty of Veterinary Medicine, University of Maiduguri for two weeks to acclimatized to laboratory condition before the commencement of the experiment. The rats were fed with growers mesh (Sanda*'s* Nig. Ltd) and water was given *ad libitum*.

Drugs used for Pharmacological Studies

Castor-oil Ultra carbon charcoal Atropine (Embassy Pharmaceuticals (Nigeria) and Loperamide (2mg) [Hovid] were used for the experiment.

Acute Toxicity Evaluation (LD_{50})

The acute toxicity LD_{50}) of the methanol stem bark extract of *Cassia singueana* was determined using standard conventional procedure as described by Lorke [19]. In this study, two different routes of administration; oral and intra peritoneal were used. In phase I, rats were divided into 3 groups of three rats each for each route (a total of 18 rats) and then treated with methanol extract at doses of 10, 100, 1000, mg/kg body weight intraperitoenally and orally. They were observed for 24 hrs. For mortality In phase II, the animals in each group (for each route) were divided into three groups of one each. The methanol extract was administrated at doses that were determined after the phase I. The final LD_{50} value was calculated as shown in the equation below.

$LD_{50} = \sqrt{a} \times b$

Where a = least dose that killed a rat

b = highest dose that killed a rat

Antidiarrhoeal Studies of *Cassia singueana*

Effect of Methanol Stem Bark Extract on Castor Oil- induced Diarrhoea

The method of Williamson *et al*. [20]. Was used to evaluate the effect of the methanol stem bark extract on castor oil induced- diarrhoea. Twenty-five (25) Wistar strain albino rats of both sexes weighing between 120-200 g was used for the experiment. The rats were denied food for 12hr but was provided with water. They were divided into five groups of five rat each. Group B, C and D were dosed orally with 400mg/kg, 800mg/kg and 1600 mg/kg of the extract respectively. Group A was given 2 ml normal saline orally. Group E was given 5 mg/kg loperamide intraperitoneally as the standard drug. The rat was separated singly in cages lined with white blotting paper. After one hour, each rat was given 1ml castor oil, orally and observed for 6 hour for wet or watery faeces. The wet faeces of each rat was counted and recorded at the end of the experiment. The percentage (%) protection was calculated using the formula below

$$\% \text{ Protection} = \frac{\text{Mean defection of control} - \text{mean defecation of treated group}}{\text{Mean defecation of control group}} \times 100$$

Effect of the Extract on Gastrointestinal Transit of Charcoal Meal in Rats

The method described by Williamson et al. [20] and Chitme et al. [21] was used to study the effect of the methanol extract oil gastro intestinal transit of charcoal meal in rats. Twenty-five (25) albino rats weighing between 120 - 200 g were used for the experiment. The rats were denied food for eighteen (18) hours but were allowed access to water. They were divided into five groups of five rats each. Group A served as the –ve control and was given 2 ml of normal saline orally. Groups B, C and D were given 200 mg/kg, 400 mg/kg and

800 mg/kg of the methanol extract respectively. Group E was treated with 3mg/kg atropine intraperitoneally as the standard drug. After 10 minutes, 1 ml of charcoal meal (5% activated charcoal) suspension in 10% aqueous solution of cassia powder was given orally to each rat. The rats were sacrificed after 30 minute and the abdomen opened. The distance travelled by the charcoal was measured and expressed as percentage of the total of the length of the intestine. The percentage intestinal transit of the charcoal meal was calculated using the formula:

$$\% \text{ Intestinal transit} = \frac{\text{Movement of charcoal (cm)} \times 100}{\text{Total length of intestine (cm)}}$$

A reduction in the gastro intestinal propulsion of the charcoal meal was indicated as an antidiarhoeal effect [22]; while increase in the gastrointestinal propulsion was indicative of a laxative effect

Effect of Extract on Castor Oil-Induced Enteropooling

The method of Robert *et al.* [23] was used to evaluate the effect of the methanol extract on intraluminal fluid accumulation in rats. Twenty-five (25) albino rats weighing between 120 – 200g was used in this experiment. The rats were fasted overnight and then divided into five groups of five rats each. Group A were served as the –ve control and was given 2 ml normal saline per body weight orally. Groups B, C and D were given 200, 400 and 800 mg/kg orally of the extract respectively. Group E was treated with 3 mg/kg, atropine sulphate intrapentioneally to serve as the +ve control. After one hour, all the rats were sacrificed and the intestine the weight of the empty intestine and the content were measured.

$$\% \text{ Protection} = \frac{(\text{Mean control} - \text{Mean test}) \times 100}{\text{Mean control}}$$

Data Analysis

The statistical analyses were carried out using Graph Pad Prism (computer package). Percentage inhibition and antidirrhoeal effects were expressed as mean ± SEM. Values in all groups were compared using the analysis of variance (ANOVA) followed by Newman Keul post hoc test. For all analyses the level of statistical significance were fixed at $p<0.05$.

RESULTS

Extraction Profile of Powdered Stem Bark of *Cassia singueana*

Table 1shows the profile of the extraction carried out on the stem bark of *Cassia singueana* (1500 kg). Extraction of the stem bark using methanol yielded 367.4g.

Phytochemical Constituents Present in Methanol Extract of *Cassia singueana*

Table 2 shows the result of the phytochemical screening of the extract obtained from the stem bark of *Cassia singueana.* The crude methanol stem bark extract revealed the presence ofcarbohydrate, cardiac glycoside, steroid, saponins, flavonoids and terpenes.

Table 1: The Extraction Profile of Air dried Powdered Stem Bark of *Cassia singueana*

S/N	Fraction	Mass of Air dried Extract (g)	% Yield ($^{w}/_{w}$)	Colour	Texture
1.	Methanol	367.44	24.50	Brown	Powdery

$$\% \text{ Yield} = \frac{\text{Mass of air dried extract} \times 100}{\text{Mass of plant material}}$$

Table 2: Phytochemical Screening of Methanol Extract of *Cassia singueana*

S/No	Active component/ Test	CCM	Observation
1.	**Carbohydrates**		
i.	Molish's test	+	Pupple ppt.
ii.	Barfoed's test	-	No colour change
iii.	Fehling's test	+	Brick red
iv.	Combined reducing sugar	+	Brick red
v.	Ketoses test	+	Rose colouration
2.	**Soluble starch test**	-	No colour change
3.	**Glycoside**		
i.	Free anthraquinones	-	No colour change
ii.	Combined anthraquinones	-	No colour change
4.	**Cardiac glycoside**		
i.	Keller- Killiani's test	+	Purple ring at interphase
5.	**Steroids**		
i.	Salkowski test	+	Red colour at the interphase
6.	**Terpenoids test**		
i.	Lieberman Burchard's test	+	Red colour ring
7.	**Flavonoids**		
i.	Shinoida's test	+	A red colouration
ii.	Ferric chloride test	-	No colour change
iii.	Sodium hydroxide test	-	No colour change
iv.	Lead acetate	-	No colour change
8.	**Saponins**		
i.	Frothing test	+	Frothing which persist > 30 min
9.	**Phlobatannins**	-	
10.	**Tannins**		
i.	Ferric chloride	-	No colour change
ii.	Lead acetate	-	No colour change
11.	**Alkaloids**		
i.	Dragendroff's test	-	No colour change
ii.	Meyer's test	-	No colour change

Key: (+) = Present, (-) = Absent, **CCM=** *Cassia singueana* crude methanol extract, **CCL**= *Cassia singueana* chloroform extract, **CEE=** *Cassia singueana* ethyl acetateextract, **CBT=***Cassia singueana* N-butanol extract.

Antidiarrhoeal Activity of the Stem Bark Extract of *Cassia singueana*

Effect of Methanol Stem Bark Extract of *C. singueana*on Castor Oil-Induced Diarrhoea

The methanol extract of *Cassia singueana* produced a marked anti-diarrhoeal effect in rats. Both doses at 200 mg/kg, 400 mg/kg, 800 mg/kg, significantly decreased the total number of wet faeces produced upon administration of castor oil and this result is similar to the effect of the standard antidiarrhoeal drug,loperamide (3 mg/kg) (Table 3).

Effect of Methanol Stem Bark Extract of *C. singueana* on Intestinal Transit Time in Rat

The methanol extract of *Cassia singueana* also slowed down the propulsion of charcoal meal through the gastrointestinal tract when compare to the control group. Atropine part produced a marked at 3 mg/kg decrease in the propulsive movement and the intestinal length travelled by charcoal meal (Table 4).

Effect of Methanol Stem Bark Extract of *C. singueana*on Castor Oil-Induced Enteropooling

Cassia singueana methanol extractwas found to possess anti-enteropooling activity. The extract significantly decreased intestinal fluid volume in rats. However, the effect of the extract was less potent in comparison to the standard drug, loperamide (Table 5).

Table 3: Effect of Extract on Castor Oil – Induced Diarrhoea in Rats

Extract/Drug	Dose (Mg/kg)	Wt of intestine	Wt. of empty Intestine	Wt of content	%fluid accumulation	% Inhibition
Normal Saline	_	7.51 ±0.80^{a}	2.00 ±0.35^{a}	6.35±0.75^{a}	26.8	0
Crude	200	5.67±0.40^{ab}	1.26 ±0.19^{ae}	3.59±0.41^{ab}	22.04	37.81
Crude	400	5.67±0.49^{abc}	0.80±0.12b^{eg}	3.96±0.43^{abc}	13.95	60.09
Crude	800	5.02±0.30^{abcd}	0.48 ±0.14^{cegh}	3.96±0.43^{abcd}	9.61	76.09
Atropine	3	5.72 ±1.07^{abcd}	0.15 ±0.06^{dfgh}	6.35±1.38^{abcd}	2.71	92.50

Table 4: Effect of Extract on Castor Oil -Induced Enteropooling in Rats

Extract/Drug	Dose (Mg/kg bd.wt	Mean Total Stool±SEM	Mean Total Wet Stool ± SEM	% Protection
Normal Saline	_	16.40 ±1.17^{a}	15.00 ±0.7^{a}	0
Crude	200	13.00 ±0.90^{ae}	10.80 ±0.73^{bf}	28.00
Crude	400	10.40 ±0.68^{beh}	7.00 ±0.71^{cgj}	53.30
Crude	800	4.80 ±1.07^{cfik}	4.60 ±1.17^{dhjl}	69.33
Loperamide	3	0.80 ±0.37^{dgjl}	0.80 ±0.37^{eikm}	94.60

Values along same column differently superscripted differ significantly (P<0.05)

Table 5: Effect of Extract on Intestinal Transmit of Meal in Rat

Extract/Drug	Dose (Mg/kg)	TLP	DMC	% Protection	% inhibition
Normal Saline	2	97.60 ±3.91^{a}	65.60 ±3.30^{a}	66.60	0.00
Crude	200	91.40 ±3.96^{ab}	44.40 ±4.08^{bf}	48.80	27.00
Crude	400	97.80 ±2.75^{abc}	39.00±3.86^{cfi}	38.80	42.90
Crude	800	94.80±2.80^{abcd}	21.20 ±4.33^{dgjl}	22.30	66.50
Atropine	3	98.00 ±5.02^{abc}	15.60 ±4.26^{ehkl}	15.90	76.10

Values along same column differently superscripted differ significantly (P<0.05)

DISCUSSION

Preliminary phytochemical screening of the crude methanol extract of *Cassia singueana* in this study revealed the presence of cardiac glycosides, terpenoids, flavonoids and steroids While alkaloid, tannins, phlobatannin and anthraquinone are absent in the extract. These phytochemicals have been scientifically reported to be responsible for anti-diarrhoeal action by medicinal plants [24].

Flavonoids have been ascribed the ability toinhibit contractions induced by spasmogenics [25]. Flavonoids present in the plant extract are reported to inhibit release of autacoids and prostaglandins, thereby inhibiting motility and secretion induced by

castor oil [26]. The anti-diarrhoeal activity of the extract may also be due to denatured proteins forming protein tannates which make intestinal mucosa more resistant and reduce secretion. Saponin also possesses anti-diarrhoeal activity by inhibiting the release of histamine [27]. Terpenoids derivatives are known to inhibit the release of autocoids and prostaglandins, thereby inhibiting the motivity and the secretion induced by castor-Oil28. Steroid could inhibit prostaglandin induced fluid secretion in the intestine [39].

The acute toxicity (LD_{50}) of the intraperitoneal route evaluated was 2150mg/ kg body weight. Which indicates the extract is considerably not toxic to the animals for the study, while (LD_{50}) of the oral administration which was ≥ 5000 mg/kg body weight, indicates the safety of the plant to local people who use this means of administration for the treatment of diseases. Toxicity potential of a new drug is assessed in the light of the purposed for which it is to be used and the period over which it will be administered. For example, vomiting as a toxic effect will disqualify a drug which is being developed for a minor complaint, but will be accepted in a drug to be used for the treatment of cancer and other life- threatening condition. Also, a toxic effect which develops after prolonged administration may not disqualify a drug intended for acute condition or for only occasional administration [30].

The castor oil-induced diarrhoea model in rats allows the observation of measurable changes in the number of stools. The diarrhoea lasts for at least 8hr [31]. And is a consequence of the action of ricinoleic acid liberated from castor oil by lipase enzymes. Studies on enteropooling showed that the extract reduced both the weight and volume of intraluminal contents. These effects, which are direct consequences of reduced water and electrolytes secretion into the small intestine, suggest that the extract may enhance electrolyte absorption from the intestinal lumen consistent with inhibition of hyper-secretion earlier indicated. However, since electrolyte absorption determines the efficiency of nutrient absorption [32], it is likely that the enhanced electrolyte absorption by the extract may have encouraged the absorption of other intestinal contents. Also, solute absorption in any region of the intestine is a function of the rate of water uptake in that region [33].

In addition, its anti-diarrhoeal action may also be due to the presence of denatured proteins, which form protein tannates. It has been previously demonstrated that protein tannates makethe intestinal mucosa more resistant and hence, reduce secretion and peristaltic movement [34]. This may be because *Cassia singueana* increases the reabsorption of water by decreasing intestinal transit of charcoal meal. It is also possible that in the crude methanol extract may be responsible for the antidiarrhoeal activity. The phytochemicals identified in *Cassia singueana* belong to large diverse groups with varied pharmacological activities.

The methanol extract of *Cassia singueana* showed antidiarrhoeal activity due to the significant reduction in the number of wet feaces or diarrhoea induced by the castor oil. The activity increased with increase in the doses of the extract administered compared to that of the negative control group. The antidiarrhoeal activity of the plant extract was not comparable to the standard drug, loperamide, which at present is one of the most efficacious and widely employed antidiarrhoeal drug. The significant inhibition of the castor oil-induced enteropooling in rats by the extract this suggests that extract produces relief in diarrhoea by spasmolytic activity *in vivo* and also anti-enteropooling effects. The atropine and different doses of the extract decreased the propulsive movement in the charcoal meal study. This is possible due to its anticholinergic effect [35]. The effect of graded doses of *Cassia singueana* extract on intestinal transit using prepared charcoalmeal was examined using atropine as reference drugs, (which has effect on GI). The % movement of the charcoal meal was reduced by the extract ($P<0.05$) in dose dependent manner. In castor oil induced diarrhoea experiment, the extract exhibited the same characteristics as the reference, loperamide by non- production of wet faeces. Inhibition of the intestinal mortility and non- production of wet faeces could be useful in the treatment of diarrhoea.

The anti-diarrhoeal properties of some medicinal plants have been attributed to their phytochemical constituent like tannins and

some flavonoids [10]. The presence of these secondary metabolites alkaloids, saponins, sterols and terpenes are also responsible for the antidiarrhoeal property of the extract [36]. Phytochemical screening on the methanolic extract of *Cassia singueana* showed the presence of some anti-diarrhoeal secondary metabolites. These are, flavonoids, and cardiac glycoside. These phytochemicals could be responsible for the anti-diarrhoeal activities displayed by the extract.

CONCLUSION

This study has demonstrated that the methanol extract of *Cassia singueana* exhibit anti-dirrhoeal properties, which may be due to the presence of phytochemicals present. Thus, the study justifies the ethno-medicinal use of the plant in the treatment of diarrhoea. Further studies on the isolated compounds are necessary to ascertain the possible mechanism(s) of action.

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